Neutralizing antibody responses against SARS-CoV-2 in vaccinated people with multiple sclerosis

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Abstract

Background: Patients with multiple sclerosis (pwMS) are often treated with disease modifying therapies (DMT) with immunomodulatory effects. This is of particular concern following the development of several vaccines to combat coronavirus disease 19 (COVID-19), a potentially fatal illness caused by SARS-CoV-2.

Objectives: To determine the efficacy of SARS-CoV-2 vaccination in pwMS and the impact of disease modifying therapies (DMT) on vaccine response.

Methods: This is a prospective longitudinal study in pwMS. Longitudinal serum samples were obtained prior to, and after SARS-CoV-2 mRNA vaccination. A novel neutralizing antibody (nAb) assay was used to determine nAbs titres against SARS-CoV-2 spike.

Results: We observed that (1) pwMS on B-cell depleting therapies exhibited reduced response to vaccination compared to other pwMS, correlating with time from last anti-CD20 infusion, (2) prior COVID-19 illness, DMT category, and pyramidal function were significant predictors of vaccine responsiveness, and (3) circulating absolute lymphocyte count (ALC) and IgG levels correlated with nAb levels.

Conclusions: We demonstrate that pwMS exhibit reduced nAb response to mRNA vaccination dependent on DMT status and identify predictive biomarkers for vaccine efficacy. We conclude that additional vaccination strategies may be necessary to achieve protective immunity in pwMS.

Keywords: Multiple sclerosis, immunology, disease-modifying therapies, vaccination, COVID-19

Date received: 20 January 2022; accepted: 25 February 2022

Introduction

Multiple Sclerosis (MS) is an acquired CNS inflammatory demyelinating disease; it is one of the leading causes of neurological disability in young adults in the Western Hemisphere. There are currently over 20 FDA-approved disease modifying therapies (DMT) which alter the course of MS through reduction in relapse rate and slowing of disability accumulation. These DMTs employ a variety of mechanisms with immunomodulatory effects. While these drugs have been instrumental in improving the prognosis of people with MS (pwMS), they are not without consequences. The most notable risks of DMTs include infections and attenuation of responses to vaccination. Such concerns have become heightened and imminent during the on-going coronavirus disease 19 (COVID-19) pandemic.

COVID-19, a potentially fatal illness caused by SARS-CoV-2, emerged as a global pandemic in early 2020 leading to worldwide health concerns. Several vaccines, including 2 innovative mRNA vaccines, were developed against this virus and have been administered across the US and other parts of the world. Vaccines targeting the SARS-CoV-2 spike protein have shown promising efficacy in preventing COVID-19 illness, and have a favourable safety profile. Subjects with underlying autoimmune disorders and/or on immunosuppressive therapies were excluded from initial vaccine clinical trials. Therefore, the safety and efficacy of the vaccines in
these populations is not known. Several studies have shown that pwMS treated with B-cell depleting therapies and sphingosine 1-phosphate (SIP) receptor modulators have a reduced humoral response following COVID-19 illness or vaccination.[8–10] The current recommendation from the National Multiple Sclerosis Society is that pwMS should be vaccinated against SARS-CoV-2 irrespective of DMT status.[11] An important clinical issue concerns the impact of DMTs used in pwMS on the efficacy of SARS-CoV-2 vaccination, and the eventual need for booster doses.

The aims of this study are to determine the efficacy of SARS-CoV-2 vaccination in patients with CNS inflammatory conditions including MS, and the impact of DMTs on vaccine response. We used a unique assay to specifically measure neutralizing antibodies against the SARS-CoV-2 spike protein.

