# Real-world data on the Immunity Response to the COVID-19 Vaccine among Patients with Central Nervous System Immunological Diseases

**Punchika Kosiyakul, M.D.[1,2,3], Jiraporn Jitprapaikulsan, M.D.[1,2], Ekdanai Uawithya[4], Patimaporn Wongprompitak, Ph.D.[5], Chutikarn Chaimayo, M.D., Ph.D.[6], Navin Horthongkham, Ph.D.[6], Nasikarn Angkasekwinai, M.D.[7], Nanthaya Tisavipat, M.D.[1,2], Naraporn Prayoonwiwat, M.D.[1,2], Natthapon Rattanathamsakul, M.D.[1,2], Kanokwan Boonyapisit, M.D.[1], Theerawat Kumutpongpanich, M.D.[1], Onpawee Sangsai, M.Sc.[2], Kamonchanok Aueaphatthanawong, B.Sc.[2], Jirawan Budkum, M.Sc.[2], Sasitorn Siritho, M.D.[1,2,8]**

*[1]Division of Neurology, Department of Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, 10700, Thailand, [2]Siriraj Neuroimmunology Center, Division of Neurology, Department of Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, 10700, Thailand, [3]Department of Research and Development, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand, [4]Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, 10700, Thailand, [5]Department of Immunology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, 10700, Thailand, [6]Department of Microbiology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, 10700, Thailand, [7]Division of Infectious disease and tropical medicine, Department of Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, 10700, Thailand, [8]Bumrungrad International Hospital, Bangkok 10110, Thailand.*

## ABSTRACT

**Objective:** The effects of immunotherapies on the immune response to various regimens of SARS-CoV-2 vaccines in patients with autoimmune neurological disease have been demonstrated in limited data. Thus, we evaluated the immune responses in each platform of COVID-19 vaccination between patients with autoimmune neurological disease and a healthy population.

**Materials and Methods:** We conducted a prospective observational study. We collected serum from patients with autoimmune neurological diseases to perform serological methods using anti-RBD IgG assay, neutralizing antibodies assay, and interferon SARS-CoV-2 immunoassay. Serological response level was analyzed by platforms of vaccines and types of immune modifying therapy.

**Results:** Fifty-eight patients had tested for an anti-RBD IgG response, and those receiving no immunotherapy/healthy controls had the highest median anti-RBD IgG levels amongst immunotherapy statuses. Rituximab in those who received inactivated or mRNA vaccine regimens had the lowest antibody level compared with other immunotherapies. In vector-based vaccine regimens, significant reductions of anti-RBD IgG response were observed in all other immunotherapy groups except for azathioprine, with the greatest difference seen compared to rituximab. Thirty-five patients with positive anti-RBD responses were further tested for neutralizing antibodies. The mRNA vaccine regimen demonstrated the highest inhibition percentage among the Delta and Omicron variants. Twenty-two patients were tested for T cell responses, with no significant difference in T-cell activity across all groups.

**Conclusion:** We have demonstrated a significant decrease in antibody response against SARS-CoV-2 in patients with autoimmune neurological diseases receiving immunotherapies compared to a healthy population, especially for patients taking rituximab.

**Keywords:** Neuromyelitis optica spectrum disorder; multiple sclerosis; COVID-19 vaccine; immunosuppressant; humoral immune response (Siriraj Med J 2024; 76: 69-79)

*Corresponding author: Sasitorn Siritho*
*E-mail: siritho@yahoo.com*
*Received 7 December 2023 Revised 26 December 2023 Accepted 26 December 2023*
*ORCID ID:http://orcid.org/0000-0002-2562-697X*
*https://doi.org/10.33192/smj.v76i2.266638*

All material is licensed under terms of the Creative Commons Attribution 4.0 International (CC-BY-NC-ND 4.0) license unless otherwise stated.

## INTRODUCTION

The Coronavirus Disease-19 (COVID-19) pandemic has prompted mass vaccination efforts worldwide. Numerous novel vaccines against the severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2) have been engineered. The platforms used range from inactivated viruses to protein subunits, viral vectors, and messenger ribonucleic acid (mRNA). Studies in general populations have shown variable vaccine efficacy and adverse events following immunization related to the vaccine with each regimen of vaccine.[1-3] However, patients using immunosuppressive agents as a special very high risk of COVID-19 population were excluded from pivotal phase III vaccine trials.[4]

Immune-mediated neurological diseases, such as multiple sclerosis (MS), neuromyelitis optica spectrum disorder (NMOSD), and other autoimmune neurological diseases (AINDs), are rare diseases.[5,6] The cornerstone of management of immune-mediated neurological diseases is immunomodulatory and immunosuppressive therapies, which are known as immunotherapies (IMTs). Examples include azathioprine (AZA), mycophenolate mofetil (MMF), and rituximab (RTX), which are sometimes used as an alternative to immunomodulators, especially in resource-limited settings.[7,8] AZA and MMF are anti-metabolites that decrease T and B lymphocyte proliferation while RTX specifically suppresses B lymphocytes targeting CD20. While achieving disease control, these agents need to be monitored for their immunosuppressive effects, which could confer greater susceptibility to infection.[9]

Some studies have observed a lower immune response to COVID-19 vaccines in patients taking immunosuppressive agents compared to healthy controls.[10] Low immunoglobulin levels due to B lymphocyte suppression could attenuate vaccine responsiveness, particularly among those taking anti-CD20 RTX.[11] Measurement of specific immunity to SARS-CoV-2, including IgG to a receptor-binding domain (anti-RBD IgG), neutralizing antibody (NAbs), and interferon-gamma release assay (IGRA) could be quantitative surrogate markers for vaccine efficacy. Therefore, we aimed to compare the immune responses after each regimen of COVID-19 vaccination between patients with AIND and a healthy control population.

## MATERIALS AND METHODS

### *Participants and samples*

This prospective observational study was conducted at Siriraj Hospital, a university hospital referral center in Thailand. Sera from patients with AIND attending the Neurology Clinic at Siriraj Hospital from December 2021 to July 2022 were collected one week before and one month after the COVID-19 vaccination.

The inclusion criteria were age greater than 18 years and receiving at least two doses of COVID-19 vaccination. Patients lost to follow-up or not able to give post-vaccination sera were excluded. Also, those with a history of COVID-19 infection in the past 6 months or anti-RBD IgG seropositivity at baseline were excluded. Patients with AIND recruited into the study were matched with healthy controls receiving a vaccine against COVID-19 to compare their responsiveness to the vaccine regimens.

Clinical data, including sex, diagnosis, age at disease onset, disease duration, the total number of previous attacks before the first COVID-19 vaccination, current IMT, the number of prior IMTs used, and the most recent follow-up Expanded Disability Status Scale (EDSS) were collected. COVID-19 vaccination status, platforms used, and doses received were documented. We performed matching healthy controls by age, sex, and vaccination regimen.

Participants in the healthy control group were healthy adults aged ≥ 18 years in whom sera were collected from two prospective cohort studies of vaccination of either two doses of CoronaVac or two doses of ChAdOx1[12], and third-dose booster vaccination either BBIBP-CorV, ChAdOx1, 30µg-BNT162b2, or 15µg-BNT162b2.[13] All patients were informed the information about each vaccine and their possible adverse events according to the government brochure.

