RESEARCH PAPER

Neurologia i Neurochirurgia Polska
Polish Journal of Neurology and Neurosurgery
2022, DOI: 10.5603/PJNNS.a2022.0067

ISSN: 0028-3843, e-ISSN: 1897-4260

Antibodies against SARS-CoV-2 S and N proteins in relapsing-remitting multiple sclerosis patients treated with disease-modifying therapies

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ABSTRACT

Clinical rationale for the study. The course of COVID-19 in people with multiple sclerosis (PwMS) has been described, while the serological status after SARS-CoV-2 infection or vaccination, especially in patients treated with disease-modifying therapies (DMT), is still under investigation. This is a significant clinical problem, as certain DMTs may predispose to a severe course of viral infections.

Aim of the study. We analyzed the presence of antibodies against spike (S) and nucleocapsid (N) proteins of SARS-CoV-2 in relapsing-remitting PwMS treated with DMT, especially dimethyl fumarate, interferon beta, and glatiramer acetate, in a single multiple sclerosis (MS) centre in north-eastern Poland (the Department of Neurology, Medical University of Bialystok).

Material and methods. The presence of antibodies against S and N proteins in PwMS was assessed twice: on visit one (between May and June 2020) (n = 186) and on visit two (between May and June 2021) (n = 88). Samples were taken from 68 individuals on both visits. Demographic and clinical data was collected: duration of MS, Expanded Disability Status Scale Score (EDSS), type of DMT, history of COVID-19 (positive PCR or antigen test in the past), vaccination status, and the type of vaccine.

Results. It was shown that on visit one: 3.7% (n = 7) PwMS were positive for IgA against S protein (IgA-S), 3.2% (n = 6) for IgG against S (IgG-S) protein, and none of those examined was positive for IgG against N protein (IgG-N). On visit two, the most common detected antibodies were IgG-S (71.3%; n = 62), then IgA-S (65.1%; n = 55), and the least common was IgG-N (18.2%; n = 16). On visit two: 20.45% of PwMS had a history of a positive SARS-CoV-2 PCR or antigen test during the last year. By the time of visit two, 42.05% (n = 37) of patients who participated in visit two had been full-course vaccinated against COVID-19. It was demonstrated that vaccination against SARS-CoV-2 significantly induces the production of IgG-S and IgA-S ($p < 0.0001$), while no difference between vaccinated and unvaccinated patients was shown in the detection of IgG-N. There was no correlation between COVID-19 infection and antibodies against proteins S and N in the study group. Moreover, the presented study did not show any relationship between the ability to produce antibodies against the S protein with any of the used DMTs.

Conclusions and clinical implications. According to our study, PwMS treated with dimethyl fumarate, interferon beta, or glatiramer acetate can efficiently produce antibodies against SARS-CoV-2 both after infection and after vaccination.

Key words: COVID-19, SARS-CoV-2, multiple sclerosis, disease-modifying therapies, antibodies, vaccines, serology

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Received: 29.08.2022 Accepted: 19.10.2022 Early publication date: 24.11.2022

Introduction

The coronavirus disease (COVID-19) pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was announced by the World Health Organisation on 11 March, 2020 [1]. In COVID-19 diagnostics, molecular (real-time polymerase chain reaction; RT-PCR) and serological tests to detect specific antibodies against the virus are used [2].

Since the introduction of vaccines against COVID-19, the assessment of antibodies against SARS-CoV-2 is a highly important element of a post-vaccine immune response evaluation. SARS-CoV-2 belongs to the beta-coronavirus group, and is one of the five pathogenic human coronaviruses. The virus is made up of the following proteins: spike protein (S), membrane protein (M), envelope protein (E), and nucleocapsid protein (N). The virus uses angiotensin-converting enzyme (ACE) receptors, which are present in many human organs, to penetrate the host cells.