Methods

Patient Cohort: This is a prospective longitudinal study in subjects with inflammatory CNS disorders including MS at the Ohio State University (OSU) MS Center. All enrolled subjects provided written informed consent for the study which was approved by the OSU institutional review board (study ID: 2020H0585). Subjects 18 years and older with MS were eligible for enrolment into the study. Patients were diagnosed by MS fellowship trained neurologists based on clinical presentation, radiological data, serum/CSF studies and electrophysiological data. All pwMS satisfied the 2017 McDonald’s criteria. The pwMS enrolled in the study were either scheduled to receive or had received one of the current FDA authorized SARS-CoV-2 vaccines, BNT162b2 from Pfizer/BioNTech (n = 36), mRNA-1273 from Moderna (n = 15), or Ad26.COV2 from Johnson and Johnson (n = 1); mean age of 42.9 years (standard deviation of 11.6 years, range 24–71 years). Serum samples were collected from subjects at least 4 weeks prior to (n = 42) and/or 4–8 weeks after (n = 52) completion of vaccination course between January 15th 2021 and August 31st 2021. All samples were collected under approved IRB protocols (2020H0228 and 2020H0527). HCW samples were collected under approved IRB protocols (2020H0228 and 2020H0527). HCW were vaccinated with Comirnaty/BNT162b2 from Pfizer/BioNTech (n = 26) or mRNA-1273 from Moderna (n = 12) and were collected 4 weeks post-vaccination with the second dose.

Data: Additional collected data included subject demographics (age, sex, race, ethnicity), MS history, DMT history (current and prior), other active medications, COVID-19 status, recent use of corticosteroids, smoking history, comorbid medical conditions, Expanded Disability Status Scale (EDSS) score, ambulatory status, timed 25ft walk, 9-hole PEG test and vaccination type, date, and side effects. Clinical and laboratory data collected from electronic medical records included the most recent MRI scan reports, circulating white blood cell count (WBC), absolute lymphocyte count (ALC), CD20 count and serum immunoglobulin levels (IgA, IgG, IgM).

Conducts for Pseudotyped Virus Production: We utilized an HIV-1 pNL4-3-inGluc construct,[13] originally a gift from David Derse’s lab at NIH (National Cancer Institute, Frederick, Maryland, USA), for production of the pseudotyped lentivirus. This construct is based on ΔEnv pNL4-3 HIV-1 vector and bears a CMV promoter driving a Gaussia luciferase reporter gene in antisense orientation relative to the HIV-1 genome. The Gaussia luciferase gene then contains a sense orientation intron to prevent expression of Gaussia luciferase in virus producer cells. SARS-CoV-2 spike construct pcDNA3.1-SARS-CoV-2-Flag-S-Flag was produced from a pcDNA3.1-SARS-CoV-2-S construct originally obtained from Jason McLellan’s lab at the University of Texas at Austin (Austin, Texas, USA). N- and C-terminal flag tags were added by overlap PCR and subsequently cloned into pcDNA3.1 by restriction enzyme cloning using Kpn I and BamH I.[12]

Cell Lines and Maintenance: HEK293T (ATCC CRL-11268, CVCL_1926) and HEK293T-ACE2 (BEI NR-52511) were maintained in Dulbecco’s Modified Eagles Medium (Gibco, 11965-092) supplemented with 10% fetal bovine serum (Sigma, F1051) and 1% penicillin-streptomycin (HyClone, SV30010), and were kept at 37°C and 5% CO2.

Pseudotyped Virus Production and Titering: HEK293T cells were transfected with HIV-1 pNL4-3-inGluc and pcDNA3.1-SARS-CoV-2-Flag-S-Flag in a 2:1 ratio using polyethylenimine (PEI) transfection. Virus was collected 24 h, 48 h, and 72 h after transfection and was pooled and stored at –80°C.

Virus Neutralization Assay: Patient serum was assessed for neutralizing antibody titres as previously described[12] Briefly, SARS-CoV-2 pseudotyped
lentivirus was incubated for 1 h with serial dilutions of patient serum (1:40, 1:160, 1:640, 1:2560, 1:10240, and no serum). This neutralized virus was then used to infect HEK293T-hACE2 cells. *Gaussia* luciferase secreted into the cell culture media was then assayed 48 h and 72 h after infection by combining 20 μL cell culture media with 20 μL *Gaussia* luciferase substrate (0.1 M Tris (MilliporeSigma, #T6066) pH 7.4, 0.3 M sodium ascorbate (Spectrum, S1349), 10 μM coelenterazine (GoldBio, CZ2.5)). Luminescence was measured by a BioTek Cytation5 plate-reader. Neutralization titre 50% (NT50) values were calculated from VNA output using a non-linear regression with least-squares fitting in GraphPad output using a non-linear regression with least-squares fitting in GraphPad software (San Diego, California, USA).

