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Supplementary appendix

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PATIENTS AND METHODS

Study Cohort

Details of patients included in this study and data on the first 90 SLE patients after the initial vaccine series have been previously published.[1] In brief, a convenience sample from the established New York University (NYU) Lupus Cohort was recruited. Inclusion in the NYU Lupus cohort required 1) fulfilling at least one of the following classification criteria: a) the American College of Rheumatology (ACR) revised classification criteria[2]; b) the Systemic Lupus International Collaborating Clinics (SLICC) classification criteria[3]; c) the European League Against Rheumatism (EULAR)/ACR classification criteria[4]; and 2) age ≥ 18 years. Having met one if the above criteria, enrollment required 1) receipt of two doses of an mRNA vaccine (BNT162b2 [Pfizer/BioNTech] or mRNA-1273 [Moderna]) or one dose of a vector based COVID-19 vaccine (Ad26.COV2.S [Johnson & Johnson]); 2) willingness to provide blood samples after vaccination; 3) clinical follow-up ≥ 6 months after initial vaccination series or until SARS-CoV-2 infection if earlier than 6 months; 4) clinical follow up on or after February 4, 2022. All patients signed an informed consent available in English, Spanish and Mandarin. Patients consented to the study as approved by the NYU IRB #s14-00487.

Data Collection

The following clinical information was recorded as previously described:[1] 1) demographic factors (age, sex, race/ethnicity); 2) history of lupus nephritis; 3) use of SLE specific medications, including antimalarials, glucocorticoids and immunosuppressants.

COVID-19 infections after vaccination were evaluated in 163 subjects from patient encounters at regularly scheduled visits and chart review of the NYU and Bellevue Hospital Center electronic medical record with last patient follow-up recorded April 24 2022. Positive polymerase chain reaction (PCR) or antigen-based testing was required for documentation, either performed at the clinical site or self-reported. Data collected on COVID-19 disease outcomes included 1) hospitalization; 2) death; 3) COVID-19 specific treatments, including high flow oxygen, mechanical ventilation, glucocorticoids, monoclonal antibody, and/or antiviral use; 4) type of testing (if discordant between PCR and antigen testing, only PCR positive cases were counted); 5) time of infection in relation to the most recent vaccination dose.

Fifty-seven patients receiving additional vaccine doses were followed longitudinally with blood samples available after the initial vaccine series (time point (TP) 2, mean 30·2, range 5-86 days after the second dose of the mRNA vaccines or the single dose of the vector vaccine) and after the additional dose (TP4, mean 44·7 (range 5-147) days after the additional dose). A subset of these patients (N=26) had blood obtained prior to the initial (TP 1) and additional dose (TP3, mean 175·5 (range 90-270) days after the initial vaccine series).

ELISA-Recombinant SARS-CoV-2 Spike Protein

The test antigen was recombinant SARS-CoV-2 spike receptor binding domain (RBD) (# BT10500; R&D Systems), coated onto a 96-well microtiter plate for 2 hours at 37 degrees Celsius. Plate blocking occurred at room temperature for 1 hour. All subject sera were applied at dilutions ranging from 1:200-1:1000 for 1·5 hours at room temperature. Samples were run in duplicate. For each 96-well plate there were 2 high positive controls from TP2, 1 low positive

and 1 calibrator. Detection relied on established use of a secondary antibody (enzyme-labeled secondary antibody, alkaline phosphatase–conjugated rabbit anti-human IgG (γ-chain–specific) (Sigma #A3312) at 1:3000), which was incubated for 30 minutes at room temperature. An alkaline phosphate reagent was used for development in 5 minute intervals. Developing occurred within 15-25 minutes to report results. Reactions readings were stopped when the calibrator reached an optical density (OD) of 1. The test sera titers were retrieved within a range from 0·3-1·0 OD. The sera titers were multiplied by the dilution factor. Importantly, the series of sera from each patient were always run on the same plate. Data are shown as units per ml (u/mL).

Statistical Analysis

Categorical variables were summarized by computing counts and proportions of patients (%). Continuous variables are expressed as mean, median with interquartile range (IQR), or range, as appropriate. Two group comparisons were performed using the Chi-square or Fisher's exact tests for categorical variables and the two-sample Mann-Whitney U tests for continuous variables. Low post-vaccine ELISA antibody response was defined as ≤100 units/ml, the lowest value seen in controls in our previous study.[1] All statistical analyses were performed using IBM SPSS Statistics version 25.

Role of the Funding Source

The funding sources for this study did not have any role in the study design, data collection, analysis, interpretation, writing of the report, or decision to submit the paper for publication.