We defined groups of vaccines for comparison. We defined inactivated vaccine regimen as those having received two doses of an inactivated vaccine. We defined vector-based vaccine regimen as those having received two doses of vector-based vaccines, one dose of each of an inactivated and a vector-based vaccine, and vector-based vaccine as the third dose. Finally, we defined mRNA vaccine regimen as those having received two doses of mRNA vaccine and mRNA vaccine as the third dose.

All participants received their COVID-19 vaccination regimens according to the availability of the vaccines at that time, each patient's preference, and the Thai Ministry of Public Health's recommendations.[14] Most Thai people received a mix-and-match, prime-boost immunization strategy.

The Siriraj Institutional Review Board approved this study (COA no. Si 707/2021). All patients gave written informed consent. The study was performed in accordance with the Declaration of Helsinki of 1975.

### *Serum immunologic testing*

Serum immunologic tests were anti-RBD IgG assay[15],

NAbs assay[16], and interferon SARS-CoV-2 immunoassay.[17,18] We defined an anti-RBD IgG assay titer level of greater or equal to 7.1 BAU/mL as seropositive. The cut off value for inhibition response was set at 30%. The cut-off value for Ag1-Nil and Ag2-Nil was set at 0.2 IU/mL.[18] The details for each test are described in the Appendix.

### *Statistical analysis*

We analyzed the data descriptively. Categorical data are displayed as frequency (%), and continuous data are displayed as median (interquartile range [IQR]). Categorial data was analyzed by the $\chi^2$ test or Fisher's exact test if less than 75% of cells had expected frequencies of greater than five. Continuous data were compared by nonparametric, rank-based Mann-Whitney U test for pairwise testing or the Kruskal Wallis test if there were more than two groups to compare. Pre- and post-vaccination anti-RBD IgG levels were compared by Wilcoxon signed-rank test for non-parametric, non-independent data. All data were analyzed on PASW Statistics for Windows version 18.0 (SPSS Inc., Chicago, IL) and Graphpad Prism version 9 (GraphPad Software, San Diego, CA).[19] A *p*-value of less than 0.05 was considered significant. No correction for multiplicity was applied.

## RESULTS

Sixty-one patients with AIND were recruited. Three were excluded due to evidence of prior COVID-19 infection (history of COVID-19 infection three months before eligibility assessment n = 1 and anti-RBD-IgG seropositivity n = 2). Thus, 58 patients were included in the final analysis (having received inactivated vaccine regimen n = 15, having received a vector-based vaccine regimen n = 31, and having receive a mRNA vaccine regimen n = 22). Of the 58 patients, 46 (78.3%) were female. All 58 patients were evaluated for anti-RBD IgG. The T cell response test was performed in only 22 patients due to limited availability. Thirty-five patients with anti-RBD IgG positivity received further testing for NAbs. Fourteen patients were tested for all the three immunity assays (Fig 1).

Of the 58 patients, the frequencies of immunotherapy status were no immunotherapy (n = 6), azathioprine (n = 15), MMF (n = 13), rituximab (n = 18), fingolimod (n = 4), glatiramer acetate (n = 1), and prednisolone (n = 1). Diagnoses included were NMOSD (n = 27), MS (n = 15), myasthenia gravis (MG) (n = 5), clinically isolated syndrome (n = 4) [idiopathic single transverse myelitis (n = 2), isolated demyelinating brainstem syndrome (n = 1), and single optic neuritis (n = 1)], autoimmune encephalitis (n = 3) [anti-N-methyl D-aspartate encephalitis (n = 2) and seronegative autoimmune encephalitis (n = 1)], pachymeningitis (n = 3) [eosinophilic granulomatosis with polyangiitis with pachymeningitis (n = 1), idiopathic pachymeningitis (n = 1), and IgG4-related pachymeningitis (n = 1)], and myelin oligodendrocyte glycoprotein antibody-associated disease (MOGAD) (n = 1). All groups of patients had similar age at onset,

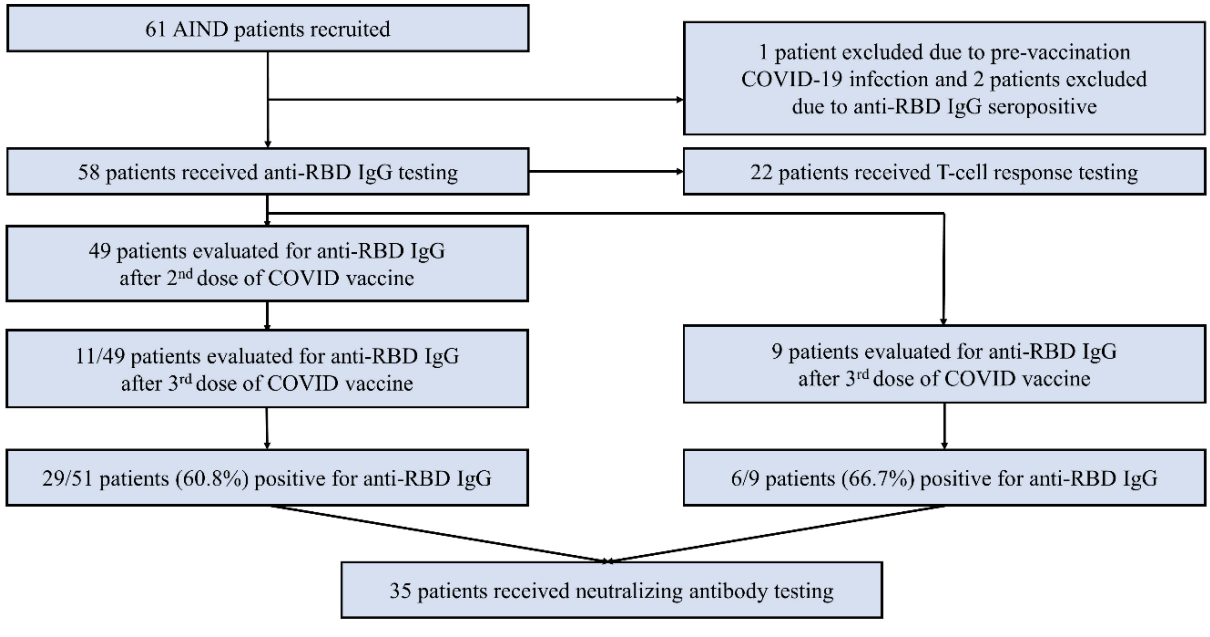


**Fig 1.** Flowchart of patients with serum immunologic testings

*Notes:* Data were available as the following: 58 patients had anti-RBD IgG level, 35 patients had neutralizing antibodies, and 22 patients had T cell responses.

**Abbreviations:** AINDs, autoimmune neurological diseases; RBD, receptor-binding domain

EDSS scores, interval from disease onset to the third dose of vaccination, interval from the most recent treatment initiation to the third dose of vaccine administration, and concomitant diseases. Conversely, interval from disease onset to the second dose vaccination and interval from the latest treatment initiation to the second vaccine were significantly different (both $p < 0.05$) (Table 1).