**Statistics:** Pairwise comparisons for pre- and post-vaccine titres were made using unpaired, two-tailed student t-test. All other comparisons of two groups were made using unpaired, two-tailed student's t-test. Comparisons between multiple groups were made using one-way analysis of variance (ANOVA) with Bonferroni’s multiple comparisons correction.

Univariate linear regression models were used to examine associations between each potential correlate and post-vaccine NT50 values. NT50 values were natural log transformed in these models to better approximate normality of residuals. Backward elimination was then performed starting with all variables significant at the 0.1 levels in the univariate analysis and forced factors that were considered clinically important (age, gender, history of COVID19 infection, days post-2nd vaccination) to be included in final model; elimination proceeded until all remaining variables were significant at the 0.05 level. All analyses were conducted using SAS v9.4 (SAS institute, Cary, North Carolina).

**Results**

Eighty-three subjects were enrolled for the study (71% female), 51 of these subjects had post-mRNA vaccination serum samples analyzed. We first sought to determine the strength of the nAb response to mRNA vaccination in pwMS and healthy controls across our cohorts. When compared to HCW samples, pwMS exhibited ~2.4-fold lower NT50 values (Figure 1A). The reduced humoral response of pwMS to mRNA vaccines may be secondary to their course of treatment and/or underlying conditions. Of the 51 pwMS with post-vaccination samples, 40 had pre-vaccination samples collected. Collectively, these patients had a significant increase in nAb titres following vaccination (Figure 1B). However, a subset of patients (n = 10) exhibited NT50 values below background (NT50 < 40) (Figure 1A and B).

To better understand the factors impacting responsiveness to mRNA vaccination, we stratified the pwMS pool by DMT status at the time of vaccination. There were 16 patients on no DMT, 6 on interferon beta-1a (INF-β1a), 9 on fumarates (dimethyl and diroximel fumarate), 1 on glatiramer acetate, 2 on natalizumab, 13 on B-cell depleting therapies (ocrelizumab and rituximab), and 4 on S1P receptor modulating therapies (fingolimod, siponimod and ozanimod). B-cell depleting therapies and S1P modulating therapies (which lead to sequestration of lymphocytes in lymphoid tissues) are of particular concern due to their impact on adaptive immunity. In fact, we observed a >9-fold reduction in the nAb response of patients treated with B-cell depleting agents compared to other therapies or no therapy (p < 0.001), with 61.5% (8/13) showing no detectable levels of nAb (Figure 1C and D). S1P modulating therapies also showed a lower mean NT50 value than the other therapies, albeit not statistically significant (Figure 1C and D), potentially due to insufficient statistical power.

We next examined the impact of prior COVID-19 status on patients’ neutralizing antibody responses. Of the 40 patients with both pre- and post-vaccination samples, 5 reported a prior positive COVID-19 test. Indeed, these individuals exhibited higher pre-vaccination NT50 values (mean = 1201) (Figure 1E) compared to those patients who had never been tested for COVID-19 or had only tested negative (mean = 16). Notably, these 5 patients showed only a subtle increase in their nAb titres following vaccination, although this appeared to be dependent on their pre-vaccination titres. Two individuals with low pre-vaccination titres showed a dramatic increase, but 3 individuals with high pre-vaccination titres even experienced a slight decrease or plateau (Figure 1E).

