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Anti–severe acute respiratory syndrome coronavirus 2 spike receptor-binding domain antibody levels in patients with systemic lupus erythematosus based on vaccination status and related factors in Indonesia

Laniyati Hamijoyo[1,2], Patrick Philo[2,3], Daniel Setiawan Nathan[3,4], Marita Restie Tiara[4], Sofie Razyanti Mardiana[2], Evan Susandi[3], Nadia Gita Ghassani[2], Bachti Alisjahbana[4,5]

[1]Rheumatology Division, Internal Medicine Department, [2]Lupus Study Group, Immunology Study Center, [3]Internal Medicine Department, [4]Research Center for Care and Control of Infectious Disease, and [5]Tropical Infectious Disease Division, Internal Medicine Department, Faculty of Medicine, Padjadjaran University, Bandung, Indonesia

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Corresponding author: Laniyati Hamijoyo, MD
Rheumatology Division, Internal Medicine Department, Faculty of Medicine, Padjadjaran University, Bandung, Indonesia
Tel: +62-222034955, Fax: +62-222032216
E-mail: hamijoyo@yahoo.com; laniyati.hamijoyo@unpad.ac.id

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Purpose: We aim to analyze the proportion and level of coronavirus disease 2019 (COVID-19) seropositivity in patients with systemic lupus erythematosus (SLE) and explore factors associated with lower anti–severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike receptor-binding domain (S-RBD) antibody levels.

Materials and Methods: A cross-sectional study involving patients with SLE was conducted. We included those aged 18–60 years, either unvaccinated or had received inactivated vaccine (CoronaVac; Sinovac Biotech Ltd., China). Furthermore, participants were tested for anti–SARS-CoV-2 S-RBD antibody levels and SARS-CoV-2 surrogate virus neutralization test, and comparative test analysis was employed.

Results: This study included 159 subjects of whom 92 and 67 were SLE subjects and controls, respectively. Significantly higher seropositive results were noted in patients with SLE receiving vaccine (96.9% versus 3.1%). Unvaccinated SLE patients receiving cyclophosphamide (CYC) had higher anti-RBD levels compared to unvaccinated SLE patients not receiving CYC (23.81 [interquartile range (IQR), 2.26–78.85] versus 2.13 [IQR, 0.1–12.5]), whereas vaccinated SLE patients receiving CYC had lower anti-RBD levels compared to vaccinated SLE patients not receiving CYC (15.5 [IQR, 6.62–35.09] and 69.77 [IQR, 17.48–201]). In the vaccinated SLE group, a lower value of anti–SARS-CoV-2 RBD antibody levels was observed in patients receiving mycophenolate mofetil and those with chronic kidney disease. No correlation was noted between disease activity and organ involvement with lower antibody response.

Conclusion: The increase in COVID-19 antibody levels in patients with SLE may be affected by exposure to hospital settings and vaccine. Furthermore, CYC treatment is associated with lower antibody response after receiving vaccine.

Keywords: Systemic lupus erythematosus, Anti–SARS-CoV-2 S-RBD antibody, Cyclophosphamide, Neutralization test, CoronaVac

Introduction

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease characterized by its relapsing and remitting nature, systemic inflammation, and multiple organ involvement, thereby requiring the use of immunosuppressant drugs. Owing to the aberrant

immune responses inherent to the disease, predisposing them to more severe disease and worse prognosis, patients with SLE are prone to infection, including coronavirus disease 2019 (COVID-19) [1,2]. A previous study from Global Rheumatology Alliance showed that SLE is the second most common diagnosis in patients with rheumatic disease with COVID-19 infection, with a higher number of patients with SLE with COVID-19 infection requiring hospitalization (11% not hospitalized versus 17% hospitalized) [3].

Currently, vaccination against COVID-19 is the preventive measure for controlling its spread and reducing associated complications. Patients taking immunosuppressants (those receiving transplantation or those diagnosed with chronic inflammatory disease) are more susceptible to severe COVID-19 manifestations, and this population might benefit from COVID-19 vaccination. However, vaccine immune responses can be affected by several factors, including intrinsic host factors (age, sex, genetics, nutritional status, gut microbiota, obesity, and immune status), extrinsic factors (drug consumption), infectious agents (genetic variation), and vaccine factors (vaccine type, adjuvant, dose, route of administration, and schedule) [4,5]. The use of immunosuppressants including mycophenolate mofetil (MMF) and corticosteroids frequently noted in patients with SLE may induce apoptosis of T cells, inhibit cytokine production by T cells, and decrease B cell proliferation. These drugs are associated with immune response impairment, thereby leading to inadequate antibody production. The response level is needed to develop a good strategy for vaccinating this patient group [6,7]. There are several modalities commonly used to measure antibody response in vaccinated patients, including spike protein receptor-binding domain (RBD), surrogate virus neutralization test (SVNT), and antigen-specific T cell production of interferon-γ (IFN-γ) [8].

Studies evaluating the immunogenicity of the COVID-19 vaccine in patients with SLE and studies investigating factors affecting antibody levels in patients with SLE receiving COVID-19 vaccination remain lacking. Therefore, this study aimed to analyze the rate of COVID-19 seropositivity in patients with SLE and explore their related factors.