***Anti-RBD IgG Response to the SARS-CoV2 Virus***

Forty-nine patients had anti-RBD antibodies testing after their second dose of COVID-19 vaccination, of which 15 patients had received two doses of an inactivated vaccine (either CoronaVac or BBIBP-CorV COVID-19 vaccine), 27 patients had received two doses of the vector-based ChAdOx1 nCoV-19 vaccine, 4 patients had received two doses of an mRNA vaccine (either the mRNA-1273 SARS-CoV-2 vaccine or the BNT162b2 nCoV-19 vaccine), and 3 patients had received combinations of CoronaVac and ChAdOx1 nCoV-19. Of the 49 patients, 11 patients had additional anti-RBD antibody testing after the third vaccination. The remaining 9 of the 58 patients had anti-RBD antibody testing performed after the third vaccination. (Fig 1)

*Comparison of Anti-RBD IgG Responses by Vaccine Regimen*

Only 13 patients had data on pre-vaccination anti-RBD antibodies levels. Of these, 10 (76.9%) demonstrated a 23-fold rising in antibody titer compared with the baseline value with a median anti-RBD level of 0.21 BAU/mL (IQR 0.16-0.43) pre-vaccination and 4.81 BAU/mL (IQR 0.17- 33.41) post-vaccination ($p = 0.013$). (Supplementary Fig S1)

After the second vaccination, 29 of 49 (59.2%) patients had rising antibody titers above the cut point for seropositivity with a median level of 15.25 BAU/mL (IQR 0.47-152.11).

mRNA vaccine regimens seemed to induce higher anti-RBD levels than the vector-based vaccine regimens, and inactivated vaccine regimen demonstrated the least immunogenicity. For all 68 sera of 58 patients included in the final analysis, the seropositive rates for inactivated vaccine, vector-based vaccine, and mRNA vaccine regimens for anti-RBD were 26.7% (4 of 15), 71% (22 of 31), and 72.7% (16 of 22), respectively. For inactivated vaccine, vector-based vaccine, and mRNA vaccine regimens, the median levels were 0.58 BAU/mL (IQR 0.24-7.50), 52.82 BAU/mL (IQR 5.61-171.79), and 969.77 BAU/mL (IQR 3.41-2382.53) BAU/mL, respectively ($p < 0.001$). (Fig 2A)

The NMOSD group demonstrated the highest anti-RBD IgG level for mRNA vaccine regimens [1062.87 BAU/mL (IQR 93.29-2151.47)], followed by vector-based vaccine regimens [106.22 BAU/mL (IQR 51.39-298.21)], and inactivated vaccine regimens [3.01 BAU/mL (IQR 0.18-91.50)], respectively ($p = 0.030$) (Fig 2B). Conversely, MS patients did not demonstrate any significant difference among mRNA regimens, vector-based regimens, and inactivated vaccine regimens with median levels at 2.78 BAU/mL (IQR 0.39-3803.75), 5.61 BAU/mL (IQR 0.20-14.00), and 0.36 BAU/mL (IQR 0.21-7.50), respectively ($p = 0.666$) (Fig 2C). Similarly, patients in the other AINDs group did not demonstrate any significant differences across all vaccine regimens with median levels at 937.88 BAU/mL (IQR 51.30- 5196.40) for mRNA regimens, 28.41 BAU/mL (IQR 2.51-135.64) for vector-based regimens, and 1.33 BAU/mL (IQR 0.41-34.93) for inactivated vaccine regimens ($p = 0.069$) (Fig 2D).

*Comparison of Anti-RBD IgG Responses by Type of Immunotherapy*

Compared to the no IMT/heathy control group, patients treated with RTX having received inactivated vaccine regimen showed a significant reduction of anti-RBD response [112.54 BAU/mL (IQR 53.30-171.32) vs. 0.26 BAU/mL (IQR 0.12-0.36); $p = 0.010$]. (Fig 2E)

For those having received a vector-based vaccine regimen, compared to the no IMT/healthy control group, significant reductions of anti-RBD IgG responses were observed in those treated with RTX, FGM, and MMF [no IMT/healthy controls 235.03 BAU/mL (IQR 96.07-405.77) vs. each of RTX 0.42 BAU/mL (IQR 0.06-61.21); $p = 0.002$, FGM 4.81 BAU/mL (IQR 0.20-5.61); $p < 0.001$, and MMF 50.91 BAU/mL (IQR 8.18-71.86); $p = 0.001$]. However, no significant reduction was seen comparing no IMT/healthy controls to AZA. (Fig 2F)

For those having received an mRNA vaccine regimen, the no IMT/healthy controls had a remarkably higher anti-RBD-IgG level [4633.13 BAU/mL (IQR 2256.71-7415.94)]. In comparison to the no IMT/healthy controls, RTX showed a significant reduction in anti-RBD level [0.34 BAU/mL (IQR 0.14-49.58); $p = 0.010$] while the FGM group showed some reduction without significance [0.77 BAU/mL (IQR 0.01-1.53); $p = 0.133$]. (Fig 2G)

*Neutralizing antibodies for delta and omicron variants*

Amongst the 35 patients seropositive for anti-RBD IgG, 41 serum samples were further evaluated for NAbs against specific COVID-19 variants.

**TABLE 1.** Demographic and characteristics of patients with AINDs

| Characteristics | No immunotherapy (n = 6) | Azathioprine (n = 15) | MMF (n = 13) | RTX (n = 18) | Fingolimod (n = 4) | Other: Glatiramer acetate, Prednisolone (n = 2) | P-value |
|---|---|---|---|---|---|---|---|
| **Female gender, n (%)** | 6 (100) | 14 (93.3) | 13 (100) | 10 (55.6) | 2 (50) | 1 (50) | 0.003 |
| **Age at onset, median (range), y** | 53.5 (29.3, 63.8) | 45.0 (35.0, 55.0) | 44.0 (26.5-50.5) | 35.5 (21.3, 43.8) | 38.5 (28.5, 47.0) | 40.5 | 0.447 |
| **EDSS†¥, median (range)** | 1.25 (1.00, 5.63) | 1.0 (1.0, 1.5) | 1.25 (1, 4.12) | 1 (0, 3.38) | 1.0 (0.25, 2.50) | 1.0 (1.0, 1.0) | 0.799 |
| | Duration from disease onset to vaccine administration, median (interquartile range), month | | | | | | |
| **To 2nd dose** | 119.34 (NA) | 93.8 (29.3, 141.4) | 123.98 (50.3, 206.1) | 47.9 (21.2, 87.6) | 81.9 (36.2, 137.9) | 4.73 (NA) | 0.027 |
| **To 3rd dose** | 23.1 (20.4, 109.5) | 129.94 (NA) | 149.8 (59.3, 240.2) | 47.2 (32.2, 74.4) | 92.76 (NA) | 5.82 (NA) | 0.166 |
| | Duration from the latest treatment initiation to vaccine administration‡, median (interquartile range), month | | | | | | |
| **To 2nd dose** | NA | 2.72 (1.77, 3.73) | 2.72 (0.46, 3.79) | 6.80 (3.22, 7.52) | 1.91 (1.47, 2.07) | 1.97 (NA) | 0.002 |
| **To 3rd dose** | NA | 7.92 (NA) | 7.56 (5.63, 8.95) | 9.96 (8.81, 23.2) | 5.90 (NA) | 7.00 | 0.055 |
| **Comorbidities¶, n (%)** | 4 (66.7) | 9 (60) | 7 (53.8) | 6 (33.3) | 4 (100) | 1 (50) | 0.198 |
| | Current disease, n (%) | | | | | | |
| **MS** | 1 (16.7) | 2 (13.3) | 2 (15.4) | 5 (27.8) | 4 (100) | 1 (50) | 0.018 |
| **NMO** | 1 (16.7) | 9 (60) | 8 (61.5) | 9 (50) | 0 | 0 | 0.089 |
| **Others AIND*** | 4 (66.7) | 4 (26.7) | 3 (23.1) | 4 (22.2) | 0 | 1 (50) | 0.239 |
| **Number of attacks#, median (range)** | 1.0 (1.0, 2.75) | 1.0 (1.0, 5.0) | 3.0 (2.0, 10.5) | 2.0 (1.0, 3.25) | 2.0 (2.0, 3.5) | 1.0 (1.0, 1.0) | 0.094 |
| | Vaccine regimen, n (%) | | | | | | |
| **Inactivated vaccine regimen** | 0 | 4 (26.7) | 4 (30.8) | 6 (33.3) | 1 (25.0) | 0 | 0.194 |
| **Vector-based vaccine regimen** | 2 (20.0) | 9 (60.0) | 7 (53.8) | 8 (44.4) | 3 (75.0) | 2 (100) | 0.035 |
| **mRNA vaccine regimen** | 4 (80.0) | 4 (26.7) †† | 6 (46.2) ‡‡ | 6 (33.3) §§ | 2 (50.0) | 0 | 0.848 |
| | Serum immunologic testing, n (%) | | | | | | |
| **Tested for RBDs** | 6 (100) | 15 (100) | 13 (100) | 18 (100) | 4 (100) | 2 (100) | 1 |
| **Tested for NAbs**** | 5 (83.3) | 15 (100) | 8 (61.5) | 6 (33.3) | 0 (0) | 1 (50.0) | <0.001 |
| **Tested for IGRA** | 3 (50.0) | 3 (20.0) | 6 (46.2) | 7 (38.9) | 2 (50.0) | 1 (50.0) | 0.623 |