APPENDIX REFERENCES

1 Izmirly PM, Kim MY, Samanovic M, *et al.* Title: Evaluation of Immune Response and Disease Status in SLE Patients Following SARS-CoV-2 Vaccination. DOI:10.1002/ART.41937.

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3 Petri M, Orbai AM, Alarcõn GS, *et al.* Derivation and validation of the systemic lupus international collaborating clinics classification criteria for systemic lupus erythematosus. *Arthritis Rheum* 2012; **64**: 2677–86.

4 Aringer M, Costenbader K, Daikh D, *et al.* 2019 European League Against Rheumatism/American College of Rheumatology Classification Criteria for Systemic Lupus Erythematosus. *Arthritis Rheumatol* 2019. DOI:10.1002/art.40930.

Appendix Table 1: Clinical Demographics and Medication History

	SLE at Time Of Initial Vaccine (n = 163)	**SLE at Time Of Additional Vaccine Dose (n = 125)**
Age, mean (range)	44.1 (22-87)	45.2 (22-87)
Sex		
Female	149 (91.4%)	116 (92.8%)
Male	14 (8.6%)	9 (7.2%)
Race		
White	64 (39.3%)	53 (42.4%)
Black	39 (23.9%)	28 (22.4%)
Asian	39 (23.9%)	27 (21.6%)
Other	21 (12.9%)	17 (13.6%)
Ethnicity		
Hispanic/Latino	56 (34.4%)	44 (35.2%)
Initial and additional COVID-19 vaccine		
Pfizer	122 (74.8%)	96 (76.8%)
Moderna	35 (21.5%)	27 (21.6%)
Janssen/Johnson & Johnson	6 (3.7%)	0 (0%)
Unknown	0 (0%)	2 (1.6%)
History of COVID-19 (PCR or IgG) prior to initial vaccination	28 (17.2%)	19 (15.2%)
History of LN	80 (49.1%)	63 (50.4%)
Medications at time of initial or additional vaccine		
Hydroxychloroquine	139 (85.3%)	104 (83.2%)
Mean dose (mg)	324.9	351.9
Chloroquine	1 (0.6%)	0 (0%)
Mean dose (mg)	250.0	0
Glucocorticoids	52 (31.9%)	39 (31.2%)
Mean dose, prednisone equivalent (mg)	8.7	6.8
At least 1 immunosuppressant	82 (50.3%)	62 (50.4%)
Azathioprine	17 (10.4%)	9 (7.2%)
Mean dose (mg)	98.5	104.2
Mycophenolate mofetil	43 (26.4%)	37 (29.6%)
Mean dose (mg)	1924.2	2007.9
Mycophenolic acid	2 (1.2%)	2 (1.6%)
Mean dose (mg)	900.0	1080
Tacrolimus	10 (6.1%)	9 (7.2%)
Mean dose (mg)	3.9	4.1

Methotrexate	14 (8.6%)	7 (5.6%)
Mean dose (mg)	15.5	15.7
Belimumab	18 (11.0%)	10 (8.0%)
Cyclophosphamide within 6 months	1 (0.6%)	0 (0%)
Rituximab within 6 months	4 (2.5%)	3 (2.4%)
Other*	7 (4.3%)	8 (6.4%)
Combination immunosuppressants	26 (16.0%)	18 (14.4%)

SLE, systemic lupus erythematosus; PCR, polymerase chain reaction; IgG, Immunoglobulin G; LN, lupus nephritis; mg (milligrams)

*Other immunosuppressants included leflunomide, abatacept, adalimumab, obinutuzumab (within 6 months of vaccine), eculizumab, ravulizumab, voclosporin, tofacitinib and SLE clinical trial drug vs. placebo

Appendix Table 2: Patients Hospitalized with COVID-19 Breakthrough Infection After Vaccination

Age	Gender	Lupus Medications	Initial Vaccine Type	Additional Vaccine Type	Date of Infection	Reason for Hospitalization	COVID Treatment
33	Female	None	Pfizer	N/A	1/17/22	SOB, chest tightness, myalgia	Glucocorticoids, Remdesivir, Monoclonal Antibodies, Supplemental oxygen
56	Female	HCQ, Prednisone, Mycophenolic acid, Tacrolimus	J&J	12/7/2021 (Pfizer)	1/24/22	SOB	Supplemental oxygen

HCQ, hydroxychloroquine; J&J, Johnson and Johnson; SOB, shortness of breath

Appendix Table 3: Factors Associated with SARS-CoV-2 Spike Antibody Titer in SLE Patients After Additional COVID-19 Vaccination Dose