Materials and Methods

Study design and participants

This was a cross-sectional observational study involving patients with SLE who visited the rheumatology outpatient clinic at Dr. Hasan Sadikin General Hospital, Bandung, Indonesia, from November 2021 to February 2022. We used total sampling, wherein all patients who met the inclusion criteria that were patients with SLE aged 18–60 years who were either unvaccinated or received inactivated vaccine (CoronaVac; Sinovac Biotech Ltd., Beijing, China) and attended the rheumatology outpatient clinic during the study period. The exclusion criteria were patients diagnosed with coronary heart disease with chronic coronary syndrome class III–IV; heart failure New York Heart Association class III–IV; diabetes mellitus with fasting blood glucose levels of <130 mg/dL or glycated hemoglobin levels of >7.5% in the last 3 months; chronic obstructive pulmonary disease with history of exacerbations in the last 3 months; had fever, cough, or acute shortness of breath during their visits; those who were currently pregnant; and those who received other vaccines besides CoronaVac. CoronaVac vaccination was done between June to November 2021 for control groups and between November 2021 to February 2022 for SLE subject groups. Participants were divided into the unvaccinated and post-vaccinated subjects and controls groups.

Variable definition

SLE diagnosis was assessed on the basis of the 2019 European League Against Rheumatism and the American College of Rheumatology Classification Criteria for Systemic Lupus Erythematosus [9]. Hemolytic anemia was defined as erythrocyte destruction due to the presence of autoantibodies against the erythrocyte, which is characterized by the evidence of hemolysis, including reticulocytosis, low haptoglobin levels, elevated indirect bilirubin levels, elevated lactate dehydrogenase levels, and positive Coomb's (direct antiglobulin) test. Thrombocytopenia was defined as platelet counts of <100,000/mm3 [9]. Serositis was defined as typical pleurisy for more than 1 day or pleural effusions or pleural rub, typical pericardial pain (pain with recumbency improved by sitting forward) for more than 1 day or pericardial effusion or pericardial rub, or pericarditis by electrocardiogram in the absence of other causes, such as infection or uremia [10]. Disease activity was defined using the Systemic Lupus Erythematosus Disease Activity Index-2000 (SLEDAI-2k). A SLEDAI-2k score of ≥4 is considered the presence of active disease activity [11].

Data collection

Eligible patients with SLE who visited our rheumatology outpatient clinic and controls were enrolled. Baseline characteristics including epidemiological, clinical, and laboratory data were collected. These data included age, sex, COVID-19 his-

tory, serologic test results and anti–severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike receptor-binding domain (S-RBD) antibody levels, SARS-CoV-2 SVNT status. For SLE subject groups, we also collected data of disease duration, SLEDAI-2k score, organ involvement, medication status, and smoking status. Medication status was assessed by asking about the patient's daily immunosuppressant intake during clinic visits (methylprednisolone, hydroxychloroquine, cyclosporine, cyclophosphamide [CYC], azathioprine, methotrexate [MTX], and MMF). Organ involvement was assessed from the medical record data on the visit (chronic kidney disease [CKD], neuropsychiatric SLE, hemolytic anemia, thrombocytopenia, and serositis).

All participants were tested for anti–SARS-CoV-2 S-RBD antibody levels. The anti–SARS-CoV-2 total S-RBD antibody level was determined using fluorescence immunoassay technology (FastBioRBD) for the quantitative detection of S-RBD antibody levels of SARS-CoV-2 in human serum or plasma specimen. FastBioRBD anti–SARS-CoV-2 S-RBD antibody test produced by Wondfo, Guangzhou Biotech, Guangzhou, China, and rebranded for distribution by PT Biofarma (Persero), Bandung, Indonesia [12]. The anti-RBD result was expressed in arbitrary unit (AU)/mL. The fluorescent nature of the complex was directly related to anti-RBD results, and the antibody concentration in the sample, with a cutoff value of >1 AU/mL, was defined as positive. Then, we multiplied the result by 20 to obtain the standard World Health Organization (WHO) binding antibody unit [13,14].

For comparison, the GenScript cPass SARS-CoV-2 SVNT (Genscript Biotech, Leiden, the Netherlands) was performed as the gold standard test for measuring the inhibition percentage of antibodies. The test was performed according to the manufacturer's instructions [14]. A positive SVNT result was defined by the cut-off value of ≥30% inhibition. The percentage of signal inhibition to the negative control was calculated as follows [15]: inhibition (%)=[1–(sample optical density [OD]450/average negative control OD450)]×100.

Statistical analysis

Baseline characteristics data were presented as percentages, medians, and interquartile ranges (IQRs). The normality test was performed using the Shapiro-Wilk test for continuous data. Parametric tests were used for normally distributed data, while non-parametric tests were applied for data that did not follow a normal distribution. The Mann-Whitney U test and chi-square were used to analyze the comparisons between baseline characteristics of subject and control groups, medications and anti–SARS-CoV-2 S-RBD antibody levels, organ involvement and anti–SARS-CoV-2 S-RBD antibody levels, disease activity and anti–SARS-CoV-2 S-RBD antibody levels, medications and SARS-CoV-2 SVNT levels, organ involvement and SARS-CoV-2 SVNT levels, and disease activity and SARS-CoV-2 SVNT levels. Furthermore, to test the correlation between anti–SARS-CoV-2 RBD antibody level and anti–SARS-CoV-2 SVNT, rho Spearman correlation analysis was performed. A two-way analysis of variance (ANOVA) test was used to determine whether two independent variables interact (vaccination and CYC) affecting the dependent variable (anti–SARS-CoV-2 S-RBD antibody level). A p-value of <0.05 was considered statistically significant. Statistical analyses were performed using IBM SPSS ver. 23.0 (IBM Corp., Armonk, NY, USA). GraphPad PRISM ver. 9.0 (GraphPad Software, San Diego, CA, USA) was used for creating figures.