**Abbreviations:** AINDs, autoimmune neurological diseases; EDSS, Expanded Disability Status Scale; IGRAInterferon SARS-CoV-2; IS, immunosuppressive; MS, multiple sclerosis; NAbs, neutralizing antibodies; NMOSD, neuromyelitis optica spectrum disorder; RBDs, anti-RBD IgG level

*Clinical findings of other patients consisted of myasthenia gravis (n = 5), autoimmune encephalitis (n = 3), MOGAD (n = 1), pachymeningitis (n = 2), and clinically isolated syndromes (n = 5)

†EDSS scores were evaluated in MS and NMOSD patients at the last visit before blood drawing for assay testings¥EDSS scores available in 44 patients

‡For patients receiving immunotherapy

§Of 51 patients, 2 received no immunotherapy

‖Of 20 patients, 4 received no immunotherapy

¶Comorbidities included diabetes mellitus, hypertension, dyslipidemia, vitamin D deficiency, obstructive sleep apnea, obesity, cirrhosis, systemic lupus erythematosus, Hashimoto thyroiditis, coronary artery disease, thymoma, herpes keratoconjunctivitis, and osteoarthritis

#The number of attacks was evaluated in MS and NMOSD patients

**Only patients with positive anti-RBD responses further analysis for neutralizing antibody response

††2 patients received vector-based vaccine as the first two doses and mRNA vaccine for third vaccine dose.

‡‡4 patients received vector-based vaccine as the first two doses and mRNA vaccine for third vaccine dose.

§§1 patient received vector-based vaccine as the first two doses; 1 patient received inactivated vaccine as the first two doses; and both received mRNA vaccine for third vaccine dose.

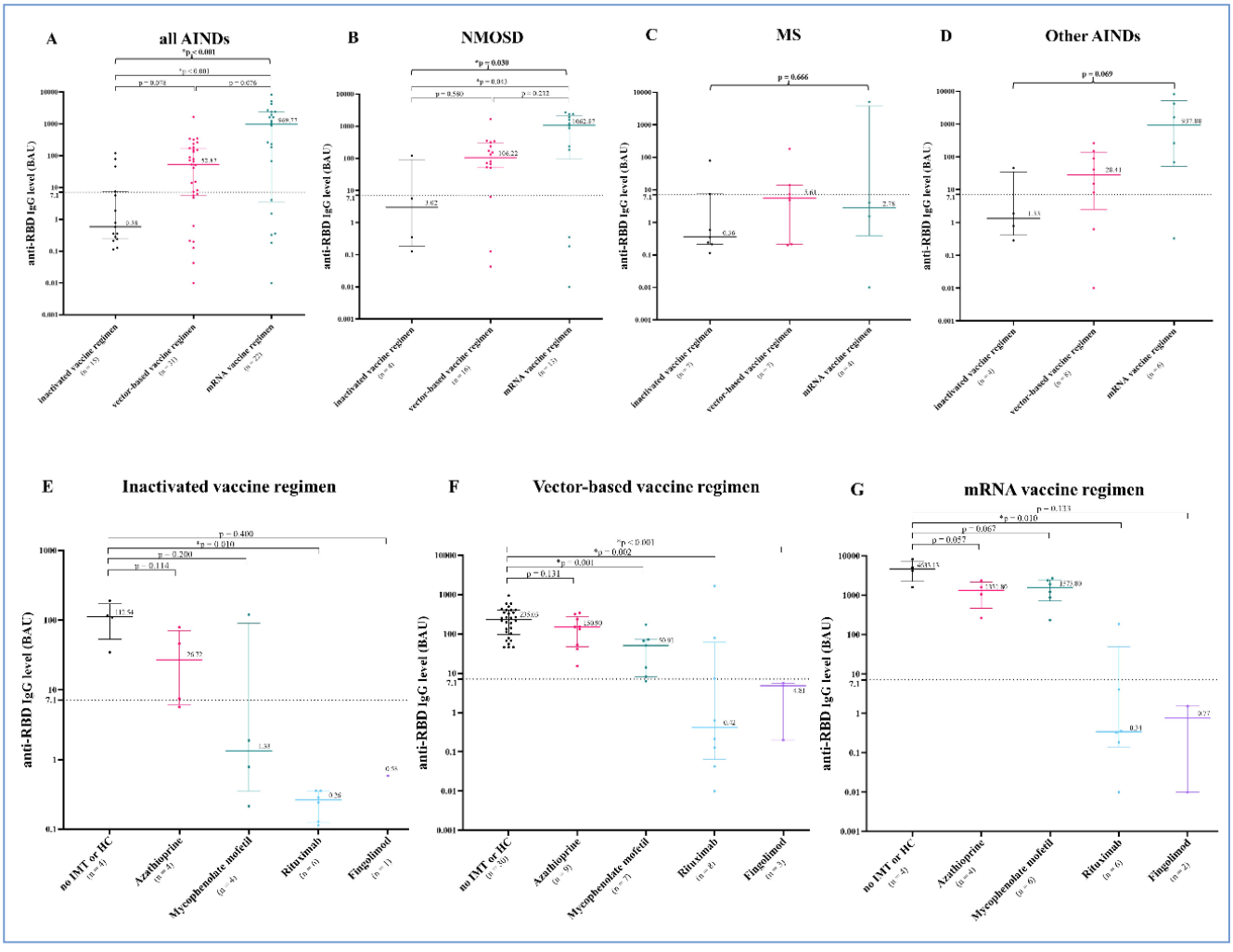


**Fig 2.** Anti-RBD IgG spike values categorized by types of vaccine regimens and immunotherapies

**Fig 2A-2D:** Nonparametric distributions are displayed as median (interquartile range) by bar and error bar, respectively. Anti-RBD IgG spike values categorized by type of vaccine regimen in different autoimmune neurological disease groups.

