Immunogenicity and risk of disease flare after a three-dose regimen of SARS-CoV-2 vaccination in patients with systemic lupus erythematosus: results from the prospective cohort study COVAC-SLE

E.S. Larsen[1-3], A.C. Nilsson[2,3], S. Möller[4,5], A.B. Voss[1,2], I.S. Johansen[2,6]

[1]Department of Rheumatology, Odense University Hospital, Odense; [2]Department of Clinical Research, University of Southern Denmark, Odense; [3]Department of Clinical Immunology, Odense University Hospital, Odense; [4]Open Patient data Explorative Network, Odense University Hospital, Odense; [5]The OPEN Research Unit, Department of Clinical Research, University of Southern Denmark, Odense; [6]Department of Infectious Diseases, Odense University Hospital, Odense, Denmark.

Abstract

Objective

To investigate the humoral immune response and risk of disease flare in systemic lupus erythematosus (SLE) patients following three-doses of SARS-CoV-2 vaccines.

Methods

In adult patients with SLE, we measured SARS-CoV-2 spike IgG in blood samples drawn three weeks after the 1st dose (baseline), four and eight weeks after the 2nd dose and after the 3rd dose. A sufficient antibody response was ≥54BAU/mL. SLEDAI-2K, SLAQ and SDI were assessed at baseline and eight weeks after the 2nd dose along with adverse events. Demographic and treatment data were collected from hospital records.

Results

Of 123 patients, 115 (93.5%) received the BNT162b2 vaccine, the remaining received the 1st dose of ChAdOx-1 followed by a 2nd and 3rd dose of mRNA-1273. After the 2nd dose 102 (83%) patients had a sufficient antibody response (median 559.2, IQR 288.8–1180.5 BAU/mL), increasing to 115 (93.5%) (median 2416.9, IQR 1289–4603.8 BAU/mL) patients after the 3rd dose. Eight weeks after the 2nd dose patients treated with high dose prednisolone (p=0.034) and DMARDs (p<0.001) had significantly lower antibodies; however, this difference was not significant following the 3rd dose. Disease activity and damage were stable during the study period. Adverse events were more frequent in patients with a sufficient response. Breakthrough infections were reported in 39 (31.7%) patients; all with mild symptoms.

Conclusion

A 3rd dose improved the humoral response to SARS-CoV-2 vaccines in patients with SLE to the level of healthy individuals. Vaccination did not affect SLE disease activity. Subsequent breakthrough infections were mild and did not require hospitalisation.

Key words

COVID-19, SARS-CoV-2 vaccine, systemic lupus erythematosus, immunogenicity, safety, antibody response

Emilie S. Larsen, MD
Anna Christine Nilsson, MD
Sören Möller, MSc, PhD
*Anne B. Voss, PhD**
*Isik S. Johansen, DMSc**

**These authors share senior authorship.*

Please address correspondence to:
Emilie Stavnsbjerg Larsen
Department of Rheumatology,
Odense University Hospital,
JB Winsløws Vej,
5000 Odense C, Denmark.
E-mail:
emilie.stavnsbjerg.larsen2@rsyd.dk
ORCID iD: 0000-0002-8459-1682

Received on April 7, 2022; accepted in revised form on May 23, 2022.

Funding: the study was supported by a grant from The Danish Rheumatism Association for the funding of blood analyses.

Competing interests: none declared.

Introduction

A two-dose regimen of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike messenger RNA (mRNA) vaccines prevents COVID-19 in 94-95% of healthy adults (1, 2). Patients receiving immunosuppressive therapy were excluded from these studies, but new studies have shown an impaired humoral immune response against SARS-CoV-2 virus after a two-dose regimen of mRNA vaccines in patients with systemic lupus erythematosus (SLE) (3-7).

Patients with SLE have an increased risk of infections partly due to the use of immunosuppressive treatment, but the inherent immune dysfunction in SLE also seems to increase the risk of severe infections (8-10). A Danish study found an increased risk of hospitalisation with COVID-19 in patients with connective tissue diseases including SLE (11). Furthermore, a survey from Northern Italy found that COVID-19 was three times more frequent in patients with SLE compared to the general population (12), emphasising the need for preventive strategies in this group of patients.

A meta-analysis has previously found a reduced immune response to the influenza vaccine among SLE patients; however, vaccination did not affect the SLE disease activity (13). Interestingly, patients treated with mild immunomodulatory drugs such as hydroxychloroquine, seem to have a preserved immune response (14). Risk factors for a diminished humoral response to influenza and pneumococcal vaccination were treatment with high doses of prednisolone, high SLE disease activity and treatment with rituximab (13, 14).

The durability of a humoral vaccine response against SARS-CoV-2 in SLE patients remains to be determined, including the effects of a third vaccine dose. In one report, patients with autoimmune rheumatic diseases demonstrated good effect of a third dose of Sinovac-CoronaVac COVID-19 vaccine (15). The currently dominating SARS-CoV-2 variant, Omicron, seems to evade the immune defence of previously vaccinated individuals and cause COVID-19 (16), but it remains to be assessed if this also poses an increased risk of infection among patients with SLE.

Our primary aim was to investigate the humoral immune response in patients with SLE following a three-dose regimen of the SARS-CoV-2 vaccines. Secondary aims were to identify risk factors for an insufficient vaccine response, to evaluate whether COVID-19 vaccination had an impact on SLE disease activity and to evaluate the risk of breakthrough infection due to the dominating SARS-CoV-2 variant Omicron.

Methods

Population and setting

This study is an observational prospective, single-centre cohort study of the humoral immune response in patients with SLE following SARS-CoV-2 vaccination. All adult patients aged ≥18 years with SLE in the Region of Southern Denmark, fulfilling the revised classification criteria for SLE by the American College of Rheumatology (ACR), were invited to participate. COVID-19 disease prior to enrolment was not an exclusion criterion for participation.

The BNT162b2 (Pfizer) and mRNA-1273 (Moderna) are approved for use in Denmark by the Danish health authorities. The ChAdOx-1 (AstraZeneca) was offered in the vaccination program between February 4, and March 11, 2021, in Denmark. Patients who received the first dose of ChAdOx-1 subsequently received two doses of mRNA-1271. Patients vaccinated with BNT162b2 received three doses of this vaccine.