This study was approved by the Research Ethics Commission of Dr. Hasan Sadikin General Hospital (410/UN6.KEP/EC/2021), and was conducted in accordance with the Declaration of Helsinki. Written informed consent was waived by the Ethics Committee because of the secondary use of medical data. All data were kept anonymous.

Results

Patient characteristics

A total of 168 subjects were enrolled for the study, with 101 patients with SLE met the inclusion criteria, but nine were excluded owing to having been vaccinated with vaccines besides CoronaVac; therefore, 92 participants were finally included in the subject group, while 67 subjects for the control group. The baseline characteristics for vaccinated and unvaccinated SLE subject and control groups are shown in Table 1. Female patients were significantly higher in proportion for both unvaccinated SLE (95% versus 36.4%, $p<0.001$) and vaccinated SLE (96.9% versus 52.2%, $p<0.001$) compared to control group. We also found Anti SARS-CoV-2 RBD antibody and SARS-CoV-2 SVNT were found higher in vaccinated control group compared to vaccinated SLE subject group without any significant difference (Fig. 1).

The baseline characteristics of the patients in both the unvaccinated and CoronaVac-vaccinated groups are shown in Table 1. Female patients predominated both the groups with a median age of 36 years (IQR, 28.0–43.8) and 37.5 years (IQR, 26.0–44.8) for the unvaccinated and vaccinated groups, re-

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Table 1. Characteristics of the unvaccinated and Coronavac vaccinated SLE and healthy control subjects

Characteristic (n=92)	SLE subjects		Healthy controls		p-value[a]	p-value[b]	p-value[c]
	Unvaccinated (n=60)	Vaccinated (n=32)	Unvaccinated (n=44)	Vaccinated (n=23)			
Age (yr)	36.0 (28.0–43.8)	37.5 (26.0–44.8)	29.5 (25.0–39.8)	40.8 (30.4–43.8)	0.03*	0.543	0.834
Female sex	57 (95.0)	31 (96.9)	16 (36.4)	12 (52.2)	0.001**	0.001**	1.000
Disease durations (yr)	5.3 (3.0–9.4)	4.5 (2.6–16.5)					0.496
SLEDAI-2k score	4.5 (2.0–10.0)	4 (0.3–8.0)					0.328
SLEDAI-2k ≥4	34 (56.7)	17 (53.1)					0.369
COVID-19 history	6 (10.0)	3 (9.4)	9 (20.5)	8 (34.8)	0.163	1.00	1.000
Anti–SARS-CoV-2 RBD antibody (BAU/mL)	4.0 (0.1–20.5)	54.3 (12.2–150.9)	4.7 (0.3–49.4)	72 (13.8–179.7)	0.06	0.73	0.000**
High (>25.25)	11 (18.3)	21 (65.6)	17 (38.6)	16 (69.6)			
Moderate (8.25–25.25)	11 (18.3)	6 (18.8)	1 (2.3)	3 (13.0)			
Low (1.00–8.25)	13 (21.7)	4 (12.5)	11 (25.0)	3 (13.0)			
Negative (<1.00)	25 (41.7)	1 (3.1)	15 (34.1)	1 (4.4)			
Anti–SARS-CoV-2 RBD antibody seropositive	35 (58.3)	31 (96.9)	29 (65.9)	23 (95.7)	0.54	0.40	0.000**
SARS-CoV-2 SVNT	17.76 (0.1–46.9)	67.7 (34.9–93.2)	67.7 (35.0–93.2)	84.4 (57.7–96.8)	0.93	0.12	0.000**
Positive inhibition	21 (35.0)	25 (78.1)	25 (78.1)	12 (92.3)			0.000**
Organ involvement							
Chronic kidney disease	30 (50.0)	18 (56.3)					0.568
NPSLE	14 (23.3)	9 (28.1)					0.613
Hemolytic anemia	18 (30.0)	8 (25.0)					0.612
Thrombocytopenia	9 (15.0)	3 (9.4)					0.532
Serositis	8 (13.3)	1 (3.1)					0.115
Medication							
Methylprednisolone ≥6 mg/day	19 (31.7)	4 (12.5)					0.043*
Hydroxychloroquine	41 (68.3)	18 (56.3)					0.250
Cyclosporin	10 (16.7)	8 (25.0)					0.337
Cyclophosphamide	13 (21.7)	6 (18.8)					0.742
Azathioprine	38 (63.3)	18 (56.3)					0.507
Methotrexate	6 (10.0)	3 (9.4)					1.000
Mycophenolic mofetil	31 (51.7)	12 (37.5)					0.195

Values are presented as median (interquartile range) or number (%) unless otherwise stated.
SLE, systemic lupus erythematosus; SLEDAI-2k, Systemic Lupus Erythematosus Disease Activity Index-2000; COVID-19, coronavirus disease 2019; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; RBD, receptor-binding domain; BAU, binding antibody unit; SVNT, surrogate virus neutralization test; NPSLE, neuropsychiatric systemic lupus erythematosus.
*p<0.05. **p<0.001. [a]p-value comparing unvaccinated SLE subjects and unvaccinated controls. [b]p-value comparing vaccinated SLE subjects and vaccinated controls. [c]p-value comparing unvaccinated and vaccinated SLE subjects.