*Notes for figures A-D:-* (A) All AINDs, (B) NMOSD, (C) MS, and (D) other AINDs.

**Fig 2E-2G:** Nonparametric distributions are displayed as median (interquartile range) by bar and error bar, respectively Anti-RBD IgG responses in different vaccination categorized by different immunotherapy agents

*Notes for figures E-G:-* Groups compared were healthy control or no immunotherapy, azathioprine, mycophenolate mofetil, rituximab, and fingolimod(E) two doses of any vaccines, (F) three doses of any vaccines. Statistical significance was calculated by the Mann–Whitney U test. The anti-RBD IgG cut-off value (dash line) was 7.1 BAU/mL. *denotes a statistically significant result at the $p < 0.05$ level.

**Abbreviations:** RBD, receptor-binding domain; AINDs, autoimmune neurological diseases; NMOSD, neuromyelitis optica spectrum disorder; MS, multiple sclerosis; HC, healthy control; IMT, immunotherapy

*Inhibition percentage categorized by vaccine regimen*

The overall median inhibition percentages were higher in the Delta variant than the Omicron one regardless of vaccine regimen [53.84% (IQR 20.69-94.40) vs. 8.59% (IQR 6.05-23.09); $p < 0.001$].

The median NAbs inhibition percentage for the Delta variant was the highest in mRNA vaccine regimens [97.63% (80.77, 98.00)], followed by vector-based vaccine regimens [30.76% (IQR 13.48-80.32)], and inactivated vaccine regimens [22.3% (IQR 2.94-38.92)], respectively ($p < 0.001$). There were significant pairwise differences between mRNA and inactivated vaccine regimens ($p = 0.004$) along with between mRNA and vector-based vaccine regimens ($p < 0.001$). (Fig 3A)

The same pattern was mostly seen in the Omicron variant. mRNA vaccine regimens had the highest median inhibition percentage [25.94% (IQR 11.15-70.84)], followed by vector-based regimens [7.69% (IQR 5.44-11.67)] and inactivated vaccine regimens [6.05 (IQR 5.77-8.13)] ($p = 0.005$). Also, there was a significant pairwise difference between mRNA vaccines and vector-based vaccines ($p = 0.010$) and a trend to significance between mRNA vaccine regimens and inactivated vaccine regimens ($p = 0.056$). None of the regimens showed an inhibition percentage greater than 30% for the Omicron variant. (Fig 3B)

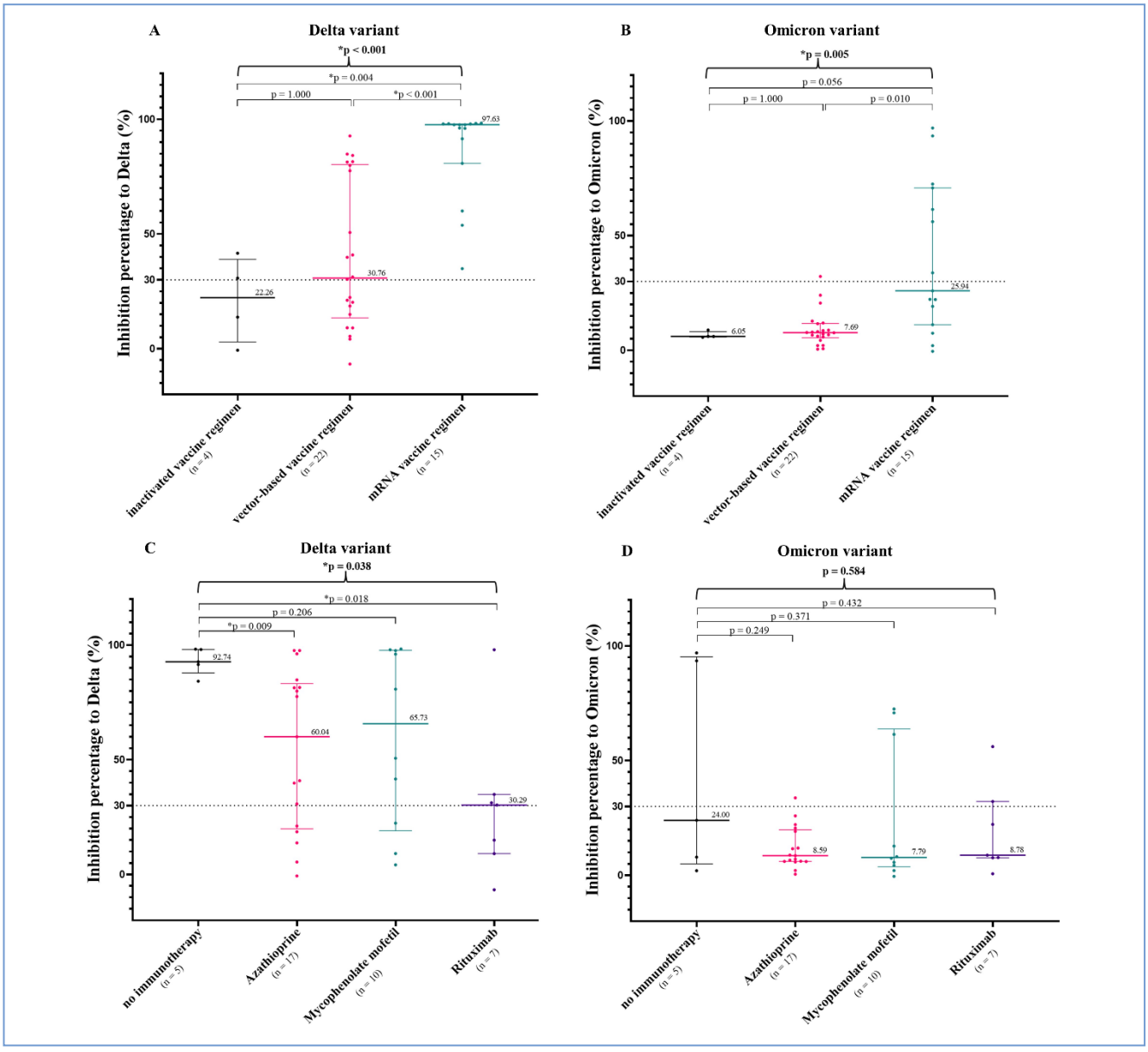


**Fig 3.** Neutralizing antibody inhibition percentage categorized by each vaccine regimen and each type of immunotherapy

**Fig 3A & 3B:** Inhibition percentage of neutralizing antibody categorized by each vaccine regimen in different COVID-19 variants for (A) Delta variant, (B) Omicron variant.

*Notes for figures A-B:-* Nonparametric distributions are displayed as median (interquartile range) by bar and error bar, respectively. The cut off value (dashed line) for inhibition response was set at 30%. Statistical significance was calculated by the Kruskal-Wallis test. *denotes a statistically significant result at the $p < 0.05$ level.