The element of the S protein: S1 subunit – the receptor-binding domain (RBD) binds to the ACE receptor, which leads to cell infection [3]. The antibodies against the S protein have neutralising properties. Antibodies against N protein do not have neutralising properties, but indicate a past infection [4, 5]. The SARS-CoV-2 virus attacks the mucous membranes. Therefore, the production of IgA starts early and persists for about two months after infection. IgG antibodies are detectable c.14 days after the infection and are present for at least a few months [4, 6]. The sensitivity of IgG detection after at least 14 days from the onset of COVID-19 is 100% for the Enzyme-Linked Immunosorbent Assay (ELISA), Chemiluminescent Immunoassay (CLIA), and Lateral Flow Immunoassay (LFIA) methods [7]. Current reports indicate that the cumulative assessment of anti-S antibodies and anti-N protein antibodies, assessed in two classes of immunoglobulins (IgG and IgA), constitute both a sensitive and specific serological diagnostic approach to SARS-CoV-2 infection [8].

Multiple sclerosis (MS), as a chronic autoimmune disease of the central nervous system, may, under certain circumstances, predispose to a more severe course of COVID-19 [9]. In MS pathogenesis, activation of circulating T and B lymphocytes and excessive production of pro-inflammatory cytokines (e.g. IL-17, IL-6) plays an important role [10]. In treating MS, immunomodulatory and immunosuppressive medications (disease-modifying therapies or DMTs) are used [11]. Certain DMTs may predispose to the development of viral infections and worsen the prognosis [9, 12]. On the other hand, studies evaluating the course of COVID-19 in people with MS (PwMS), including those treated with DMTs, have indicated a relatively mild course of infection in most cases [13, 14].

Clinical rationale for study

To the best of our knowledge, no analysis of antibodies against the SARS-CoV-2 protein (S and N) has been carried out in north-eastern Poland or indeed north-eastern Europe. Moreover, the current literature does not provide data on the presence of antibodies as well against N and S proteins in other MS populations.

Here we assess the presence of anti-S antibodies in class IgG (IgG-S), IgA (IgA-S), and anti-N protein antibodies in class IgG (IgG-N) in PwMS treated with different DMTs in a single MS centre in north-eastern Poland i.e. the Department of Neurology, Medical University of Bialystok (MUB).

Material and methods

Study group

PwMS who had the relapsing-remitting course of disease (RR PwMS) diagnosed according to the McDonald's 2010 and 2017 criteria, and who were being treated with DMTs at the Department of Neurology, MUB, were included in this study. Blood samples were collected during two routine visits to the MS centre. The first visit (visit 1) was between May and June 2020, and the second one (visit 2) one year later, between May and June 2021. 186 patients participated in visit 1, 88 patients participated in visit 2, and 68 individuals took part in both visits. On each visit, the following survey data was collected: duration of MS, Expanded Disability Status Scale score (EDSS), type of used DMT, COVID-19 history (positive PCR or antigen test in the past), vaccination against SARS-CoV-2 status, and the type of used vaccine against COVID-19. During both visits, each study participant was examined by a neurologist. All individuals signed informed consent to participate in this study. The study was approved (approval No. APK.002.230.2020) by the Bioethics Committee at the Medical University of Bialystok, Poland. The clinical characteristics of the study group are set out in Table 1.

Laboratory tests

Assessment of antibodies against the SARS-CoV-2 virus was conducted: IgG-S, IgA-S, IgG-N. Serum levels of the IgG-S and IgA-S antibodies were assessed with the ELISA using the anti-SARS-CoV-2 IgA and IgG kits (Euroimmun, Medizinische Labordiagnostika AG, Germany) adhering to the manufacturer's instructions. The results were assessed semi-quantitatively by calculating the value of the ratio of the extinction of the person sample to the extinction of the calibrator. Results were interpreted according to the manufacturer's recommendation: ratio values < 0.8 were considered negative, ≥ 0.8 to < 1.1 as borderline, and ≥ 1.1 as positive.