spectively. Most-vaccinated patients showed positive results for anti–SARS-CoV-2 S-RBD antibodies (96.9% versus 3.1%) and SARS-CoV-2 SVNT (78.1% versus 21.9%), with a significant difference observed between both groups (p<0.000). Meanwhile, unvaccinated participants showed a higher proportion of negative anti–SARS-CoV-2 S-RBD antibodies (n=25, 41.7%), with more than half of them showing negative SARS-CoV-2 SVNT (n=39, 65%). Regarding organ involvement, kidney involvement (50% versus 56.3%) was observed higher in the vaccinated group. According to data on medication, the unvaccinated group had more participants who consumed high-dose methylprednisolone (≥6 mg/day) (31.7% versus 12.5%), CYC (21.7% versus 18.8%), and MMF (51.7% versus 37.5%) than the vaccinated group. Furthermore, we performed a correlation study between anti–SARS-CoV-2 S-RBD antibody levels and SARS-CoV-2 SVNT, as the gold standard procedure for antibody measurement. The correlation showed a significant result (p<0.001, r=0.903) (Fig. 2).

Comparison between medications and anti–SARS-CoV-2 S-RBD antibody levels or SARS-CoV-2 neutralization antibody based on SVNT in the unvaccinated and post-vaccinated participants

We compared medications and anti–SARS-CoV-2 S-RBD anti-

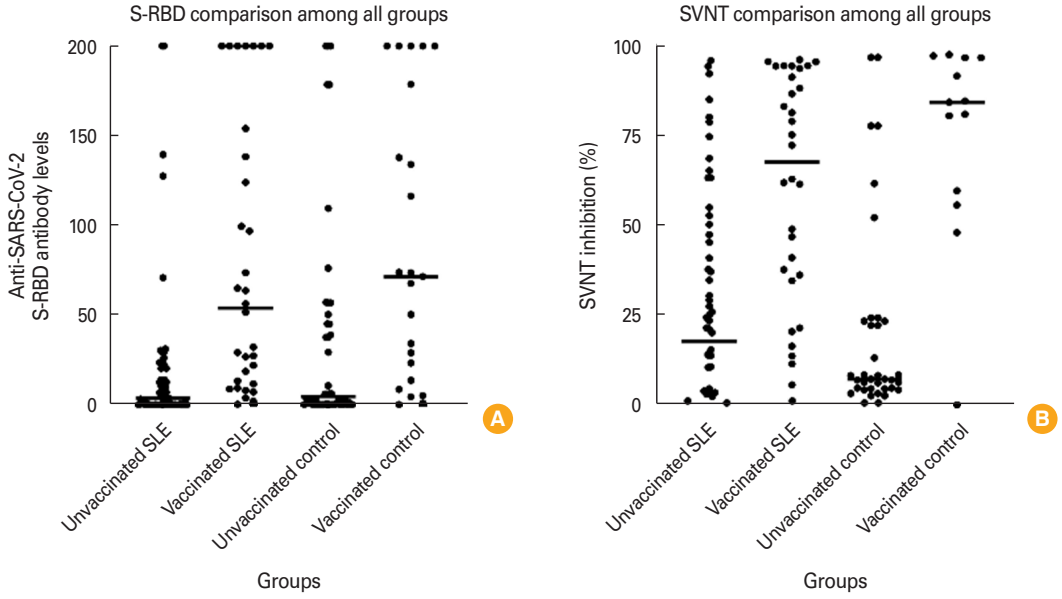

Fig. 1. Comparison between anti–severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) S-receptor-binding domain (RBD) antibody levels **(A)** and SARS-CoV-2 surrogate virus neutralization test (SVNT) **(B)** in systemic lupus erythematosus (SLE) patients and healthy controls.

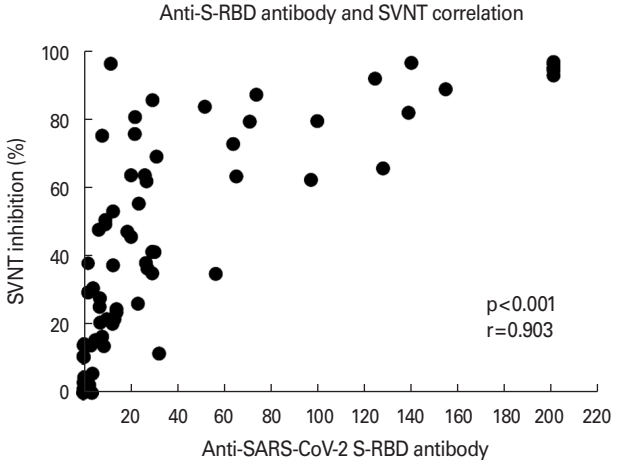

Fig. 2. Spearman correlation between anti–severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike receptor-binding domain (S-RBD) antibody and SARS-CoV-2 surrogate virus neutralization test (SVNT) (inhibition percentage) in participants with systemic lupus erythematosus (SLE).

body levels in both groups. In both unvaccinated and vaccinated groups, CYC was the only drug that showed significant results (unvaccinated group, p=0.007; post-vaccinated group, p=0.029). However, CYC intake showed a conflicting result in both groups, with anti–SARS-CoV-2 S-RBD antibody levels being higher in those receiving CYC in the unvaccinated group (23.81 [IQR, 2.26–78.85] versus 2.13 [IQR, 0.1–12.5]), whereas anti–SARS-CoV-2 S-RBD antibody levels were lower in those receiving CYC in the vaccinated group (15.5 [IQR, 6.62–35.09] and 69.77 [IQR, 17.48–201.00]) (Fig. 3).