We previously reported that Moderna mRNA-1273 vaccinated healthy individuals generally exhibit higher nAb titres than those vaccinated with Pfizer/BioNTech BNT162b2.[13] In this patient cohort, we observed the same trend, but with no statistically significant difference in nAb titres elicited by the two vaccines (Figure 1F). The nAb titre in male MS patients was relatively high (Figure 1G), similar to some previous reports on response to COVID-19 status,[14] but this did not reach statistical significance.

We further sought to examine the univariate association of clinical outcome measures, DMT treatment,
Figure 1. Neutralizing antibody response to mRNA vaccination in patients with multiple sclerosis. SARS-CoV-2 pseudotyped lentivirus bearing a *Gaussia* luciferase reporter gene was incubated with serial dilutions of sera collected from pwMS (pre- and post-vaccination) or HCWs (collected post-vaccination). Neutralized virus was then used to infect HEK293T-ACE2 cells and the resulting luciferase readout at 48 h and 72 h after infection was used to calculate neutralizing titres 50% (NT50). (A) Post-mRNA vaccination NT50 values for pwMS and HCWs are displayed. (B) For 40 of the 51 pwMS, whose pre-vaccination samples were collected, the NT50 values for pre- and post-vaccination are displayed. (C) NT50 values for pwMS stratified by treatment group including no therapy, INF-β1a, fumarates (dimethyl and diroximel fumarate), glatiramer acetate, natalizumab, B-cell depleting therapies (ocrelizumab and rituximab), and S1P modulating therapies (fingolimod, siponimod and ozanimod). (D) Percentage of subjects within given NT50 ranges for HCWs, all pwMS, and pwMS treatment groups. (E) Of the 40 pwMS with pre-vaccination samples, 5 self-reported prior positive COVID-19 testing. Pre- and post-vaccination NT50 values for these 5 pwMS are displayed and two patients on B-cell depleting therapies are indicated with arrows. (F) Patient NT50 values were examined based on type of mRNA vaccine received, either Moderna or Pfizer. (G) Patient NT50 values were examined based on gender. P-values are represented as ***p < 0.001. (H) Scatter plot between log10 transformed post-vaccine NT50 values and days from last infusion to the 1st dose of vaccination. Mean and n values are displayed at the top of all plots, and the background level (NT50 < 40) is indicated with a dashed line. Statistical significance was determined by unpaired two tailed t-test (A, F, G), paired two tailed t-test (B, E), one-way ANOVA (C), or linear regression (H).
| Predictor                              | Raw NT50 values, Mean (SD) | Least Square Means | SE   | 95% CI     | P-value |
|---------------------------------------|-----------------------------|--------------------|------|------------|---------|
| Race                                  |                             |                    |      |            | 0.58    |
| Non-White \(N = 9\)                   | 801.91 (1005.44)            | 4.90               | 0.94 | 3.01–6.79  |         |
| White \(N = 42\)                      | 1475.27 (1609.72)           | 5.81               | 0.44 | 4.93–6.69  |         |
| Gender                                |                             |                    |      |            | 0.06    |
| Male \(N = 12\)                       | 1526.78 (1034.18)           | 6.98               | 0.79 | 5.38–8.57  |         |
| Female \(N = 39\)                     | 1304.04 (1665.97)           | 5.24               | 0.44 | 4.36–6.13  |         |
| Smoking history                       |                             |                    |      |            | 0.54    |
| Never smoker \(N = 34\)               | 1525.36 (1729.04)           | 5.48               | 0.49 | 4.50–6.45  |         |
| Past/current smoker \(N = 17\)       | 1018.62 (1003.76)           | 6.00               | 0.69 | 4.61–7.38  |         |
| Ambulatory Status                     |                             |                    |      |            | 0.24    |
| Fully ambulatory \(N = 42\)          | 1478.44 (1603.46)           | 5.96               | 0.42 | 5.12–6.81  |         |
| Walk with assistance \(N = 8\)       | 885.38 (1071.40)            | 4.72               | 0.96 | 2.79–6.65  |         |
| Steroid treatment within the last 2 months |                     |        |    |           | 0.46    |
| No \(N = 38\)                        | 1337.79 (1589.97)           | 5.48               | 0.46 | 4.55–6.40  |         |
| Yes \(N = 13\)                       | 1410.97 (1417.12)           | 6.15               | 0.79 | 4.57–7.73  |         |