Serum levels of the IgG antibodies against the N protein of the SARS-CoV-2 were measured by Chemiluminescent Microparticle Immunoassay (CMIA) on the automatic Alinity system (Abbott, Chicago, IL, USA). The obtained chemiluminescent reaction was assessed as relative light units (RLU). The level of serum antibodies was directly proportional to the RLU detected by the system optics. The S/C (serum/cut-off)

Table 1. Study group characteristics

Group visit 1					
DMT	DMF	INF	GA	Other*	All
n	(n = 79)	(n = 49)	(n = 37)	(n = 21)	(n = 186)
Sex					
Female	67.09% (n = 53)	63.27% (n = 31)	62.16% (n = 23)	80.95% (n = 17)	66.67% (n = 124)
Male	32.91% (n = 26)	36.73% (n = 18)	37.83% (n = 14)	19.05% (n = 4)	33.33% (n = 62)
Age					
Mean	38	46	45	44	42
SD	10.5	12.2	12.4	13.7	12.1
MS duration					
Mean	8.6	9.0	9.1	10.0	9.0
SD	5.79	5.41	6.39	5.72	5.78
EDSS					
Mean	1.6	1.8	2.5	2.5	1.9
SD	1.18	1.01	1.17	1.62	1.25
Group visit 2					
DMT	DMF	INF	GA	Other**	All
n	(n = 29)	(n = 34)	(n = 18)	(n = 7)	(n = 88)
Sex					
Female	58.62% (n = 17)	67.65% (n = 23)	44.44% (n = 8)	71.42% (n = 5)	60.23% (n = 53)
Male	41.38% (n = 12)	32.35% (n = 11)	55.56% (n = 10)	28.57% (n = 2)	39.77% (n = 35)
Age					
Mean	37	42	45	48	41
SD	10.1	12.1	11.6	8.5	11.5
MS duration					
Mean	6.5	7.6	10.4	9.1	8.0
SD	6.03	5.00	9.31	6.82	6.59
EDSS					
Mean	1.5	1.8	2.3	2.6	1.8
SD	1.26	1.10	1.23	1.88	1.28
History of COVID-19					
Yes	13.79% (n = 4)	17.65% (n = 6)	33.33% (n = 6)	28.57% (n = 2)	20.45% (n = 18)
No	86.21% (n = 25)	79.41% (n = 27)	66.67% (n = 12)	71.43% (n = 5)	78.41% (n = 69)
Questionable	0% (n = 0)	2.94% (n = 1)	0% (n = 0)	0% (n = 0)	1.14% (n = 1)
Vaccine against COVID-19 (full vaccine course)					
None	58.62% (n = 17)	58.82% (n = 20)	61.11% (n = 11)	42.86% (n = 3)	57.95% (n = 51)
Pfizer (2 doses)	31.03% (n = 9)	29.41% (n = 10)	33.33% (n = 6)	28.57% (n = 2)	30.68% (n = 27)
Astra Zeneca (2 doses)	3.45% (n = 1)	2.94% (n = 1)	0% (n = 0)	28.57% (n = 2)	4.55% (n = 4)
Moderna (2 doses)	3.45% (n = 1)	8.82% (n = 3)	5.56% (n = 1)	0% (n = 0)	5.68% (n = 5)
Johnson&Johnson (1 dose)	3.45 (n = 1)	0%(n = 0)	0% (n = 0)	0% (n = 0)	1.14% (n = 1)
Group visit 1+2					
DMT	DMF	INF	GA	Other***	All
n	(n = 23)	(n = 26)	(n = 15)	(n = 4)	(n = 68)
Sex					
Female	17.39% (n = 14)	65.38% (n = 17)	46.67% (n = 7)	75.00% (n = 3)	60.29% (n = 41)
Male	39.13% (n = 9)	34.62% (n = 9)	56.33% (n = 8)	25.00% (n = 1)	39.71% (n = 27)