Mann-Whitney analysis showed a significant interaction between CYC intake and vaccination (p=0.0082), resulting in a reduction in S-RBD antibody levels. In the vaccinated group, a lower value of anti–SARS-CoV-2 S-RBD antibody levels was noted in those who received MMF (40.72 [IQR, 10.42–71.90] and 82.08 [IQR, 13.52–201.00]); however, it did not show a significant result.

The neutralizing antibody (nAB) showed a similar trend with anti–SARS-CoV-2 S-RBD antibody level analysis, which showed a higher inhibition percentage in unvaccinated patients with SLE receiving CYC, whereas in the vaccinated group, a lower inhibition percentage was noted in patients with SLE receiving CYC (Table 2).

Comparison between organ involvement and anti–SARS-CoV-2 S-RBD antibody levels or SARS-CoV-2 neutralization antibody based on SVNT in unvaccinated and post-vaccinated participants

Analysis between organ involvement and anti–SARS-CoV-2 S-RBD antibody levels in both groups showed no significant results. However, a lower value of anti–SARS-CoV-2 S-RBD antibody levels among patients with CKD in the post-vaccinated group (40.72 [IQR, 11.17–104.24] and 82.08 [IQR, 22.12–201.00]) was noted. On SARS-CoV-2 SVNT analysis, no significant results were noted (Table 3).

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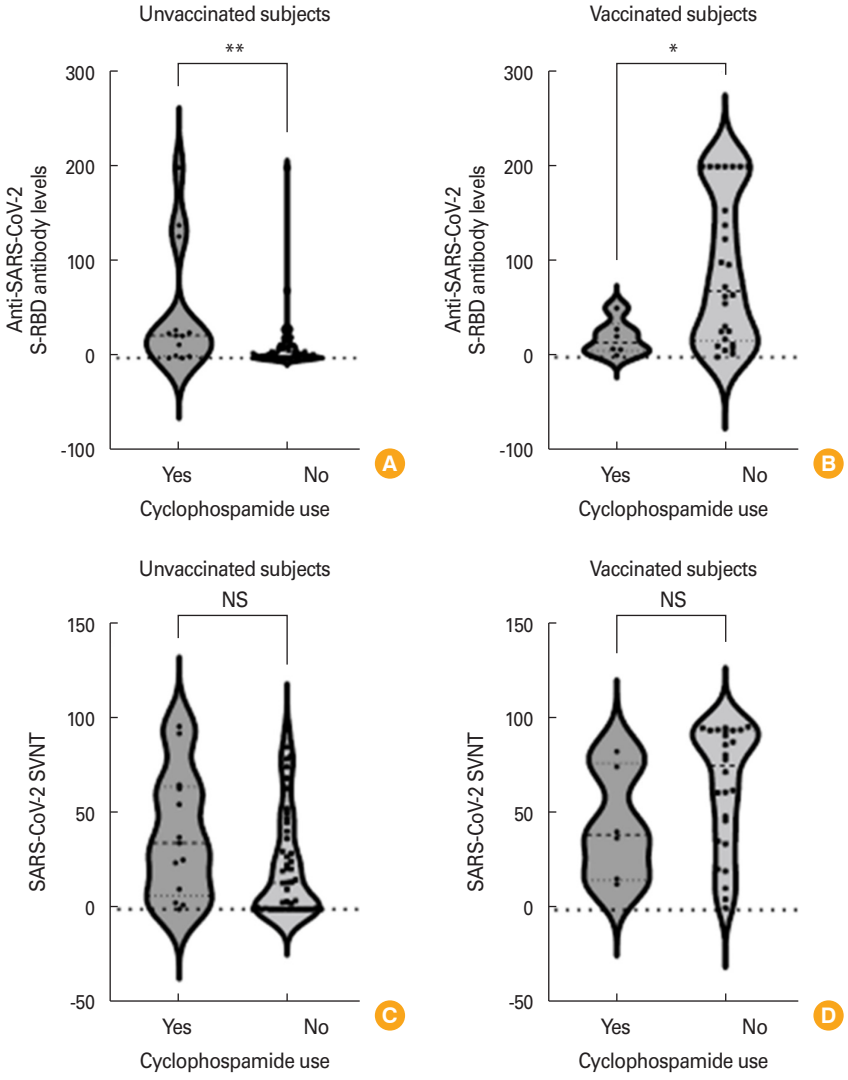

Fig. 3. (A–D) Comparison between anti–severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike receptor-binding domain (S-RBD) antibody levels and SARS-CoV-2 surrogate virus neutralization test (SVNT) in participants with and without cyclophosphamide in the unvaccinated and post-CoronaVac-vaccinated groups. NS, not significant. *p<0.05. **p<0.001.

Comparison between disease activity and anti–SARS-CoV-2 S-RBD antibody levels or SARS-CoV-2 neutralization antibody based on SVNT in unvaccinated and post-vaccinated participants

Comparative analysis between disease activity and anti–SARS-CoV-2 S-RBD antibody levels in both groups showed no significant results. Patients with lower SLEDAI-2k scores (<4) had a lower trend of anti–SARS-CoV-2 RBD antibody levels in both unvaccinated (0.24 [IQR, 0.07–20.68] and 5.41 [IQR, 0.24–15.79]) and post-vaccinated (22.11 [IQR, 8.14–201.00] and 64.14 [IQR, 28.48–139.81]) groups than those with higher SLEDAI-2k scores (≥4). Meanwhile, on SARS-CoV-2 SVNT analysis, no significant results were observed (Table 3).