| Visual function                       |                             |                    |      |            | 0.87    |
| 0 \(N = 19\)                         | 1265.93 (1762.35)           | 5.61               | 0.66 | 4.27–6.94  |         |
| >0 \(N = 26\)                        | 1581.17 (1486.55)           | 5.76               | 0.57 | 4.61–6.90  |         |
| BrainStem function                    |                             |                    |      |            | 0.99    |
| 0 \(N = 41\)                         | 1437.11 (1642.91)           | 5.69               | 0.45 | 4.78–6.60  |         |
| >0 \(N = 4\)                         | 1560.36 (1186.28)           | 5.70               | 1.45 | 2.78–8.61  |         |
| Pyramidal function                    |                             |                    |      |            | 0.02    |
| 0 \(N = 27\)                         | 1775.31 (1802.47)           | 6.49               | 0.52 | 5.44–7.55  |         |
| >0 \(N = 18\)                        | 957.21 (1099.28)            | 4.49               | 0.64 | 3.20–5.78  |         |
| Cerebellar score                      |                             |                    |      |            | 0.14    |
| 0 \(N = 36\)                         | 1604.48 (1685.24)           | 6.01               | 0.47 | 5.06–6.96  |         |
| >0 \(N = 9\)                         | 822.42 (1027.03)            | 4.44               | 0.94 | 2.54–6.33  |         |
| Sensory score                         |                             |                    |      |            | 0.12    |
| 0 \(N = 31\)                         | 1656.44 (1733.11)           | 6.14               | 0.50 | 5.12–7.16  |         |
| >0 \(N = 14\)                        | 986.67 (1170.33)            | 4.70               | 0.75 | 3.18–6.21  |         |
| Bowel bladder score                   |                             |                    |      |            | 0.28    |
| 0 \(N = 31\)                         | 1497.84 (1659.79)           | 5.97               | 0.52 | 4.92–7.01  |         |
| >0 \(N = 13\)                        | 1341.89 (1563.81)           | 4.93               | 0.80 | 3.32–6.54  |         |
| Cerebral score                        |                             |                    |      |            | 0.0062  |
| 0 \(N = 26\)                         | 1884.72 (1848.36)           | 6.66               | 0.52 | 5.61–7.71  |         |
| >0 \(N = 19\)                        | 850.54 (915.64)             | 4.36               | 0.61 | 3.14–5.59  |         |
| Ambulation score                      |                             |                    |      |            | 0.07    |
| 0 \(N = 37\)                         | 1635.84 (1668.60)           | 6.05               | 0.46 | 5.13–6.97  |         |
| >0 \(N = 8\)                         | 579.65 (817.05)             | 4.04               | 0.98 | 2.05–6.02  |         |
| EDSS step score                       |                             |                    |      |            | 0.24    |
| 0 \(N = 9\)                          | 2275.65 (2264.23)           | 7.09               | 0.94 | 5.19–8.99  |         |
| 1–3 \(N = 28\)                       | 1304.70 (1432.53)           | 5.46               | 0.53 | 4.38–6.54  |         |
| 3.5–6 \(N = 8\)                      | 1017.83 (1042.51)           | 4.94               | 1.00 | 2.92–6.96  |         |
| History of Covid-19 infection         |                             |                    |      |            | 0.095   |
(continued)
and laboratory parameters with vaccine responsiveness. We first examined the association between degree of neurological disability, measured using the functional system score (FSS), and natural log-transformed NT50 values. We found that NT50 values negatively correlated with pyramidal (motor) and cerebral (cognitive) function. NT50 values were significantly lower in pwMS on DMT (B-cell depleting, S1P modulating, or other) versus those not on therapy. There were borderline associations with gender, ambulation score, and history of COVID-19 illness (Table 1). Furthermore, we found a significantly positive correlation between NT50 values and lymphocyte count or IgG levels (Table 2). At the conclusion of backward elimination (Table 3), only history of COVID-19, pyramidal function, and DMT category significantly impacted nAb titres, with B-cell depleting therapies (p < 0.0001) and S1P modulating therapies (p = 0.0378) resulting in significantly reduced NT50 values.