**Fig 3C & 3D:** Inhibition percentage of neutralizing antibody categorized by immunotherapy in different COVID-19 variants (C) Delta variant, (D) Omicron variant

*Notes for figures C and D:-* Nonparametric distributions are displayed as median (interquartile range) by bar and error bar, respectively. Statistical significance was calculated by the Mann–Whitney U test. *denotes a statistically significant result at the $p < 0.05$ level.
The cut-off value (dashed line) for inhibition response was set at 30%.

*Inhibition percentage categorized by type of immunotherapy*

Patients tested for the Delta variant showed a significantly greater median inhibition percentage than the Omicron variant across all IMTs ($p < 0.001$). Compared to the no IMT group [92.74% (IQR 87.88-98.15)], AZA and RTX groups showed significant attenuation of inhibition percentages against the Delta variant [AZA 60.04% (IQR 19.87-83.22); $p = 0.009$] and RTX 30.29% (IQR 9.06-34.93); $p = 0.018$] (Fig 3C). For the Omicron variant, the no IMT group showed no significant difference in inhibition percentages compared with either IMT use groups. (Fig 3D)

### *T cell Responses to the SARS-CoV2 Virus*

Twenty-two patients with 23 serum samples were tested for T cell responses with seven samples tested after the second vaccination, and 16 samples tested after the third vaccination. One patient was tested after the second and third dose of vaccination.

The T cell response did not significantly differ between the second and third vaccination; Ag1-Nil 0.21 IU/mL (IQR -0.01-1.88) vs. 0.23 IU/mL (IQR -0.03-3.59), $p$ = 0.343 and Ag2-Nil 0.22 IU/ml (IQR 0.00-2.41) vs. 0.57 IU/mL (IQR -0.05-8.67), $p$ = 0.131, respectively.

*T cell Responses Categorized by Vaccine Regimen*

T cell response measured by Ag1-Nil and Ag2-Nil were no significantly different across all vaccine regimes ($p$ = 0.346 for Ag1-Nil and $p$ = 0.297 for Ag2-Nil). The mRNA vaccine regimens had the highest positive rates for Ag1-Nil response at 64.71% (11 of 17) compared with 60% (3 of 5) in vector-based regimens and 0% (0 of 1) in inactivated vaccine regimens. However, the positive rate for Ag2-Nil response was the highest in vector-based vaccine regimens at 80% (4 of 5), followed by 12 of 17 (70.59%) in mRNA regimens, and 0% (0/1) in inactivated vaccine regimen. (Fig 4A-4B)

*Anti-CD20 Therapy Demonstrates Similar T cell Responses to Non-anti-CD20 Treatment*

Among the group treated with no IMTs (n = 3), anti-CD20 RTX (n = 7), and non-anti-CD20 therapy (n = 13), both Ag1-Nil and Ag2-Nil revealed no significant differences (1.37 IU/mL [IQR 0.47-1.88], 0.19 IU/mL [IQR 0.08-0.98], and 0.24 IU/mL [IQR 0.01-0.50]; $p$ = 0.210 for Ag-1 Nil, and 2.41 IU/mL [IQR 1.32-2.42], 0.40 IU/mL [IQR 0.11-1.52], and 0.38 IU/mL [IQR 0.04-1.03]; $p$ = 0.193 for Ag2-Nil, respectively). (Fig 4C-4D)

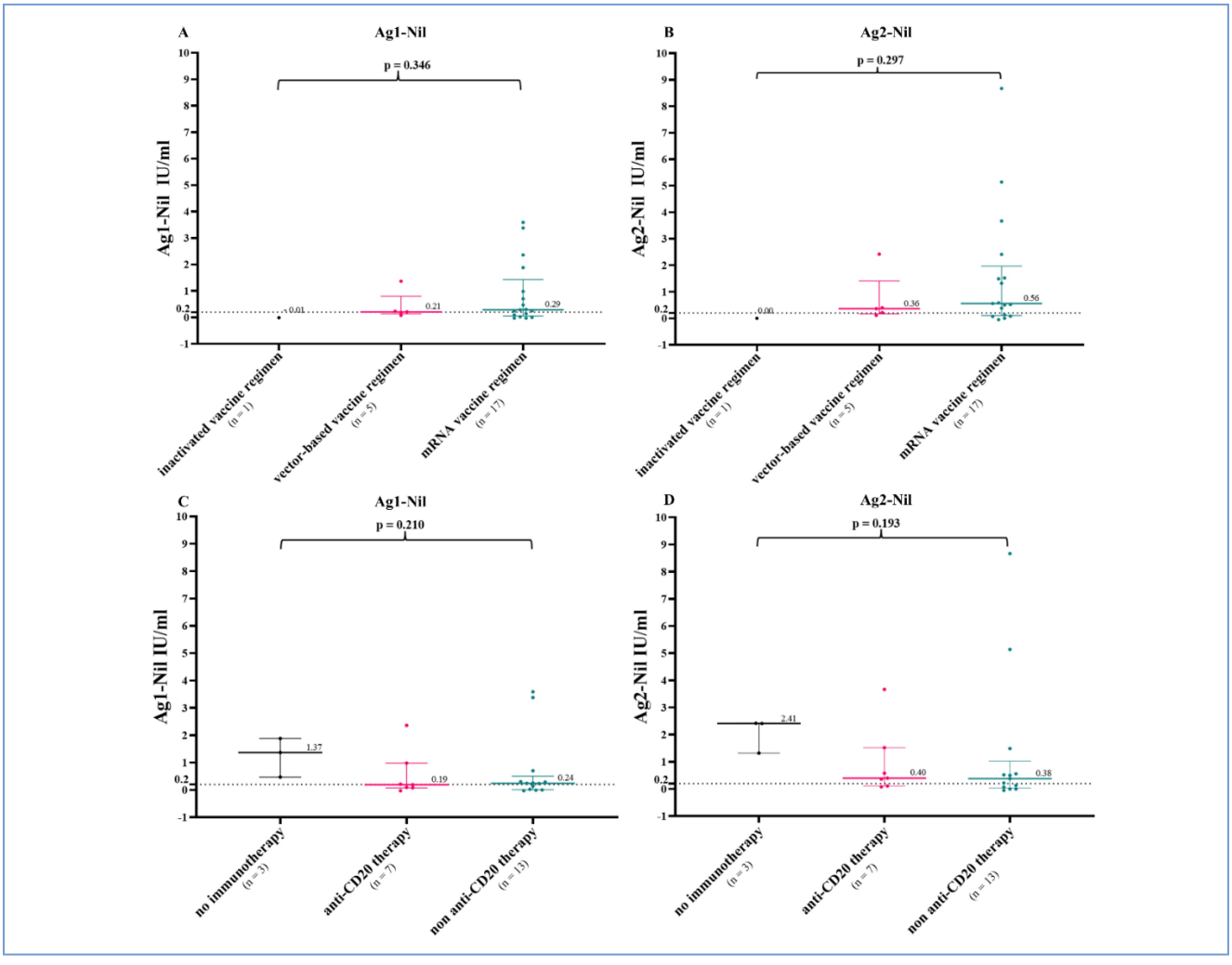


**Fig 4.** T cell responses for each vaccine regimen and each type of immunotherapy
**Fig 4A & 4B:** T cell responses for each vaccine regimen (A) Ag1-Nil, (B) Ag2-Nil
**Fig 4C & 4D:** T cell responses categorized by type of immunotherapy (C) Ag1-Nil, (D) Ag2-Nil
*Notes for figures 4A-4D:-* Nonparametric distributions are displayed as median (interquartile range) by bar and error bar, respectively. The cut-off value (dashed line) for Ag1-Nil and Ag2-Nil was set at 0.2 IU/mL. Statistical significance was calculated by the Kruskal-Wallis test. *denotes a statistically significant result at the $p < 0.05$ level.