Discussion

Our study aimed to analyze the rate of COVID-19 seropositivity in vaccinated and unvaccinated patients with SLE and explore their related factors. Based on the findings, vaccinated patients with SLE showed a higher proportion of seropositive results for both anti–SARS-CoV-2 S-RBD antibody and SARS-CoV-2 SVNT than unvaccinated ones. Regarding medication intake, a significant difference in anti–SARS-CoV-2 RBD antibody levels was noted in those who received CYC in the vaccinated group, with a higher antibody level shown in patients who did not receive CYC. The unvaccinated group showed a contrasting result, with a higher anti–SARS-CoV-2 S-RBD antibody level noted in those receiving CYC.

Table 2. Comparison between drug consumption and anti–SARS-CoV-2 RBD antibody level and SARS-CoV-2 SVNT in unvaccinated and vaccinated research subjects

Drug consumption	Anti-SARS-CoV-2 RBD antibody				SARS-CoV-2 SVNT			
	No.	Unvaccinated subjects (n=60)	No.	Vaccinated subjects (n=32)	No.	Unvaccinated subjects (n=60)	No.	Vaccinated subjects (n=32)
Hydroxychloroquine								
Yes	41	3.2 (0.11–17.35)	18	42.04 (8.70–132.28)	41	14.24 (0.50–41.59)	18	54.91 (20.18–89.08)
No	19	7.12 (0.06–22.22)	14	63.01 (23.24–201.00)	19	20.12 (0.00–63.28)	14	82.33 (47.04–94.46)
Cyclosporin								
Yes	10	12.67 (0.76–22.75)	8	114.25 (9.46–201.00)	10	21.78 (1.75–60.85)	8	65.73 (25.14–94.52)
No	50	3.48 (0.10–20.95)	24	54.29 (13.52–118.57)	50	14.82 (0.00–42.58)	24	67.69 (36.20–87.92)
Cyclophosphamide								
Yes	13	23.81 (2.26–78.85)[a]	6	15.5 (6.62–35.09)[a]	13	34.81 (7.06–64.32)	6	39.35 (15.66–77.25)
No	47	2.13 (0.10–12.50)	26	69.77 (17.48–201.00)	47	13.84 (0.00–40.94)	26	75.71 (35.79–94.46)
Azathioprine								
Yes	38	2.91 (0.98–20.91)	18	47.48 (12.28–166.40)	38	14.01 (0.00–45.93)	18	69.13 (32.51–91.96)
No	22	6.17 (0.22–21.25)	14	54.29 (11.17–123.30)	22	23.69 (3.43–53.55)	14	67.19 (31.06–94.48)
Methotrexate								
Yes	6	8.01 (0.59–13.18)	3	100.01 (3.92)	6	18.61 (2.66–41.05)	3	79.03 (5.62)
No	54	3.98 (0.11–22.52)	29	51.98 (12.58–146.96)	54	17.78 (0.00–48.20)	29	62.98 (35.40–92.56)
Mycophenolic mofetil								
Yes	31	3.75 (0.10–23.41)	12	40.72 (10.42–71.90)	31	10.64 (0.00–55.04)	12	45.03 (34.99–78.14)
No	29	7.02 (0.20–14.23)	20	82.08 (13.52–201.00)	29	21.15 (1.46–43.18)	20	80.25 (27.04–94.46)
Methylprednisolone								
≥6 mg/day	19	5.22 (0.10–23.81)	4	17.76 (10.02–99.09)	41	20.12 (0.00–47.86)	28	67.69 (36.57–94.29)
<6 mg/day	41	3.75 (0.14–17.35)	28	60.37 (13.52–189.47)	19	15.39 (0.41–47.49)	4	48.36 (15.56–87.33)

Values are presented as number or median (interquartile range) unless otherwise stated. Statistical analysis was performed with the Mann-Whitney test.
SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; RBD, receptor-binding domain; SVNT, surrogate virus neutralization test.
[a]Anti SARS-CoV-2 RBD Antibody shows a significant difference in systemic lupus erythematosus patients receiving cyclophosphamide for both unvaccinated (p=0.007) and vaccinated group (p=0.029).

In this study, there is a significantly higher proportion of female subjects in SLE groups in comparison to control groups. This could be explained by estrogen, as a sex hormone, which play a role in the susceptibility to SLE, and also the X-linked genetic contribute to this gender factor [16].

We found a lower antibody response to vaccination in SLE group compared to control group. This result is in line with previous study by Izmirly et al. [8] who reported a significantly lower anti–SARS-CoV-2 S-RBD antibody in SLE patient receiving vaccination compared to control. Besides, previous studies also reported decreased vaccine-induced seroreactivity in patients with rheumatic diseases. This reduced antibody response might be caused by immunosuppressive therapy which identified as an independent predictor for poor vaccine response [8].