Baseline characteristics

We collected clinical information from the patients' hospital records regarding SLE and comorbidities such as malignancy or metabolic diseases, body mass index (BMI), immunosuppressive treatment, dates of SARS-CoV-2 vaccinations, type of vaccine and results of PCR testing for SARS-CoV-2. If the patients received prednisolone treatment, daily doses were registered as <7.5 mg or ≥7.5, the latter was defined as high dose prednisolone.

Blood samples

A total of 4 blood-samples were drawn from each patient: sample 1 was drawn

three weeks after the 1st vaccine dose (baseline), sample 2 and 3 were drawn four and eight weeks after the 2nd dose and sample 4 was drawn four to eight weeks after the 3rd vaccine dose (Fig. 1).

Serological analysis of SARS-CoV-2 antibodies

SARS-CoV-2 spike receptor-binding domain (RBD) IgG antibodies were determined in plasma samples, using the SARS-CoV-2 IgG II Quant assay (Abbott Laboratories). This assay has shown excellent correlation with the first WHO International Standard for anti-SARS-CoV-2 immunoglobulin (NIBSC code 20/136), enabling issuing of immunogenicity results in standardised units, binding antibody units (BAU)/mL. The mathematical relationship of the Abbott arbitrary units (AU)/mL unit to the WHO BAU/mL unit follows the equation BAU/mL=0.142xAU/mL, corresponding to a detection cut-off at ≥7.1 BAU/mL.

To identify patients who were convalescent after COVID-19 infection prior to the study, SARS-CoV-2 nucleocapsid IgG antibodies were determined in baseline plasma samples, using the qualitative SARS-CoV-2 IgG assay (Abbott Laboratories).

Definition of responders and a sufficient humoral vaccine response

Based on the description of the analysis above, responders to the vaccine had a spike IgG ≥7.1 BAU/mL; thus, non-responders had an undetectable spike IgG <7.1 BAU/mL.

However, this cut-off does not predict protection against infection. Based on a study from Khoury *et al*. (17), a sufficient protective level of spike IgG antibodies was defined as IgG ≥54 BAU/mL, correlating to a reduced risk of infection of 50%. Thus, sufficient responders are defined as patients with a spike IgG ≥54 BAU/ml, while insufficient responders had spike IgG <54 BAU/mL. The stratification of patients as either sufficient or insufficient responders is based on the spike antibody response after the second dose of vaccine.

Assessment of SLE disease activity and damage

The patients had two visits at an outpatient clinic during the study: visit one at baseline between the 1st and 2nd dose of vaccine and visit two eight weeks after the 2nd dose. A Systemic Lupus Activity Questionnaire (SLAQ) was sent to the included patients via e-mail in order to assess self-reported disease activity within a few days after both visits (18). SLEDAI-2K (Systemic Lupus Erythematosus Disease Activity Index 2000) and SDI (Systemic Lupus International Collaborating Clinics (SLICC)/American College of Rheumatology (ACR) Damage Index) were assessed by a medical doctor at both visits. The SLEDAI-2K score allows the clinician to quantify the range of disease activity and predicts survival, thus suitable for use in clinical trials (19). The SDI records damage in patients with SLE regardless of its cause predicts survival and is a reproducible tool to access damage (20-22).

Data on adverse events following vaccination were obtained by interview at visit two and reported as either local or systemic adverse events. Occurrence of adverse events are reported collectively; we did not differentiate whether the events occurred after the 1st or 2nd vaccine dose. We have no data on adverse events after the 3rd dose of vaccine.

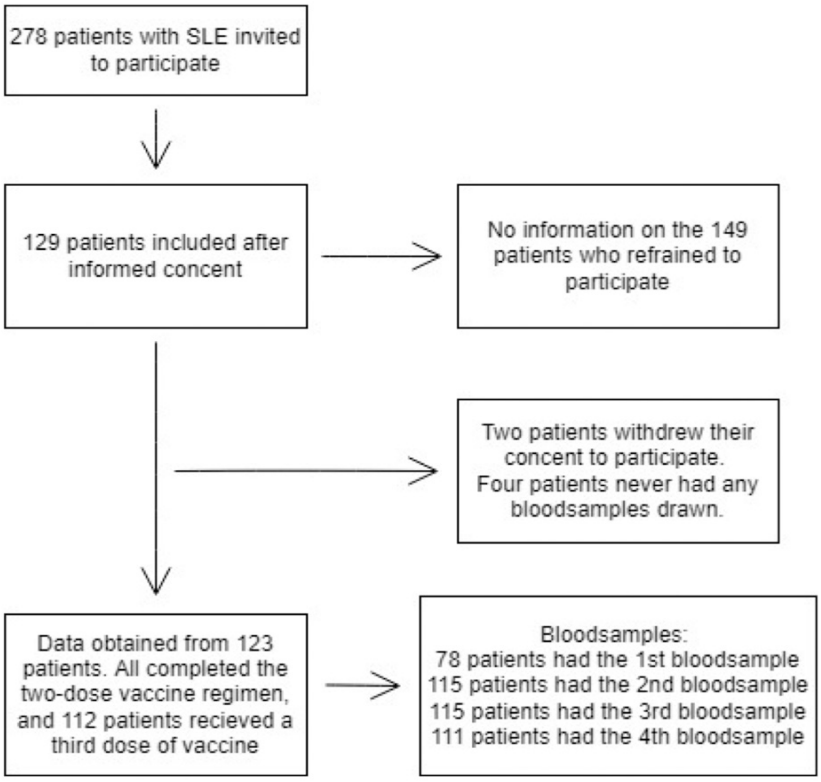

Fig. 1. Enrolment flowchart.

Statistical analysis

Continuous variables were described as medians with interquartile ranges (IQR) or described as means and compared using an unpaired t-test when the observations were normally distributed. Categorical variables were described as proportions (percentages) and compared using χ^2 test or Fischer's exact test for low counts. Spike IgG concentrations were analysed by mixed effects linear regression models, including a random intercept to consider repeated measurements from the same patient. All statistical tests were two-tailed and considered significant at $p<0.05$. Statistical analyses were carried out using Stata, version 17 (StataCorp LP, Collage Station, Texas).

Ethical considerations

The study was approved by The Danish Data Protection Agency (21/13088) and the Danish Ethical Committee in the Region of Southern Denmark (111801-21). We conducted the study according to the declaration of Hel-

sinki and reported data according to STROBE (Strengthening the reporting of observational studies in epidemiology) guidelines.