**Immune Response Categorized by Disease; MS, NMOSD, and Other AINDs**

All anti-RBD IgG assays, neutralizing antibody assays, and interferon SARS-CoV-2 immunoassays were categorized by disease. The details for each immune response are described in the Results section and Supplementary Fig S2 in the Supplementary Appendix.

## DISCUSSION

Our study shows COVID-19 vaccination augments the immune system in both T cell functions and B cell functions, demonstrated by anti-RBD IgG, surrogate NAbs, and interferon SARS-CoV-2 immunoassay. The response differed depending on the vaccine regimen, the IMT type, and the variant of COVID-19.

Similar to previous studies, vaccination platforms played a role in the responsiveness of antibodies.[20] Our study also showed inactivated vaccine regimens induced the lowest response as in Fig 2 and 3. mRNA vaccine regimens showed a significantly higher antibody response compared with inactivated and vector-based vaccine regimens among patients with central nervous system immunological diseases measured by the anti-RBD IgG antibody.

Compared to those with no IMT or healthy controls, there was a significant reduction of anti-RBD IgG levels in patients treated with RTX in all vaccine regimens.

The present study also showed neutralizing activity against SARS-CoV-2 measured by NAbs depended on the variant. The overall median inhibition percentages were higher for the Delta variant than for the Omicron variant regardless of vaccine regimen group and type of IMT used. However, for the Omicron variant, those having received an inactivated vaccine, a vector-based vaccine, or an mRNA vaccine regimen all showed a median inhibition percentage of no more than 30%. This supports a previous study showing the estimated effectiveness of two doses of a COVID-19 vaccine was high against symptomatic Delta infection while it was lower against symptomatic Omicron infection.[21]

Although mRNA vaccines seem to stimulate the highest response for the Delta variant, RTX and AZA showed a significant attenuation of the inhibition percentages to the Delta variant compared to the no IMT group.

The T cell response showed no significant difference among vaccine platforms after the second or third dose vaccination or among the groups treated with RTX, non-anti-CD20 therapy, and no IMTs.

These findings support the evidence that anti-CD20 therapy, such as RTX, attenuates vaccine responsiveness due to B lymphocyte suppression, concordant with previous reports.[10,11] A cohort study[10] showed patients with chronic inflammatory disease receiving B cell depletion therapy would have lower humoral responsiveness measured by spike IgG, NAb, and circulating S-specific plasmablast after COVID vaccination. Some studies[22,23] measured antibody response for the mRNA vaccine regimens in neurological patients, showing similar results. FGM and anti-CD20 therapy, such as RTX, showed no detectable immune response to the COVID-19 vaccines. Based on the drug mechanism, RTX can mediate B cell depletion by binding to the CD20 receptor on the B cell surface, resulting in eliciting fewer B cells immune responses.[24] FGM might play a role in inhibiting germinal center formation, resulting in fewer immune responses and less recall of antigens.[25] Theoretically, anti-CD-20 depleting agents, such as ocrelizumab and RTX, might cause a lower protective response to COVID-19 vaccines.[26] Nevertheless, several studies showed anti-CD20 treatments decreased the humoral response, but did not interfere with the cellular response.[27,28] B cell immunity and T cell response are essential for fighting COVID-19 infection, and the T cell level is accepted to be one of the predictors of COVID-19 severity.[29] We only evaluated one modality of B-cell function, which was the antibody response as levels of anti-RBD and NAbs.[30,31] The NAb assay in the present study is a surrogate neutralizing antibody assay (sVNT), which is a blocking ELISA detection kit that detects the presence of neutralizing antibodies against SARS-CoV-2 RBD.[32,33] However, NAbs do not necessarily correlate with anti-RBD activity. It should also be noted that immunoglobulin classes other than IgG could be important, especially in patients who received anti-CD20 therapy.

This prospective observational study had several limitations. The sample sizes in the compared groups were small, so comparisons may have been underpowered to detect true differences. Since our study is a real-life practice amongst consecutive cases, we could not test all the patients on all three tests, immunoglobulin classes, and adverse events of each patient due to time and resource limitations and the lack of correlation with clinical outcomes.

In conclusion, we have investigated the immunity response to COVID-19 vaccination in Thai patients with autoimmune neurological disease. In general, all DMDs/ISs may slightly decrease antibody level response, but they may not decrease cellular immunity. This cellular immunity may be sufficient to reduce the severity of COVID-19 infection. However, in MS patients on RTX or FGM therapy, choosing an mRNA vaccine regimen might be desirable (if possible) because of its superior

immunogenicity evidenced by a higher B cell response. To date, there has been no consensus on suggestions or recommendations for specific COVID vaccine platforms for patients with autoimmune neurological disease. Therefore, generalizing the results of the present study to other diseases and geographic areas requires caution.

## ACKNOWLEDGMENTS

We would like to thank Khemjira Karaketklang for data analysis.

### Declarations of interest

None.

### Data availability statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

### Funding

This research project was supported by Siriraj Research Development Fund, Grant number (IO) R016533016, Faculty of Medicine Siriraj Hospital, Mahidol University.