→

Table 1. cont. Study group characteristics

Group visit 1					
Age					
Mean	37	43	46	52	42
SD	9.7	12.6	10.4	8.2	11.5
MS duration					
Mean	6.7	8.7	10.2	4.2	8.1
SD	5.24	4.69	7.49	2.63	5.65
EDSS					
Mean	1.3	1.8	2.6	1.2	1.8
SD	1.27	0.92	1.18	0.96	1.18
History of COVID-19					
Yes	4.35% (n = 1)	19.23% (n = 5)	33.33% (n = 5)	50.00% (n = 2)	19.12% (n = 13)
No	95.65% (n = 22)	76.92% (n = 20)	66.67% (n = 10)	50.00% (n = 2)	79.41% (n = 54)
Questionable	0.00% (n = 0)	3.85% (n = 1)	0.00% (n = 0)	0.00% (n = 0)	4.47% (n = 1)
Vaccine (full vaccine course)					
None	52.17% (n = 12)	46.15% (n = 12)	53.33% (n = 8)	25.00% (n = 1)	48.53% (n = 33)
Pfizer (2 doses)	34.78% (n = 8)	38.46% (n = 10)	40.00% (n = 6)	25.00% (n = 1)	36.76% (n = 25)
Astra Zeneca (2 doses)	4.35% (n = 1)	3.85% (n = 1)	0.00% (n = 0)	50.00% (n = 2)	5.88% (n = 4)
Moderna (2 doses)	4.35% (n = 1)	11.54% (n = 3)	6.67% (n = 1)	0.00% (n = 0)	7.35% (n = 5)
Johnson&Johnson (1 dose)	4.35% (n = 1)	0.00% (n = 0)	0.00% (n = 0)	0.00% (n = 0)	1.47% (n = 1)

Group visit 1 — patients participated in first visit; group visit 2 — patients participated in second visit; group visit 1+2 — patients participated in both first and second visits; DMT — disease-modifying therapy; DMF — dimethyl fumarate; INF — interferon; GA — glatiramer acetate; MS — multiple sclerosis, EDSS — Expanded Disability Status Scale; SD — standard deviation; yes — positive antigen/PCR test in past; no — no positive antigen/PCR test in past
*other: teriflunomide (n = 11); fingolimod (n = 5); cladribine (n = 3); natalizumab (n = 2) **other: teriflunomide (n = 5); fingolimod (n = 1); cladribine (n = 1) ***other: teriflunomide (n = 4)

index was determined based on the above relationship. A titre ≥ 1.4 was considered a positive result.

Statistical analysis

The statistical analysis was based on a description of groups of patients by DMT and demographic variables (sex, age, MS duration, EDSS). This was followed by estimating the Odds Ratio for positive Ig bioassays based on logistic regression models (logit link function). The Wald test evaluated the presence of antibodies, vaccination status, vaccine type, multiple sclerosis disease duration, EDSS, DMTs, and COVID status. Tukey's multiple comparison test was conducted to account for all paired comparisons. Graphical representations supported these statistical models, mainly forest plots comparing the least mean square estimations of the odds ratio and the 95% confidence interval. The whole analysis was realised with R software (V4.1.0, R Core Team 2021).

Results

Visit 1

On visit 1, 186 PwMS were enrolled in the study, of whom 66.67% (n = 124) were women. The mean age of the PwMS was 42 years (± 12.1). The mean disease duration was 9 years (± 5.78). The most commonly used DMT was dimethyl fumarate (42.47%; n = 79), followed by interferon-beta (26.34%; n = 49), and then glatiramer acetate (19.89%; n = 37). Detailed data on the characteristics of the study group during visit 1 are set out in Table 1.

During visit 1, 6.98% (n = 13) of the PwMS had positive antibodies to the S protein: 3.22% (n = 6) IgG-S and 3.76% (n = 7) IgA-S. No IgG-N was detected in any of the tested samples. At the time of visit 1, no patient had a history of positive antigen or PCR test for SARS-CoV-2. In addition, on visit 1, vaccinations against COVID-19 were not yet available. Detailed data on the analysis of anti-SARS-CoV-2 antibodies are set out in Table 2.