Subgroup analyses of pwMS on B-cell depleting agents revealed a significant relationship between post-vaccine NT50 values and time from last infusion to vaccine administration (Figure 1H). For every additional day from the last infusion to vaccine administration, post-vaccine NT50 values increased by 3.7% (p = 0.0032). The duration of B-cell depleting drug exposure ranged between 1–41 months with an average of 24 months and a median of 25 months. There was no statistically significant correlation between duration of drug exposure with NT50 values, Spearman correlation coefficient 0.35 (p = 0.24).

Discussion

We report reduced neutralizing humoral responses to the SARS-CoV-2 mRNA vaccination in a subset of patients with MS. Treatment with either B-cell depleting or S1P receptor modulating therapies, both of which reduce the frequency of circulating lymphocytes, was associated with reduced nAb responses to SARS-CoV-2 vaccination. Our findings are similar to the results of other published studies that measured COVID-19 vaccine responses in patients with MS,[8–10] although those studies used antibody binding, as opposed to neutralizing assays. A number of studies, conducted prior to the COVID-19 pandemic, demonstrated reduced humoral responses to other vaccine types in pwMS treated with ocrelizumab[15] and fingolimod.[16] Our subgroup analyses of patients treated with anti-CD20/ B-cell depleting therapies (rituximab and ocrelizumab) revealed that nAb levels correlated directly with the length of time between the most recent drug infusion and SARS-CoV-2 vaccination nAb levels, i.e., longer interval between the infusion and vaccine administration resulted in higher nAb titre. This corroborated our previous findings in hematological cancer patients on similar B-cell depleting therapies.[17] Other studies have shown similar correlations between vaccine induced antibody levels and the time since last dose of anti-CD20 therapy.[18] Consistent with these observations, circulating CD20 cell levels also correlate with vaccine-induced antibody responses.[19] B-cell repopulation in anti-CD20 treated patients has been shown to display high inter- and intra-individual variability.[20] Collectively, these data suggest there may be a benefit in administering SARS-CoV-2 vaccination towards the later portion of anti-CD20 treatment cycle, and

Table 1. Continued.

| Predictor                  | Raw NT50 values, Mean (SD) | Least Square Means | SE  | 95% CI   | P-value |
|----------------------------|---------------------------|--------------------|-----|----------|---------|
| Diagnosed and recovered (N = 8) | 1804.87 (1493.25)         | 7.18               | 0.98| 5.21–9.15|         |
| Never diagnosed (N = 43)    | 1273.02 (1544.35)         | 5.37               | 0.42| 4.52–6.21|         |
| Vaccine                    |                           |                    |     |          | 0.79    |
| Moderna (N = 15)           | 1596.47 (1750.72)         | 5.81               | 0.74| 4.33–7.29|         |
| Pfizer (N = 36)            | 1256.44 (1449.65)         | 5.58               | 0.47| 4.63–6.54|         |
| Therapy                    |                           |                    |     |          | <.0001  |
| B-cell Depleting (N = 13)  | 199.69 (305.0)            | 2.48               | 0.56| 1.35–3.62|         |
| Other (N = 18)             | 2372.31 (1918.11)         | 7.26               | 0.48| 6.30–8.23|         |
| S1P Mod (N = 4)            | 738.71 (1321.51)          | 4.30               | 1.02| 2.26–6.34|         |
| None/NA (N = 16)           | 1307.90 (866.06)          | 6.74               | 0.51| 5.72–7.77|         |
that monitoring peripheral B cell counts might be informative in determining the optimal timing of COVID19 vaccinations, even warranting a delay in the next infusion.