Results

A total of 278 SLE patients were invited to the study and 129 (46%) patients consented to participate. Details on enrolment and participation are presented in Figure 1. In total, 123 patients completed the two-dose vaccine regimen, and 112 (91.1%) patients received three vaccine doses.

None of the patients had a positive PCR test for COVID-19 before entering the study, and none of the patients had SARS-CoV-2 nucleocapsid IgG antibodies at baseline, indicating no convalescents after a subclinical COVID-19 infection prior to entering the study.

The median age was 51 years (IQR: 42–63) and most of the patients (89.4%) were females. Table I presents characteristics at baseline for all patients overall and stratified by the SARS-CoV-2 spike antibody response after the 2nd dose of vaccine. The sufficient response rate was 82.9% (102 patients). There were no significant differences in baseline demographics and clinical characteristics when comparing patients with sufficient and insufficient antibody responses, and this did not change after the 3rd dose of vaccine.

After the 2nd dose of vaccine nine (7.3%) patients did not have a detectable antibody response. Compared to patients with a detectable antibody response, these non-responders did not differ in regard of age (p=0.097), sex (p=0.23) or SLEDAI-2K at baseline (p=0.772). However, non-responders were more likely to have been vaccinated with the BNT162b2 vaccine (p=0.047) and were more frequently treated with high dose prednisolone (p=0.036).

Overall, 20 (16.3%) patients did not receive immunosuppressive or immunemodulating treatment for SLE. In total, 89 (72.4%) patients were treated with hydroxychloroquine, in 31 (25.2%) patients as monotherapy. A total of 35 (28.5%) patients were treated with prednisolone, hereof 6 (17.2%) with high daily doses of prednisolone. Patients with an insufficient antibody response were more likely to receive high doses of prednisolone (66.7% *vs.* 6.9%, p<0.001). Description of other immunosuppressive treatments are shown in Table I.

Table I. Baseline demographic and clinical characteristics of patients after the second dose of vaccine.

Characteristics	Overall (%)	Insufficient responders (%)	Sufficient responders (%)	p-value
n	123	21 (17.1)	102 (82.9)	
Age in years (median, (IQR))	51 (42-63)	60 (50-64)	51 (41-63)	0.102
Sex (females)	110 (89.4)	17 (80.95)	93(912.)	0.165
SLE disease duration in years (median, (IQR))	15 (6-25)	16.5 (8.1-26.5)	13 (6-23)	0.312
High SLE disease activity (SLEDAI ≥ 4)	49 (39.8)	9 (45)	40 (38.8)	0.606
Obesity (BMI>30)	23 (18.7)	4 (19.05)	19 (18.63)	0.964
Current smoker	19 (15.5)	5 (25)	14 (13.9)	0.415
Active malignant disease	2 (1.6)	1 (4.8)	1 (1)	0.192
Vaccine				0.623
BNT162b2	115 (93.5)	19 (90.5)	96 (94.1)	
ChAdOx-1 + mRNA-1273	8 (6.5)	2 (9.5)	6 (5.9)	
Immunosuppressive treatment				
No treatment	20 (16.3)	1 (4.8)	19 (18.6)	0.117
Prednisolone	35 (28.5)	6 (28.6)	29 (28.4)	0.990
High dose Prednisolone (≥7.5 mg daily)	6 (17.2)	4 (66.7)***	2 (6.9)***	0.000
Hydroxychloroquine monotherapy	31 (25.2)	0	31 (30.4)	0.003
Other DMARD*	58 (47.2)	14 (66.7)	44 (43.1)	0.490
Biologic treatment**	8 (6.5)	2 (9.5)	6 (5.9)	0.488

*Methotrexate, azathioprine, mycophenolic acid, mycophenolate mofetil. Not including hydroxychloroquine. Including two patients treated with tacrolimus.
**Four patients treated with TNF-α inhibitors, two patients with belimumab and two patients with rituximab. ***Of patients treated with prednisolone.

After the 3rd dose of vaccine, 115 (93.5%) patients had spike IgG antibodies ≥54 BAU/mL. In detail, among the 21 (17.7%) insufficient responders after the 2nd dose of vaccine, 13 patients mounted an antibody response ≥54 BAU/mL following the 3rd dose of vaccine, while six remained <54 BAU/mL (missing data on two patients) (Fig. 2). Of the nine non-responders after the 2nd dose, six patients achieved a detectable antibody response ≥7.1 BAU/mL after the 3rd dose of vaccine (missing data on two patients).

Blood samples were drawn in relation to vaccinations as follows: following the 1st vaccine dose baseline blood samples were drawn after median 18 days (IQR: 15.5–21) in 78 patients, following the 2nd vaccine dose two consecutive blood samples were drawn after median 31 days (IQR: 28–33) and median 63 days (IQR: 60–68) in 115 patients, and following the 3rd vaccine dose blood samples were drawn after median 50 days (IQR: 38–62) in 111 patients.

Serum concentrations of SARS-CoV-2 spike IgG antibodies are trend depicted in Figures 2, 3 and 4. The overall median antibody concentration was 975.4 BAU/mL (IQR: 319–2597.5) four weeks after the 2nd dose of vaccine. We calculated a rapid decline about 50% of antibody concentration eight weeks after the 2nd dose of vaccine. A marked increase in median antibody concentration of 1221.3 BAU/mL (IQR: 348.2–2837.1) was observed following the 3rd dose. In total, 64 (58.7%) patients had at least a three-fold increase in antibody concentration. The specific antibody concentrations are shown in Table II. Patients receiving biologic treatment at baseline showed only a minor increase in antibody concentrations after the 3rd dose of vaccine (Table II, Fig. 4).