Visit 2

On visit 2, 88 PwMS participated, of whom 60.22% were women (n = 53). The mean age of PwMS was 41 years (± 11.5), and the mean disease duration was 8.0 years (± 6.59). The most commonly used DMT was interferon-beta (38.63%; n = 34), followed by dimethyl fumarate (32.95%; n = 29), and then glatiramer acetate (20.45%; n = 18). Patients attending visit 2 did not significantly differ in sex, age, disease duration, or EDSS from those on visit 1. At visit 2, 20.45% (n = 18) of the PwMS had a history of a positive SARS-CoV-2 antigen or PCR result [hereinafter referred to as COVID-19 (+)]. One person obtained questionable results. The mean time from COVID-19 onset to antibodies determination was 151 days (SD ± 66.75). On visit 2, 42.04% (n = 37) of the PwMS had

Table 2. Analysis of anti-SARS-CoV-2 antibodies

Antibody type	IgA-S	IgG-N	IgG-S
Group visit 1			
Percentage of positive results	3.8% (n = 7)	0.0% (n = 0)	3.2% (n = 6)
Sex			
Female	4.8% (n = 6)	0.0% (n = 0)	3.2% (n = 4)
Male	1.6% (n = 1)	0.0% (n = 0)	3.2% (n = 2)
DMT			
DMF	1.3% (n = 1)	0.0% (n = 0)	2.5% (n = 2)
INF	4.1% (n = 2)	0.0% (n = 0)	4.1% (n = 2)
GA	5.4% (n = 2)	0.0% (n = 0)	2.7% (n = 1)
Other*	9.5% (n = 2)	0.0% (n = 0)	4.8% (n = 1)
History of COVID-19/Vaccine status			
COVID (+)		0.00% (n = 0)	
Vaccine (+)		0.00% (n = 0)	
Group visit 2			
Percentage of positive results	64.7% (n = 55)	18.4% (n = 16)	71.3% (n = 62)
Sex			
Female	69.2% (n = 36)	15.1% (n = 8)	73.6% (n = 39)
Male	57.6% (n = 19)	23.5% (n = 8)	67.6% (n = 23)
DMT			
DMF	62.1% (n = 18)	6.9% (n = 2)	72.4% (n = 21)
INF	63.6% (n = 21)	27.3% (n = 9)	64.7% (n = 22)
GA	68.8% (n = 11)	16.7% (n = 3)	82.4% (n = 14)
Other**	71.4% (n = 5)	28.6% (n = 2)	71.4% (n = 5)
History of COVID-19/Vaccine status			
COVID-19 (-)	61.2% (n = 41)	14.5% (n = 10)	66.7% (n-=46)
COVID-19 (+)	77.8% (n = 14)	33.3% (n = 6)	88.9% (n = 16)
Vaccine (-)	42.9% (n = 21)	18.0% (n = 9)	54.0% (n = 27)
Vaccine (+)	94.4% (n = 34)	18.9% (n = 7)	94.6% (n = 35)
Group visit 1+2			
Percentage of positive results	72.1% (n = 49)	17.6% (n = 12)	76.5% (n = 52)
Sex			
Female	78.0% (n = 32)	14.6% (n = 6)	78.0% (n = 32)
Male	63.0% (n = 17)	22.2% (n = 6)	74.1% (n = 20)
DMT			
DMF	69.6% (n = 16)	4.3% (n = 1)	78.3% (n = 18)
INF	73.1% (n = 19)	30.8% (n = 8)	76.9% (n = 20)
GA	66.7% (n = 10)	13.3% (n = 2)	73.3% (n = 11)
Other***	100.0% (n = 4)	25.0% (n = 1)	75.0% (n = 3)
History COVID-19/Vaccine status on visit 2			
COVID-19 (-)	64.81% (n = 35)	22.22% (n = 12)	70.37% (n = 38)
COVID-19 (+)	92.31% (n = 12)	38.46% (n = 5)	92.31% (n = 12)
Vaccine (-)	46.88% (n = 15)	18.75% (n = 6)	53.13% (n = 17)
Vaccine (+)	91.43% (n = 32)	17.14% (n = 6)	94.29% (n = 33)