	Median (IQR) SARS-CoV-2 Spike Ab after Additional Covid-19 Vaccine Dose +	**Median (IQR) SARS-CoV-2 Spike Ab after Additional Covid-19 Vaccine Dose -**	**P value**
COVID-19 Breakthrough Infection	1135 (675-1378.5) (N=13)	994.5 (436.25-1306.75) (N=44)	0.31
Sex			
Female	1018.0 (448.75-1326.25) (N=54)	1250 (955-) (N=3)	0.32
Race			
White	1043.5 (787.5-1306.75) (N=20)	1036 (376.5-1364.5) (N=37)	0.44
Ethnicity			
Hispanic/Latino	1067.5 (840.75-1277.5) (N=16)	1036 (389.5-1376.5) (N=41)	0.74
Initial mRNA COVID-19 vaccine	1018 (486-1350.75) (N=56)	1232 (1232-1232) (N=1)	0.63
History of LN	945 (377.5-1231.5) (N=32)	1232 (741-1389) (N=25)	0.072
Medications at time of last vaccine N (%)			
Hydroxychloroquine	1039 (548-1326.25) (N=50)	920 (397-1391) (N=7)	0.83
Glucocorticoids	945 (390.5-1274.5) (N=18)	1087 (607-1366) (N=39)	0.42
At least 1 immunosuppressant	934.5 (307.25-1241.75) (N=28)	1185 (811-1376) (N=29)	0.071
Glucocorticoids + immunosuppressant	934.5 (377.5-1230.5) (N=16)	1135 (627.5-1364.5) (N=41)	0.28
Combination immunosuppressants	820.5 (167.5-1335.75) (N=8)	1042 (593-1338.5) (N=49)	0.46

IQR, interquartile range; mRNA, messenger RNA; LN, lupus nephritis

Figure 1: SARS-CoV-2 Infections in Initially Vaccinated SLE patients Among Those That Received and Did Not Receive an Additional COVID-19 Vaccine Dose

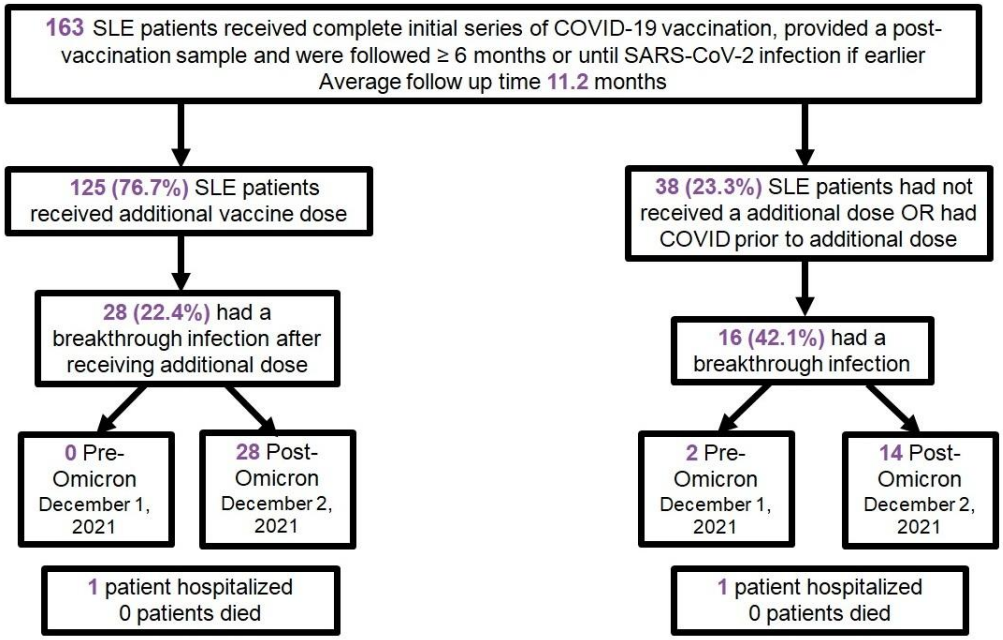

Figure 2: SARS-CoV-2 spike antibody ELISA before and after initial COVID-19 vaccine series and additional dose

Time Point 1: Pre-COVID vaccination

Time Point 2: Approximately 1-month post-second vaccine dose (mean 34.1 days after initial vaccine series in SLE patients)

Time Point 3: Prior to additional COVID-19 vaccine dose (mean 181.5 days after initial vaccine series in SLE patients)

Time Point 4: Approximately 1-month post-booster (mean 49.7 days after additional vaccine dose)