Most patients (115, 93.5%) received the BNT162b2 vaccine. The remaining eight (6.5%) patients received the first dose of ChAdOx-1 vaccine and two doses of the mRNA-1273 vaccine thereafter. There was no difference in antibody concentrations when stratified on patients receiving the BNT162b2

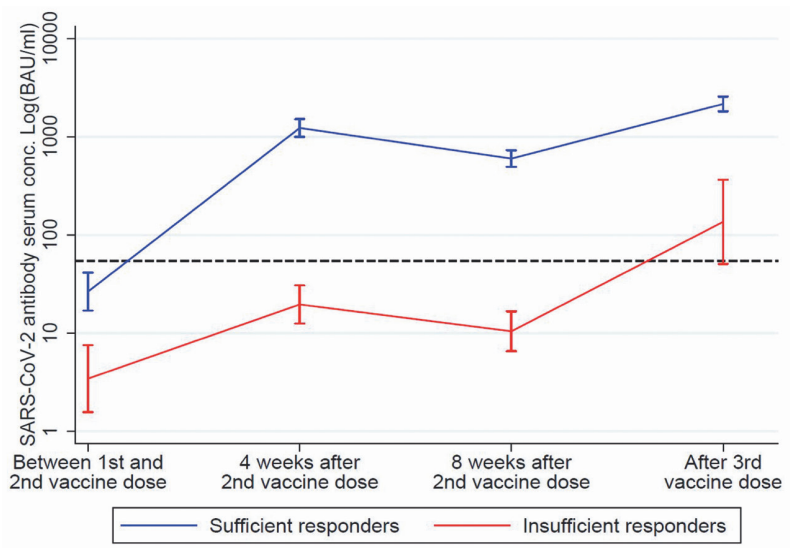

Fig. 2. SARS-CoV-2 spike IgG antibody concentrations during follow-up, stratified by patients with a sufficient (blue) and insufficient (red) SARS-CoV-2 spike IgG antibody response. The dashed line indicated the defined sufficient spike IgG cut-off at ≥54 BAU/mL.

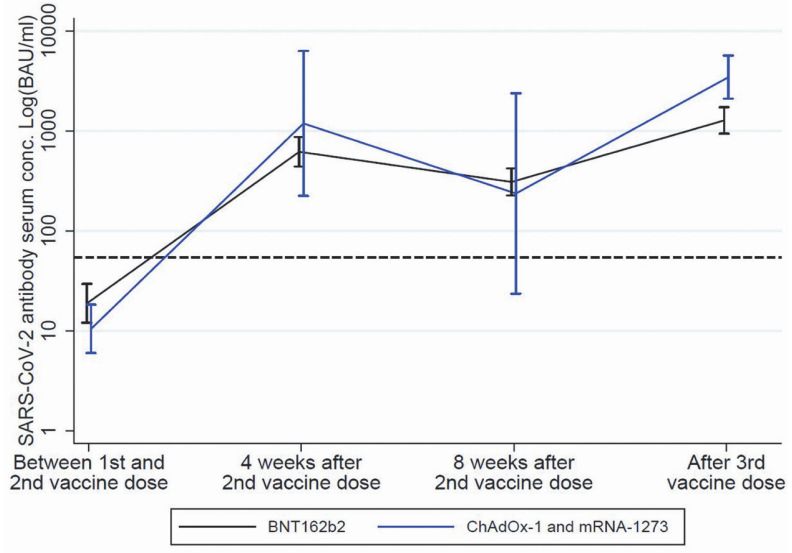

Fig. 3. SARS-CoV-2 spike IgG antibody concentrations during follow-up, stratified by patients receiving the BNT162b2 (Pfizer) vaccine compared to patients receiving a combination of ChAdOx-1 (AstraZeneca) and mRNA-1273 (Moderna) vaccines. The dashed line indicates the defined sufficient spike IgG cut-off at ≥54 BAU/mL.

vaccine compared to patients receiving a combination of ChAdOx-1 and mRNA-1273 vaccines (p=0.623). The largest reduction in spike IgG antibodies was observed in patients receiving the combination of ChAdOx-1 and mRNA-1273 vaccines (55.6%, 1478.4 BAU/mL), resulting in approximately equal spike IgG antibody concentrations between the groups eight weeks after the 2nd vaccine dose (Fig. 3) with an equal increase in spike IgG antibodies after the 3rd vaccine dose.

Patients who received either no medical treatment or hydroxychloroquine monotherapy had the highest SARS-CoV-2 spike IgG antibody concentrations, shown in Figure 4 and Table II. Patients who received biologic treatment, DMARD or high dose prednisolone had a significantly lower antibody response compared to patients with hydroxychloroquine monotherapy at four weeks after the 2nd dose of vaccine (p=0.002, p<0.001 and p<0.001). Eight weeks after the 2nd dose of vaccine this difference was only significant for high dose prednisolone (p=0.034) and DMARD (p<0.001). Similar significant differences were observed when compared to patients who did not receive medical treatment.

During the period from November 27, 2021, to March 8, 2022, 39 (31.7%) patients tested PCR positive for SARS-CoV-2. Subtyping was performed in seven patients (two patients with Delta variant, five patients with Omicron variant). There was no difference in risk of breakthrough infections between sufficient or insufficient responders (p=0.451). Patients with breakthrough infections had median spike IgG antibodies of 2114.2 BAU/mL (IQR: 1242.2–3764.8 BAU/mL) after the 3rd dose of vaccine, and this was not significantly different from patients without infection (median 1815.5 BAU/mL, IQR: 747.9–3927.6 BAU/mL) (p=0.755). In addition, there were no significant differences in demographic characteristics regarding age (p=0.060), sex (p=0.508) or treatment among patients with breakthrough infections compared to those without infection (p=0.942). One patient was treated with remdesivir for five days. None of the infected patients required hospital admission.

The SLAQ disease activity was reported by 119 patients and the score at baseline was 3 (IQR; 1–4) and remained 3 (IQR; 1–5) (reported by n=115) at the second visit 8 weeks after the 2nd vaccine dose. There was no difference between in SLAQ activity score between sufficient and insufficient responders.

The majority had low disease activity with a SLEDAI-2K score of ≤4 at baseline, with no increase after the 2nd vaccine dose. There was no difference in SDI score from baseline to the second visit.

Adverse effects after vaccination are presented in Table III. More patients with a sufficient antibody response experienced any adverse effect compared to patients with an insufficient response; statistically significant for overall adverse effects (91.3% *vs.* 75%, p=0.036), local (85.4% *vs.* 65%, p=0.029) and systemic adverse effects (67% *vs.* 40%, p=0.022).