## REFERENCES

1. Andrews N, Stowe J, Kirsebom F, Toffa S, Rickeard T, Gallagher E, et al. Covid-19 Vaccine Effectiveness against the Omicron (B.1.1.529) Variant. N Engl J Med. 2022;386(16):1532-46.
2. Jara A, Undurraga EA, Gonzalez C, Paredes F, Fontecilla T, Jara G, et al. Effectiveness of an Inactivated SARS-CoV-2 Vaccine in Chile. N Engl J Med. 2021;385(10):875-84.
3. Lopez Bernal J, Andrews N, Gower C, Robertson C, Stowe J, Tessier E, et al. Effectiveness of the Pfizer-BioNTech and Oxford-AstraZeneca vaccines on covid-19 related symptoms, hospital admissions, and mortality in older adults in England: test negative case-control study. BMJ. 2021;373:n1088.
4. Frenck RW, Jr., Klein NP, Kitchin N, Gurtman A, Absalon J, Lockhart S, et al. Safety, Immunogenicity, and Efficacy of the BNT162b2 Covid-19 Vaccine in Adolescents. N Engl J Med. 2021;385(3):239-50.
5. Tisavipat N, Jitpratoom P, Siritho S, Prayoonwiwat N, Apiwattanakul M, Boonyasiri A, et al. The epidemiology and burden of neuromyelitis optica spectrum disorder, multiple sclerosis, and MOG antibody-associated disease in a province in Thailand: A population-based study. Mult Scler Relat Disord. 2023;70:104511.
6. Hor JY, Asgari N, Nakashima I, Broadley SA, Leite MI, Kissani N, et al. Epidemiology of Neuromyelitis Optica Spectrum Disorder and Its Prevalence and Incidence Worldwide. Front Neurol. 2020;11:501.
7. Holroyd K, Vogel A, Lynch K, Gazdag B, Voghel M, Alakel N, et al. Neuromyelitis optica testing and treatment: Availability and affordability in 60 countries. Mult Scler Relat Disord. 2019;33:44-50.
8. Mathew T, John SK, Kamath V, Murgod U, Thomas K, Baptist AA, et al. Efficacy and safety of rituximab in multiple sclerosis: Experience from a developing country. Mult Scler Relat Disord. 2020;43:102210.
9. Zheng C, Kar I, Chen CK, Sau C, Woodson S, Serra A, et al. Multiple Sclerosis Disease-Modifying Therapy and the COVID-19 Pandemic: Implications on the Risk of Infection and Future Vaccination. CNS Drugs. 2020;34(9):879-96.
10. Deepak P, Kim W, Paley MA, Yang M, Carvidi AB, Demissie EG, 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.
11. Louapre C, Ibrahim M, Maillart E, Abdi B, Papeix C, Stankoff B, et al. Anti-CD20 therapies decrease humoral immune response to SARS-CoV-2 in patients with multiple sclerosis or neuromyelitis optica spectrum disorders. J Neurol Neurosurg Psychiatry. 2022;93(1):24-31.
12. Angkasekwinai N, Sewatanon J, Niyomnaitham S, Phumiamorn S, Sukapirom K, Sapsutthipas S, et al. Comparison of safety and immunogenicity of CoronaVac and ChAdOx1 against the SARS-CoV-2 circulating variants of concern (Alpha, Delta, Beta) in Thai healthcare workers. Vaccine X. 2022;10:100153.
13. Angkasekwinai N, Niyomnaitham S, Sewatanon J, Phumiamorn S, Sukapirom K, Senawong S, et al. The immunogenicity and reactogenicity of four COVID-19 booster vaccinations against SARS-CoV-2 variants of concerns (Delta, Beta, and Omicron) following CoronaVac or ChAdOx1 nCoV-19 primary series. 2022.
14. Department of Disease Control MoPH, Thailand. Guidelines for vaccination against COVID-19 in Thailand - 2021 epidemic situation 2022 [Available from: https://ddc.moph.go.th/vaccine-covid19/getFiles/11/1628849610213.pdf.
15. Hassold N, Brichler S, Ouedraogo E, Leclerc D, Carroue S, Gater Y, et al. Impaired antibody response to COVID-19 vaccination in advanced HIV infection. AIDS. 2022;36(4):F1-F5.
16. Company NGB. Instruction for use: cPass™ SARS-CoV-2 Neutralization Antibody Detection Kit 2022 [cited 2022 26 August]. Available from: https://www.genscript.com/gsfiles/techfiles/GS-SOP-CPTS001G-05_L00847-C.pdf?1109908894.
17. QIAGEN Company. Instructions for use: QuantiFERON SARS-CoV-2 ELISA Kit [Internet]. 2022 [cited 2022 Aug 26]. Available from: https://www.qiagen.com/at/resources/download.aspx?id=3d27842e-c811-442c-bcad-a9d42945e59c&lang=en.
18. Johnson SA, Phillips E, Adele S, Longet S, Malone T, Mason C, et al. Evaluation of QuantiFERON SARS-CoV-2 interferon-g release assay following SARS-CoV-2 infection and vaccination. Clin Exp Immunol. 2023.
19. GraphPad Software Company. Prism: a statistical analysis software [Internet]. 2022 [cited 2022 Aug 26]. Available from: https://www.graphpad.com/scientific-software/prism.
20. Coyle PK, Gocke A, Vignos M, Newsome SD. Vaccine Considerations for Multiple Sclerosis in the COVID-19 Era. Adv Ther. 2021;38(7):3550-88.
21. Buchan SA, Chung H, Brown KA, Austin PC, Fell DB, Gubbay JB, et al. Estimated Effectiveness of COVID-19 Vaccines Against Omicron or Delta Symptomatic Infection and Severe Outcomes. JAMA Netw Open. 2022;5(9):e2232760.
22. Mariottini A, Bertozzi A, Marchi L, Di Cristinzi M, Mechi C, Barilaro A, et al. Effect of disease-modifying treatments on antibody-mediated response to anti-COVID19 vaccination

in people with multiple sclerosis. J Neurol. 2022;269(6):2840-7.
23. Sormani MP, Inglese M, Schiavetti I, Carmisciano L, Laroni A, Lapucci C, et al. Effect of SARS-CoV-2 mRNA vaccination in MS patients treated with disease modifying therapies. EBioMedicine. 2021;72:103581.
24. Luo W, Yin Q. B Cell Response to Vaccination. Immunol Invest. 2021;50(7):780-801.
25. Han S, Zhang X, Wang G, Guan H, Garcia G, Li P, et al. FTY720 suppresses humoral immunity by inhibiting germinal center reaction. Blood. 2004;104(13):4129-33.
26. Mateen FJ, Rezaei S, Alakel N, Gazdag B, Kumar AR, Vogel A. Impact of COVID-19 on U.S. and Canadian neurologists' therapeutic approach to multiple sclerosis: a survey of knowledge, attitudes, and practices. J Neurol. 2020;267(12):3467-75.
27. Apostolidis SA, Kakara M, Painter MM, Goel RR, Mathew D, Lenzi K, et al. Cellular and humoral immune responses following SARS-CoV-2 mRNA vaccination in patients with multiple sclerosis on anti-CD20 therapy. Nat Med. 2021;27(11):1990-2001.
28. Iannetta M, Landi D, Cola G, Malagnino V, Teti E, Fraboni D, et al. T-cell responses to SARS-CoV-2 in multiple sclerosis patients treated with ocrelizumab healed from COVID-19 with absent or low anti-spike antibody titers. Mult Scler Relat Disord. 2021;55:103157.
29. Peng Y, Mentzer AJ, Liu G, Yao X, Yin Z, Dong D, et al. Broad and strong memory CD4(+) and CD8(+) T cells induced by SARS-CoV-2 in UK convalescent individuals following COVID-19. Nat Immunol. 2020;21(11):1336-45.
30. Tauzin A, Gendron-Lepage G, Nayrac M, Anand SP, Bourassa C, Medjahed H, et al. Evolution of Anti-RBD IgG Avidity following SARS-CoV-2 Infection. Viruses. 2022;14(3).
31. Lo Sasso B, Agnello L, Giglio RV, Gambino CM, Ciaccio AM, Vidali M, et al. Longitudinal analysis of anti-SARS-CoV-2 S-RBD IgG antibodies before and after the third dose of the BNT162b2 vaccine. Sci Rep. 2022;12(1):8679.
32. Pang NY, Pang AS, Chow VT, Wang DY. Understanding neutralising antibodies against SARS-CoV-2 and their implications in clinical practice. Mil Med Res. 2021;8(1):47.
33. Khoury DS, Cromer D, Reynaldi A, Schlub TE, Wheatley AK, Juno JA, et al. Neutralizing antibody levels are highly predictive of immune protection from symptomatic SARS-CoV-2 infection. Nat Med. 2021;27(7):1205-11.