Our study reported that patients receiving CYC had lower anti–SARS-CoV-2 S-RBD antibody levels following vaccination than those who did not receive CYC. Therefore, a correlation exists between low levels of antibodies formed with the use of CYC. A previous study reported a significant difference in anti–SARS-CoV-2 S-RBD antibody levels and a lower antibody level in patients with SLE with high-level immunosuppression (prednisone >20 mg/day, MMF, CYC, or combination therapy) compared with patients with SLE with no treatment to medium-level immunosuppression at days 21–28 after the second dose vaccination with Comirnaty [2]. Moreover, several previous studies reported similar results that patients with SLE taking immunosuppressants, particularly MMF and MTX, showed significantly lower anti–SARS-CoV-2 S-RBD antibody levels than those without MMF and MTX treatment. The low anti–SARS-CoV-2 S-RBD antibody levels in patients with CYC and MMF treatment history stems from the mechanism of action of these drugs. CYC is known as an immunosuppressant drug that selectively works against T cells. It is known that T helper 2 cells function in B cell activation to produce antibodies against COVID-19 [17]. This result was also

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Table 3. Comparison between organ involvement and anti–SARS-CoV-2 RBD antibody level and SARS-CoV-2 SVNT in unvaccinated and vaccinated research subjects

Variable	Anti–SARS-CoV-2 RBD antibody				SARS-CoV-2 SVNT			
	No.	Unvaccinated subjects (n=60)	No.	Vaccinated subjects (n=32)	No.	Unvaccinated subjects (n=60)	No.	Vaccinated subjects (n=32)
Organ involvement								
Chronic kidney disease								
Yes	30	5.92 (0.22–23.51)	18	40.72 (11.17–104.24)	30	22.77 (2.00–57.10)	18	75.71 (18.20–94.46)
No	30	2.07 (0.10–13.17)	14	82.08 (22.12–201.00)	30	13.81 (0.00–38.10)	14	62.48 (36.92–89.08)
NPSLE								
Yes	14	2.67 (0.15–12.05)	9	65.49 (37.05–201.00)	14	20.64 (0.55–53.32)	9	83.19 (67.69–94.50)
No	46	6.12 (0.11–20.95)	23	29.46 (9.42–139.05)	46	14.01 (0.00–35.55)	23	49.05 (21.45–88.32)
Hemolytic anemia								
Yes	18	2.26 (0.09–23.51)	8	41.49 (8.33–175.10)	18	2.91 (0.00–31.56)	8	54.41 (21.69–88.43)
No	42	6.87 (0.16–20.49)	24	54.29 (15.59–150.92)	42	21.29 (2.44–55.38)	24	77.15 (34.99–93.68)
Thrombocytopenia								
Yes	9	3.75 (0.33–25.32)	3	201.00 (8.89)	9	4.51 (0.00–46.40)	3	94.54 (13.59)
No	51	4.20 (0.10–20.47)	29	51.98 (12.58–131.90)	51	20.12 (0.41–47.49)	29	62.98 (35.40–89.84)
Serositis								
Yes	8	3.41 (0.14–12.32)	1	18.84	8	17.43 (0.58–43.24)	1	
No	52	4.49 (0.11–21.80)	31	56.59 (11.75–154.87)	52	17.76 (0.10–49.09)	31	72.39 (34.60–93.77)
Disease activity								
SLEDAI-2k score								
≥4	34	5.41 (0.24–15.79)	17	64.14 (28.48–139.81)	34	22.43 (2.88–48.81)	17	62.98 (36.94–89.84)
<4	20	0.24 (0.07–20.68)	15	22.11 (8.14–201.00)	20	2.29 (0.00–24.49)	15	75.27 (16.35–94.55)

Values are presented as number or median (interquartile range) unless otherwise stated. Statistical analysis was performed with the Mann-Whitney test.
SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; RBD, receptor-binding domain; SVNT, surrogate virus neutralization test; NPSLE, neuropsychiatric systemic lupus erythematosus; SLEDAI-2k, Systemic Lupus Erythematosus Disease Activity Index-2000.

confirmed by the two-way ANOVA analysis in our study, which showed an interaction between CYC intake and vaccination, thereby resulting in a reduction in anti–SARS-CoV-2 S-RBD antibody levels.

A significant difference was noted in unvaccinated patients with SLE who received CYC, with higher anti–SARS-CoV-2 S-RBD antibody levels. According to data from the WHO, there was a spike of confirmed COVID-19 cases in South-East Asia from July to August 2021 [18]. This may have resulted in high community exposure of COVID-19, leading to possible asymptomatic cases with high level of anti–SARS-CoV-2 S-RBD antibody levels on enrolled subjects.

Furthermore, we observed that patients receiving MMF had lower anti–SARS-CoV-2 S-RBD antibody levels following vaccination, showing a potential correlation between low levels of antibodies formed with MMF intake. This result is consistent with that of So et al. [1], whose univariate analyses showed that the use of MMF was associated with significantly lower nAB levels following vaccination in patients with SLE (mean, 52.1%±20.7% versus 68.7%±22.3%; p=0.009). Previous studies also showed that patients with SLE receiving MMF had low antibody response, inducing a 70%–80% reduction in immunogenicity following complete COVID-19 vaccination [8,18]. Mycophenolic acid (MPA), an active MMF metabolite, inhibits inosine monophosphate dehydrogenase, thereby leading to the inhibition of DNA production and lymphocyte proliferation. Meanwhile, the effects of MPA on T lymphocytes can induce the apoptosis of peripheral T cells activated by superantigens, suppress cytotoxic T lymphocyte generation, and reduce cytokine production. B cell proliferation inhibition will lead to reduced antibody production, which may reduce anti–SARS-CoV-2 S-RBD antibody levels following vaccination [19-21].