Prior COVID-19 illness was identified as a marker for significantly higher pre-vaccine nAbs levels compared to those without history of COVID-19 or never tested. This is consistent with prior studies showing rapid generation of nAbs in COVID-19 patients. While 2 of these patients still exhibited a response to mRNA vaccination, 3 patients with higher pre-vaccination NT50 values showed reduced or plateaued response after vaccination. Of these 3 patients exhibiting no response to vaccination, 2 of them were on B-cell depleting therapies which may have limited their response to vaccination and a single patient was on glatiramer acetate and exhibited very modest response.

Univariate analysis of serological data revealed a significant positive correlation between nAb levels and pre-vaccination ALC or IgG levels. S1P modulating therapies reduce circulating lymphocytes and B-cell depleting drugs cause profound B-cell lymphopenia. Long term use of rituximab has been associated with hypogammaglobulinemia, where a subset of patients with reduced IgG levels develop recurrent infections. Another study revealed that lymphopenia in patients treated with fingolimod was associated with reduced humoral response to SARS-CoV-2 vaccination, but this correlation was not observed in ocrelizumab treated subjects. Our results suggest that ALC and IgG levels may serve as predictive biomarkers for vaccine efficacy in patients treated with S1P modulating and B-cell depleting therapies, respectively. Further studies are needed to confirm and establish these correlations.

The EDSS score and ambulatory status did not correlate with nAb levels. However, there was a correlation between the pyramidal and cerebral (cognitive) function with nAb levels where patients with pyramidal and cerebral FSS greater than 0 had lower nAb levels than those with a score of 0. Notably, multiple registries have reported a correlation between COVID-19 illness severity with more severe disability in pwMS. While the reason for this correlation is not clear, a possible explanation could be the more frequent use of B-cell depleting and S1P receptor modulating therapies in pwMS with more severe disability and progressive MS phenotypes. A unique strength of our study is the use of a novel, sensitive virus neutralization to determine nAb levels in MS patients with different treatments. This assay utilizes SARS-CoV-2 pseudotyped lentivirus bearing a Gaussia luciferase reporter gene that allows for high throughput readout of virus neutralization that correlates well with an infectious SARS-CoV-2 based virus neutralization assay. Additionally, the collection of pre-vaccination samples allowed for comparison to baseline nAb titres—such comparisons are important as prior SARS-CoV-2 infection can enhance response to mRNA vaccination.

Limitations of the study include relatively small sample size particularly in subgroups of some DMTs. Additionally, examination of cell-based immunity was beyond the scope of this study, but would have provided insight into other immune pathways governing the mRNA vaccine-mediated

| Predictor                        | b    | SE    | P-value |
|----------------------------------|------|-------|---------|
| Age \( (N = 51) \)               | 0.0036 | 0.034 | 0.92    |
| BMI \( (N = 51) \)               | −0.052 | 0.048 | 0.28    |
| WBC \( (N = 44) \)              | 0.051 | 0.24  | 0.83    |
| Lymphocyte count \( (N = 44) \) | 1.91  | 0.62  | 0.004   |
| B cell count \( (N = 16) \)     | 0.007 | 0.004 | 0.097   |
| IGG test result \( (N = 27) \)  | 0.005 | 0.002 | 0.0089  |
| IGM test result \( (N = 27) \)  | 0.010 | 0.007 | 0.16    |
| IGA test result \( (N = 27) \)  | 0.0015 | 0.007 | 0.83    |
| Days post 2\textsuperscript{nd} vaccine \( (N = 39) \) | −0.00023 | 0.014 | 0.99    |

Table 2. Univariate association between natural log transformed post NT50 values and individual continuous predictors.
protection in MS patients. We plan on examining anti-SARS-CoV-2 T-cell responses in similar patient cohorts in future studies.