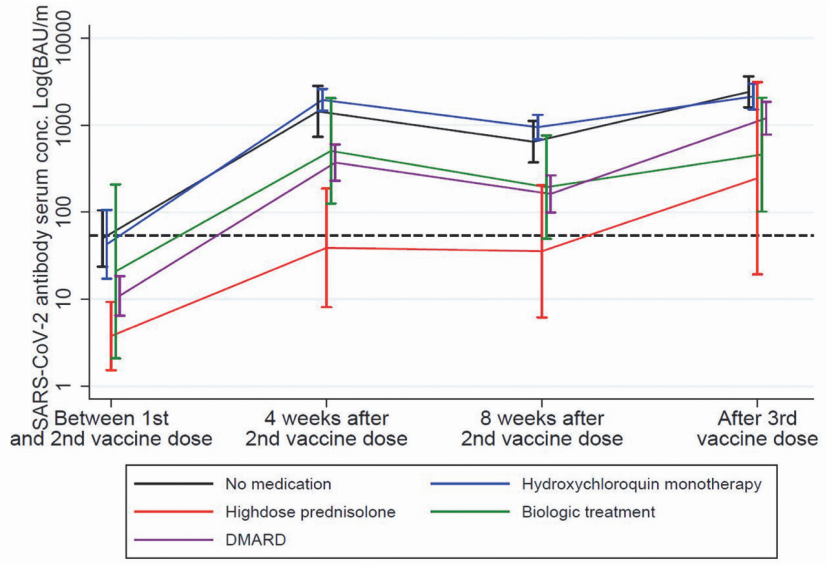

Fig. 4. SARS-CoV-2 Spike IgG antibody concentrations during follow-up, stratified by treatment for SLE at baseline. The dashed line i¡ndicates the defined sufficient spike IgG cut-off at ≥54 BAU/mL.

Discussion

In this prospective cohort study of 123 patients with SLE we found that 83.7% of the patients mounted a sufficient SARS-CoV-2 spike IgG antibody response eight weeks following the 2nd dose of vaccine. Antibody concentrations declined rapidly from four to eight weeks after the 2nd vaccine dose. However, the response rate increased to 93.5% following the 3rd dose of vaccine, comparable to the level of healthy individuals. Furthermore, we found that high dose of daily prednisolone treatment was significantly associated with a poorer antibody response, while patients treated with hydroxychloroquine monotherapy had an antibody response at least as good as patients without treatment after the 2nd dose.

It is assumed that antibody reactivity correlates with virus neutralisation and hence immunity, but it is important to note that correlates of protection, and most importantly an absolute protective threshold, are yet to be established. Khoury *et al*. (17) estimated a 50% protective level against COVID-19 at ≥54 BAU/mL (95% CI 30–96 BAU/mL). To our knowledge, this is one of the first studies to report an estimate of protective antibody concentrations in standardised units. Recently, Feng *et al*. (23) reported that the antibody concentration associated with 80% vaccine efficacy (ChAdOx1 nCoV-19) against COVID-19 was 506 BAU/mL for an anti-spike RBD IgG assay. According to these first estimates, we chose to define a sufficient protective concentration of SARS-CoV-2 IgG antibodies, as IgG ≥54 BAU/mL.

Patients with SLE were reported to be at higher risk of SARS-CoV-2 infection and severe course of disease. Danish colleagues reported that patients with SLE had an almost threefold increase in incidence of hospitalisation with COVID-19 compared to the general population (24). This is in spite the fact that patients with inflammatory rheumatic diseases in Denmark seem to be more cautious and are more likely to self-isolate than others of the same age (25). In addition, Ammitzbøll *et al*. (3) found that 23% of patients with rheumatoid arthritis (n=73) and SLE (n=61) did not have any serological detectable response after two doses of the BNT162b2 vaccine. Our results support these findings; however, only two patients were treated with Rituximab in our study, compared to 17 in the abovementioned.

Despite the hypothesis that hydroxychloroquine could inhibit SARS-CoV-2 *in vitro*, it has been thoroughly determined that SLE patients treated with hydroxychloroquine were not protected against infections (26-29). On the other

Table II. SARS-CoV-2 spike IgG antibody concentrations (BAU/ml) during the study period.

	Baseline (n=78) (median (IQR))	4 weeks after the 2nd dose vaccine (n=115) (median (IQR))	8 weeks after the 2nd dose vaccine (n=115) (median (IQR))	4-8 weeks after the 3rd dose vaccine (n=111) (median (IQR))
Overall	16.55 (ND*-66.8)	975.4 (319-2597.5)	486.5 (192.8-1049.7)	2039.8 (884.1-3927.6)
Sufficient responders	29.2 (ND*-73.1)	1322.9 (583.2-2713.1)	559.2 (288.8-1180.5)	2416.9 (1289-4603.8)
Insufficient responders	ND*	16.8 (ND*-27.5)	9.15 (ND*-14.5)	147 (21.3-1112.2)**
	Baseline (median (IQR))	4 weeks after 2nd vaccine (median (IQR))	8 weeks after 2nd vaccine (median (IQR))	4-8 weeks after the 3rd dose vaccine (median (IQR))
No treatment (n=20)	66.8 (17.4-162.3)	1660.2 (795.1-4128.8)	784.4 (409.5-1171)	2741.3 (1658.5-4664.5)
Hydroxychloroquine monotherapy (n=31)	51.9 (17.8-195.3)	1629.8 (1101.6-1629.8)	919.6 (541.5-1585)	2329.6 (1335.8-5680)
Biologic treatment (n=8)	25.6 (ND*-108.6)	1218.4 (356.5-1990.7)	393.7 (91.7-826.3)	863.6 (190.8-2584.9)
DMARDs (n=58)	ND* (ND*-40.7)	503.4 (137.7-1352.6)	270.6 (67.1-535.8)	1803.4 (766.1-3764.8)
High dose prednisolone (n=6)	ND* 26.95 (ND*-388.4)	9.3 (ND*-253.45)	469.2 (21.3-2232.4)	

*number not detectable, value below 7.1 . **13 patients who had an insufficient antibody response after the second dose of vaccine mounted an antibody response ≥54 BAU/mL after the third dose of vaccine.

Table III. Adverse effects following vaccination reported after completed two-dose vaccine regimen.