No significant difference in anti–SARS-CoV-2 S-RBD antibody levels was observed in patients receiving corticosteroids in both our study groups. However, studies conducted by Izmirly et al. [8] and Yuki et al. [19] showed that patients with SLE receiving steroids had lower antibody responses follow-

ing vaccination. Another study reported that glucocorticoids are the most associated factor with decreasing nAB levels (75% decrease) in treated SLE based on a multivariate analysis [22]. By inducing apoptosis in developing thymocytes and T cells, glucocorticoid is the choice for anti-inflammatory and immunosuppressive therapies. Furthermore, it acts as a potent suppressor of cytokine production in T cells and reduces the circulating number of B cells, which may contribute to low anti–SARS-CoV-2 S-RBD antibody levels [22-24]. The contrasting result of our study with those of previous studies may be related to the limited sample size.

Vaccination by inactivated vaccine showed increased anti–SARS-CoV-2 S-RBD antibody levels. Elevated levels of anti–SARS-CoV-2 S-RBD antibodies occur in patients with SLE with SLEDAI-2k scores of >4 or <4. On the basis of these results, it can be concluded that disease activity (SLEDAI-2k) is not correlated with anti–SARS-CoV-2 S-RBD antibody responses. The results of the present study are consistent with those of a study conducted by Izmirly et al. [8] in New York. The study was conducted on patients with SLE with a two-dose COVID-19 vaccine status to observe for antibody responses and B cell, T cell, and IFN-γ levels. The study showed an increase in anti–SARS-CoV-2 S-RBD antibody levels following vaccination and showed no change in SLEDAI-2k scores. However, patients with SLE had significantly lower antibody levels than those without comorbidities [8]. Another study conducted on patients with SLE in a cohort starting from before BNT162b2 vaccine administration to day 42 after the second dose showed that BNT162b2 was well tolerated, and no statistically significant variations in British Isles Lupus Assessment Group and SLEDAI-2k scores were observed throughout the study in patients with SLE with active and inactive disease at baseline [25]. The anti–SARS-CoV-2 S-RBD antibody is originate from the component of disease-specific autoantibody anti-double stranded (ds)-DNA in patients with SLE. Furthermore, anti-dsDNA was expected to affect the antibody formation against COVID-19 in SLE. Anti-dsDNA was affected by IFN-γ levels, with higher IFN-γ levels associated with higher anti-dsDNA levels. Anti-dsDNA can form antibody complexes that function as antiviral components and help myeloid dendritic cell maturation and B cell life prolongation. Dendritic cells are components that introduce antigens to T cells (CD4 and CD8). CD4 activates B cells, whereas CD8 destroys cells affected by antigen infection. Additionally, B cells form antibody and memory immunity against a disease; therefore, it can be concluded that the higher the level of anti-dsDNA in a patient with SLE, the more capable the patient's body is to form antibodies against COVID-19 and stimulate antibodies following COVID-19 vaccination [8,16].

In this study, kidney involvement in SLE had a lower trend of anti–SARS-CoV-2 S-RBD antibody level; however, this result is insignificant. No previous studies regarding inactivated vaccination in patients with lupus nephritis were conducted; however, a study in a population with CKD by Akpolat T reported that the number of seropositive patients with CKD is significantly lower than that of patients without CKD ($p<0.01$) following two doses of inactivated vaccination [26]. Lupus nephritis development originates from anti-dsDNA antibody immune complex deposition in the renal parenchyma. This process is followed by complement activation, immune cell infiltration, pro-inflammatory cytokine release, and oxidative damage, which lead to kidney inflammation and damage. Elevated immunoglobulin levels are frequently noted with lupus nephritis, which is parallel with the increased activity of the immune system observed in patients with SLE. Low immunoglobulin (Ig)M and IgG levels associated with lupus nephritis may be related to more severe or long-standing SLE and long-term corticosteroid or immunosuppressive therapy. A combination of corticosteroid use with an immunosuppressive agent such as MMF or CYC is the standard therapy for active lupus nephritis. The immunosuppressive effect of these drugs is related to low antibody levels in patients with lupus nephritis [26-28].

This study had some limitations. First, it had a limited number of participants. Second, as this was an observational study with a cross-sectional design, the post-vaccination antibody test and the interval between doses were not uniformly performed. Third, we only assessed one type of COVID-19 vaccine in this study, which does not have the highest stimulation effect; however, this information can add to the lack of information regarding inactivated vaccination. Fourth, we acknowledged the period of the vaccination was not similar in both control and SLE groups, because in the beginning of COVID-19 vaccination period, the vaccination regulation for autoimmune patients had not been established, so the autoimmune patients have delayed period of vaccination in this study. Therefore, future studies should focus on different types of COVID-19 vaccines for patients with SLE with a bigger sample size.

In conclusion, the increase in the levels of antibodies for COVID-19 in patients with SLE is affected by exposure in the community and inactivated vaccination. Meanwhile, CYC

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treatment is associated with a lower antibody response after receiving inactivated vaccine. The medication status of patients with SLE should be a consideration for vaccination strategies, and additional boosters may be needed for patients with SLE receiving multiple immunosuppressants, particularly for those with inactivated vaccines. Antibody level monitoring is significant for those taking immunosuppressants.

ORCID

Laniyati Hamijoyo *https://orcid.org/0000-0002-1310-674X*
Patrick Philo *https://orcid.org/0009-0005-6280-3399*
Daniel Setiawan Nathan *https://orcid.org/0009-0000-6230-3195*
Marita Restie Tiara *https://orcid.org/0000-0002-5513-7077*
Sofie Razyanti Mardiana *https://orcid.org/0000-0002-3955-7434*
Evan Susandi *https://orcid.org/0000-0002-0178-7138*
Nadia Gita Ghassani *https://orcid.org/0000-0002-4416-5208*
Bachti Alisjahbana *https://orcid.org/0000-0003-3745-8229*

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