The results of our study provide evidence that can be used for establishing guidelines for pwMS regarding SARS-CoV-2 vaccination. Future directions for this study will be to determine the effect of additional vaccine doses (boosters) on nAb in pwMS. On August 12th, 2021, the FDA authorized the administration of additional dose of both the Pfizer-BioNTech and Moderna mRNA vaccines to immunocompromised individuals. We plan to obtain longitudinal serum samples to determine nAb levels as subjects receive the booster dose of the vaccine. Findings from the extended part of this study will provide further evidence on the role of the booster dose of the vaccine in pwMS and the impact of DMTs on this response.

**Acknowledgements**

We thank the NIH AIDS Reagent Program and BEI Resources for supplying important reagents that made this work possible. S.-L.L. and R.G. were supported by the National Cancer Institute of the NIH under award no. U54CA260582. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. This work was supported by a fund provided by an anonymous private donor to OSU; additional support of S.-L.L.’s lab includes NIH grant R01 AI150473. J.P.E. was supported by Glenn Barber Fellowship from the Ohio State University College of Veterinary Medicine. B.M.S. is supported by grants from the NINDS, National Institutes of Health (R01 NS105385) and NEI (R01EY029159 and R01EY028350). B.M.S. holds the Stanley D. and Joan H. Ross Chair in Neuromodulation at the Ohio State University.

**Author contributions**

T.V.G contributed to study design and performed clinical recruitment, sample acquisition, and drafting/revision of the manuscript. J.P.E. performed the majority of the virus neutralization assays, aided in drafting and revision of the manuscript, and aided in statistical analysis. C.Z. aided in virus neutralization assays and provided discussion. J.M. and K.A. performed collection and management of clinical data. E.H.B. contributed to study design, sample acquisition and storage. J.P. performed statistical analysis. S.-L.L. contributed to study design, management of research personnel, acquired funding, and aided in manuscript revision. B.M.S. contributed to study design, supervised the study and performed critical revision of the manuscript. All authors contributed to data interpretation and manuscript review.

**Declaration of Conflicting Interests**

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: T.V. Gyang has served as consultant for Genentech, Horizon Therapeutics, Greenwich Biosciences and EMD Serono. J.P Evans reports no disclosure relevant to the manuscript. J. Miller reports no disclosure relevant to the manuscript. K. Alcorn reports no disclosure relevant to the manuscript. J. Peng reports no disclosure relevant to the manuscript. E.H. Bell reports no disclosure relevant to the manuscript. C. Zeng reports no disclosure relevant to the manuscript. R. Gumina reports no disclosure relevant to the manuscript. S-L. Liu reports no disclosure relevant to the manuscript. B.M. Segal has served as a consultant for Neurodiem, Senda Biosciences, and Banner Life Sciences, as a speaker for PRIME, Inc. and as a moderator for Neurodiem. He has served on a data safety monitoring board for Eli Lilly.

## Table 3. Multivariate model after backward elimination.

| Parameter                                      | Estimate | Standard Error | t Value | Pr > |t| |
|-----------------------------------------------|----------|----------------|---------|------|---|
| Intercept                                     | 6.04     | 1.45           | 4.17    | 0.0002|
| Age                                           | 0.0058   | 0.027          | 0.21    | 0.83 |
| Female vs. Male                               | −0.89    | 0.69           | −1.29   | 0.21 |
| Diagnosed with COVID-19 and have recovered vs. | 2.08     | 0.76           | 2.75    | 0.0093|
| Never diagnosed                               |          |                |         |      |
| Days Post 2nd Vaccine                         | −0.0019  | 0.0097         | −0.20   | 0.85 |
| Pyramidal function 0 vs. 1                    | 1.86     | 0.55           | 3.37    | 0.0018|
| B-cell Depleting vs. None/NA*                 | −4.51    | 0.78           | −5.79   | <.0001|
| Other vs. None/NA*                            | 0.18     | 0.69           | 0.25    | 0.99 |
| S1P Mod vs. None/NA*                          | −2.85    | 1.11           | −2.57   | 0.04 |

*Reported P-values were after Dunnett adjustment.
Funding
The author(s) received no financial support for the research, authorship, and/or publication of this article.

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