	Overall (%)	Insufficient responders (%)	Responders (%)	*p*-value
Local at site of injection	101 (82.1)	13 (65)	88 (85.4)	0.029
Redness	13 (10.6)	0	13 (12.6)	
Swelling	20 (16.3)	2 (10)	18 (17.5)	
Soreness	101 (82.1)	13 (65)	88 (84.4)	
Systemic	77 (62.6)	8 (40)	69 (67)	0.022
Muscle pain	34 (27.6)	3 (15)	31 (30.1)	
Joint pain	21 (17.1)	3 (15)	18 (17.5)	
Fatigue	66 (53.7)	8 (40)	58 (56.3)	
Chills	15 (12.2)	1 (5)	14 (13.6)	
Headache	39 (31.7)	6 (30)	33 (32)	
Nausea	17 (13.8)	3 (15)	14 (13.6)	
Fever	16 (13)*	4 (20)	12 (11.7)	

*Hereof, five patients with mild fever (tp. 38-38.5), five patients with moderate fever (38.5-38.9) and six patients with high fever (≥39.0).

hand, our findings suggest a preserved humoral response in patients treated with hydroxychloroquine monotherapy (Fig. 2). A similar effect was seen associated to influenza vaccination (14).

Patients treated with high doses of prednisolone are particularly at risk of a severe course of COVID-19. Glucocorticoid treatment >10 mg/day is reported to be associated with higher odds for hospitalisation with COVID-19 (30), however, an association was not observed when not adjusting for the glucocorticoid dose (31). Although we present only a small subgroup of patients treated with prednisolone, we found an increased risk of insufficient antibody response against SARS-CoV-2 vaccines among patients receiving ≥7.5 mg prednisolone daily in accordance with the results reported by Deepak *et al*. (32) in patients with autoimmune inflammatory rheumatic diseases, including 15 (11.3%) patients with SLE.

It has previously been a concern whether vaccination, especially influenza vaccination, poses a risk of disease flare in patients with SLE (13), and this potential risk has been reported as a common reason for SLE patients to refrain from vaccination (33). We found no significant change in SLE disease activity or damage after COVID-19 vaccination, which is in concordance with the results from Moyon *et al*. (4). A survey investigation among 183 SLE patients found that BNT162b2 vaccination in general was safe; >91% of patients did not experience flare-ups and adverse events were as common in SLE patients as in the general population post-SARS-CoV-2 vaccination (1, 34).

Despite the high humoral antibody response rate of 93.5% after the 3rd vaccine dose, one third of the SLE patients tested PCR positive for SARS-CoV-2 in the following 3 months. This is probably due to the Danish health authorities' strategy including widespread COVID testing of the population even among individuals without symptoms of COVID-19 infection. The fact that none of the SLE patients were admitted to hospital and the absence of a difference in frequency of positive PCR tests between responders and non-responders supports this explanation.

The currently dominating SARS-CoV-2 variant Omicron is characterised by several mutations in the RBD, which increases resistance against the neutralising spike antibodies after vaccination (35, 36). Breakthrough infections with Omicron is well described, but a high-titered antibody response after vaccination is expected to protect against severe COVID-19 (37). Likewise, all patients in this study only showed mild symptoms of COVID-19. SARS-CoV-2 virus subtyping was not performed in most patients with breakthrough infections. It is, however, reasonable to assume that these patients were infected by the Omicron variant because this was the dominating virus subtype at that time.

Duration of the antibody response following vaccination is yet to be determined. The Danish Health Authorities recommended a 4th vaccine dose to prioritised target groups in higher risk of severe COVID-19 infections, including SLE patients. The first publications about a 4th vaccine dose have been published for some immunocompromised patients with promising results (38, 39) but remains to be assessed in patients with SLE.

The humoral response against vaccines is well described in the literature since it is easy to measure specific antibodies. On the contrary, there is no clearly defined correlate of cellular responses. The importance of a humoral or a cellular response in infectious diseases varies with the specific microorganism (40) and it has been suggested that the cellular response plays a crucial role in COVID-19 (41-43). A limitation to this study is our inability to describe the T-cell response. However, a good correlation between the cellular and humoral response has been described in healthy adults (44), and in SLE patients receiving the BNT162b2 vaccine (4, 7).

A limitation of our study was selection bias as only approximately 50% of patients with SLE consented to participate. We had no information about patients who refrained from participating; however, we presumed that they might have had higher disease activity, might have been older and/or might not have wished to be vaccinated. Another limitation was the lack of an age balanced control group, since age is a factor known to have a deleterious effect on the vaccine induced antibody response.

In conclusion, patients with SLE had a compromised humoral response to the SARS-CoV-2 vaccines compared to the general population after the 2nd dose of vaccine, however, this difference was equalised after a 3rd dose. Use of high daily doses of prednisolone was found to be associated with an insufficient humoral response. Treatment with hydroxychloroquine monotherapy preserved an antibody response at least as good as in patients without immunosuppressive treatment. The vaccines did not affect disease activity or damage. Vaccine-breakthrough infections were seen frequently and mainly with the Omicron variant, however, all with mild infection.

Acknowledgment

The authors thank the patients who participated in the study, without whom these results could not be achieved.

Key messages

- Patients with SLE have an impaired antibody response to the SARS-CoV-2 vaccination after the second dose compared to the general population. The third dose improved the humoral response.
- Daily dose of prednisolone ≥7.5 mg is a risk factor for an insufficient humoral response.
- SARS-CoV-2 vaccination did not affect disease activity or damage in patients with SLE.
- Breakthrough infections were mild and did not require hospitalisation.

References

1. POLACK FP, THOMAS SJ, KITCHIN N *et al*.: Safety and efficacy of the BNT162b2 mRNA Covid-19 vaccine. *N Engl J Med* 2020; 383(27): 2603-15. https://doi.org/10.1056/nejmoa2034577
2. BADEN LR, EL SAHLY HM, ESSINK B *et al*.: Efficacy and safety of the mRNA-1273 SARS-CoV-2 vaccine. *N Engl J Med* 2021; 384(5): 403-16.
3. AMMITZBØLL C, BARTELS LE, BØGH ANDERSEN J *et al*.: Impaired antibody response to the BNT162b2 messenger RNA Coronavirus disease 2019 vaccine in patients with systemic lupus erythematosus and rheumatoid arthritis. *ACR Open Rheumatol* 2021; 3(9): 622-8. https://doi.org/10.1002/acr2.11299
4. MOYON Q, STERLIN D, MIYARA M *et al*.: BNT162b2 vaccine-induced humoral and cellular responses against SARS-CoV-2 variants in systemic lupus erythematosus. *Ann Rheum Dis* 2022; 81(4): 575-83. https://doi.org/10.1136/annrheumdis-2021-221097
5. RUDDY JA, CONNOLLY CM, BOYARSKY BJ *et al*.: High antibody response to two-dose SARS-CoV-2 messenger RNA vaccination in patients with rheumatic and musculoskeletal diseases. *Ann Rheum Dis* 2021; 80(10): 1351-2. https://doi.org/10.1136/annrheumdis-2021-220656
6. FURER V, EVIATAR T, ZISMAN D *et al*.: Immunogenicity and safety of the BNT162b2 mRNA COVID-19 vaccine in adult patients with autoimmune inflammatory rheumatic diseases and in the general population: a multicentre study. *Ann Rheum Dis* 2021; 80(10): 1330-8. https://doi.org/10.1136/annrheumdis-2021-220647
7. IZMIRLY PM, KIM MY, SAMANOVIC M *et al*.: Evaluation of immune response and disease status in systemic lupus erythematosus patients following SARS-CoV-2 vaccination. *Arthritis Rheumatol* 2022; 74(2): 284-94. https://doi.org/10.1002/art.41937
8. BARBER MRW, CLARKE AE: Systemic lupus erythematosus and risk of infection. *Expert Rev Clin Immunol* 2020; 16(5): 527-38. https://doi.org/10.1080/1744666x.2020.1763793
9. FELDMAN CH, MARTY FM, WINKELMAYER WC *et al*.: Comparative rates of serious infections among patients with systemic lupus erythematosus receiving immunosuppressive medications. *Arthritis Rheumatol* 2017; 69(2): 387-97. https://doi.org/10.1002/art.39849
10. SIMARD JF, ROSSIDES M, GUNNARSSON I, SVENUNGSSON E, ARKEMA EV: Infection hospitalisation in systemic lupus in Sweden. *Lupus Sci Med* 2021; 8(1): e000510. https://doi.org/10.1136/lupus-2021-000510
11. CORDTZ R, LINDHARDSEN J, SOUSSI BG *et al*.: Incidence and severeness of COVID-19 hospitalisation in patients with inflammatory rheumatic disease: a nationwide cohort study from Denmark. *Rheumatology* (Oxford) 2021; 60 (S1): S159-67. https://doi.org/10.1093/rheumatology/keaa897
12. BOZZALLA CASSIONE E, ZANFRAMUNDO G, BIGLIA A, CODULLO V, MONTECUCCO C, CAVAGNA L: COVID-19 infection in a northern-Italian cohort of systemic lupus erythematosus assessed by telemedicine. *Ann Rheum Dis* 2020; 79(10): 1382-3. https://doi.org/10.1136/annrheumdis-2020-217717
13. PUGÈS M, BISCAY P, BARNETCHE T *et al*.: Immunogenicity and impact on disease activity of influenza and pneumococcal vaccines in systemic lupus erythematosus: a systematic literature review and meta-analysis. *Rheumatology* (Oxford) 2016; 55(9): 1664-72. https://doi.org/10.1093/rheumatology/kew211
14. BORBA EF, SAAD CG, PASOTO SG *et al*.: Influenza A/H1N1 vaccination of patients with SLE: can antimalarial drugs restore diminished response under immunosuppressive therapy? *Rheumatology* (Oxford) 2012; 51(6): 1061-9. https://doi.org/10.1093/rheumatology/ker427
15. AIKAWA NE, KUPA LVK, MEDEIROS-RIBEIRO AC *et al*.: Increment of immunogenicity after third dose of a homologous inactivated SARS-CoV-2 vaccine in a large population of patients with autoimmune rheumatic diseases. *Ann Rheum Dis* 2022; 81(7): 1036-43. https://doi.org/10.1136/annrheumdis-2021-222096
16. KUHLMANN C, MAYER CK, CLAASSEN M *et al*.: Breakthrough infections with SARS-CoV-2 omicron despite mRNA vaccine booster dose. *Lancet* 2022; 399(10325): 625-6. https://doi.org/10.1016/s0140-6736(22)00090-3
17. KHOURY DS, CROMER D, REYNALDI A *et al*.: Neutralizing antibody levels are highly predictive of immune protection from symptomatic SARS-CoV-2 infection. *Nat Med* 2021; 27(7): 1205-11. https://doi.org/10.1038/s41591-021-01377-8
18. KARLSON EW, DALTROY LH, RIVEST C *et al*.: Validation of a Systemic Lupus Activity Questionnaire (SLAQ) for population studies. *Lupus* 2003; 12(4): 280-6. https://doi.org/10.1191/0961203303lu332oa
19. GLADMAN DD, IBAÑEZ D, UROWITZ MB: Systemic Lupus Erythematosus Disease Activity Index 2000. *J Rheumatol* 2002; 29(2): 288-91.
20. GLADMAN D, GINZLER E, GOLDSMITH C *et al*.: The development and initial validation of the Systemic Lupus International Collaborating Clinics/American College of Rheumatology damage index for systemic lupus erythematosus. *Arthritis Rheum* 1996; 39(3): 363-9. https://doi.org/10.1002/art.1780390303
21. GLADMAN DD, UROWITZ MB, GOLDSMITH CH *et al*.: The reliability of the Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index in patients with systemic lupus erythematosus. *Arthritis Rheum* 1997; 40(5): 809-13. https://doi.org/10.1002/art.1780400506
22. NIVED O, JÖNSEN A, BENGTSSON AA, BENGTSSON C, STURFELT G: High predictive value of the Systemic Lupus International Collaborating Clinics/American College of Rheumatology damage index for survival in systemic lupus erythematosus. *J Rheumatol* 2002; 29(7): 1398-400.
23. FENG S, PHILLIPS DJ, WHITE T *et al*.: Correlates of protection against symptomatic and asymptomatic SARS-CoV-2 infection. *Nat Med* 2021: 27(11): 2032-40. https://doi.org/10.1038/s41591-021-01540-1
24. CORDTZ R, KRISTENSEN S, DALGAARD LPH *et al*.: Incidence of COVID-19 hospitalisation in patients with systemic lupus erythematosus: a nationwide cohort study from Denmark. *J Clin Med* 2021; 10(17): 3842. https://doi.org/10.3390/jcm10173842
25. GLINTBORG B, JENSEN DV, ENGEL S *et al*.: Self-protection strategies and health behaviour in patients with inflammatory rheumatic diseases during the COVID-19 pandemic: results and predictors in more than 12 000 patients with inflammatory rheumatic diseases followed in the Danish DANBIO registry. *RMD Open* 2021; 7(1): e001505. https://doi.org/10.1136/rmdopen-2020-001505
26. ZURITA MF, IGLESIAS ARREAGA A, LUZURIAGA CHAVEZ AA, ZURITA L: SARS-CoV-2 infection and COVID-19 in 5 patients in Ecuador after prior treatment with hydroxychloroquine for systemic lupus erythematosus. *Am J Case Rep* 2020; 21: e927304. https://doi.org/10.12659/ajcr.927304
27. KONIG MF, KIM AH, SCHEETZ MH *et al*.: Baseline use of hydroxychloroquine in systemic lupus erythematosus does not preclude SARS-CoV-2 infection and severe COVID-19. *Ann Rheum Dis* 2020; 79(10): 1386-8. https://doi.org/10.1136/annrheumdis-2020-217690
28. MATHIAN A, MAHEVAS M, ROHMER J *et al*.: Clinical course of coronavirus disease 2019 (COVID-19) in a series of 17 patients with systemic lupus erythematosus under long-term treatment with hydroxychloroquine. *Ann Rheum Dis* 2020; 79(6): 837-9. https://doi.org/10.1136/annrheumdis-2020-217566
29. PUXEDDU I, FERRO F, BARTOLONI E *et al*.: COVID-19: the new challenge for rheumatologists. One year later. *Clin Exp Rheumatol* 2021; 39(1): 203-13. https://doi.org/10.55563/clinexprheumatol/si106e
30. GIANFRANCESCO M, HYRICH KL, AL-ADELY S *et al*.: Characteristics associated with hospitalisation for COVID-19 in people with rheumatic disease: data from the COVID-19 Global Rheumatology Alliance physician-reported registry. *Ann Rheum Dis* 2020; 79(7): 859-66. https://doi.org/10.1136/annrheumdis-2020-217871
31. FREITES NUÑEZ DD, LEON L, MUCIENTES A

et al.: Risk factors for hospital admissions related to COVID-19 in patients with autoimmune inflammatory rheumatic diseases. *Ann Rheum Dis* 2020; 79(11): 1393-9. https://doi.org/10.1136/annrheumdis-2020-217984

32. DEEPAK P, KIM W, PALEY MA *et al*.: Effect of immunosuppression on the immunogenicity of mRNA vaccines to SARS-CoV-2: a prospective cohort study. *Ann Intern Med* 2021; 174(11): 1572-85. https://doi.org/10.7326/m21-1757
33. CHEHAB G, RICHTER JG, BRINKS R, FISCHER-BETZ R, WINKLER-ROHLFING B, SCHNEIDER M: Vaccination coverage in systemic lupus erythematosus-a cross-sectional analysis of the German long-term study (LuLa cohort). *Rheumatology* (Oxford) 2018; 57(8): 1439-47. https://doi.org/10.1093/rheumatology/key120
34. BARBHAIYA M, LEVINE JM, SIEGEL CH, BYKERK VP, JANNAT-KHAH D, MANDL LA: Adverse events and disease flares after SARS-CoV-2 vaccination in patients with systemic lupus erythematosus. *Clin Rheumatol* 2022: 41(5): 1619-22. https://doi.org/10.1007/s10067-021-05963-6
35. LIU Z, VANBLARGAN LA, BLOYET LM *et al*.: Identification of SARS-CoV-2 spike mutations that attenuate monoclonal and serum antibody neutralization. *Cell Host Microbe* 2021; 29(3): 477-88.e4. https://doi.org/10.1016/j.chom.2021.01.014
36. CHEN J, WANG R, GILBY NB, WEI GW: Omicron variant (B.1.1.529): infectivity, vaccine breakthrough, and antibody resistance. *J Chem Inf Model* 2022; 62(2): 412-22. https://doi.org/10.1021/acs.jcim.1c01451
37. HAVERI A, SOLASTIE A, EKSTRÖM N *et al*.: Neutralizing antibodies to SARS-CoV-2 Omicron variant after third mRNA vaccination in health care workers and elderly subjects. *Eur J Immunol* 2022; 52(5): 816-24. https://doi.org/10.1002/eji.202149785
38. ABBASI J: Fourth COVID-19 vaccine dose increases low antibodies. *JAMA* 2022; 327(6): 517. https://doi.org/10.1001/jama.2022.0727
39. ALEJO JL, MITCHELL J, CHIANG TP *et al*.: Antibody response to a fourth dose of a SARS-CoV-2 vaccine in solid organ transplant recipients: a case series. *Transplantation* 2021; 105(12): e280-e1. https://doi.org/10.1097/tp.0000000000003934
40. POLLARD AJ, BIJKER EM: A guide to vaccinology: from basic principles to new developments. *Nat Rev Immunol* 2021; 21(2): 83-100. https://doi.org/10.1038/s41577-020-00479-7
41. KARLSSON AC, HUMBERT M, BUGGERT M: The known unknowns of T cell immunity to COVID-19. *Sci Immunol* 2020; 5(53): eabe8063. https://doi.org/10.1126/sciimmunol.abe8063
42. ELKAYAM O, BASHKIN A, MANDELBOIM M *et al*.: The effect of infliximab and timing of vaccination on the humoral response to influenza vaccination in patients with rheumatoid arthritis and ankylosing spondylitis. *Semin Arthritis Rheum* 2010; 39(6): 442-7. https://doi.org/10.1016/j.semarthrit.2008.12.002
43. BENUCCI M, INFANTINO M, MAROTTO D, ARDIZZONE S, MANFREDI M, SARZI-PUTTINI P: Vaccination against SARS-CoV-2 in patients with rheumatic diseases: doubts and perspectives. *Clin Exp Rheumatol* 2021; 39(1): 196-202. https://doi.org/10.55563/clinexprheumatol/7afn90
44. SAHIN U, MUIK A, DERHOVANESSIAN E *et al*.: COVID-19 vaccine BNT162b1 elicits human antibody and T(H)1 T cell responses. *Nature* 2020; 586(7830): 594-9. https://doi.org/10.1038/s41586-020-2814-7