Group visit 1 — patients participated in first visit; group visit 2 — patients participated in second visit; group visit 1+2 — patients participated in both first and second visits; IgG-S — IgG antibodies against S protein; IgA-S — antibodies against S protein; IgG-N — IgG antibodies against N protein; Ig — immunoglobulin; DMT — disease-modifying therapy; DMF — dimethyl fumarate; INF — interferon; GA — glatiramer acetate; history of COVID-19 — positive antigen or PCR test in past; COVID-19 (-) — no positive antigen/PCR test in past; COVID-19 (+) — positive history of COVID-19; vaccine (-) — patients who were not vaccinated against COVID-19; vaccine (+) — patients who were full-course vaccinated against COVID-19
*other: teriflunomide (n = 11); fingolimod (n = 5); cladribine (n = 3); natalizumab (n = 2)
**other: teriflunomide (n = 5); fingolimod (n = 1); cladribine (n = 1)
***other: teriflunomide (n = 4)

Table 3. Analysis of anti-SARS-CoV-2 antibodies in subgroups of patients on visit 2

Subgroup	IgA-S	IgG-N	IgG-S
COVID-19 (-) and vaccine (-) n = 46	41.5% (n = 17)	19.0% (n = 8)	50.0% (n = 21)
COVID-19 (-) and vaccine (+) n = 51	92.3% (n = 24)	7.4% (n = 2)	92.6% (n = 25)
COVID-19 (+) and vaccine (-) n = 11	50.0% (n = 4)	12.5% (n = 1)	75.0% (n = 6)
COVID-19 (+) and vaccine (+) n = 25	100.0% (n = 10)	50.0% (n = 5)	100.0% (n = 10)

IgG-S — IgG antibodies against S protein; IgA-S — antibodies against S protein; IgG-N — IgG antibodies against N protein; COVID-19 (-) — no positive antigen/PCR test in past; COVID-19 (+) — positive antigen/PCR test in past; vaccine (-) — patients who were not vaccinated against COVID-19 with any dose; vaccine (+) — patients who were full-course vaccinated against COVID-19

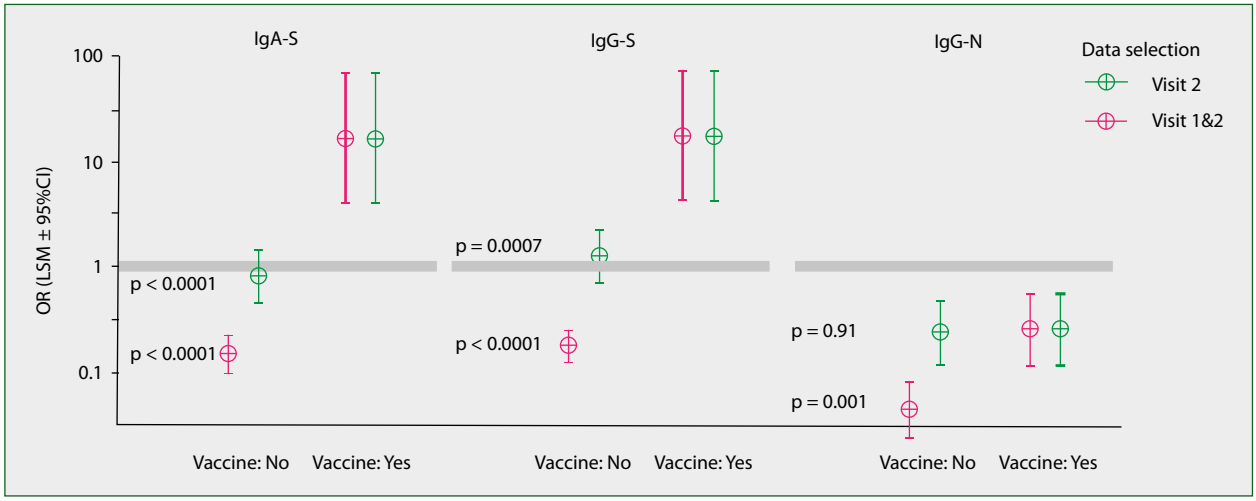

Figure 1. Odds Ratio of positive anti-SARS-COV-2 antibodies test by vaccine status at visit 2 and at visit 1 and 2

already been vaccinated with the full anti-SARS-CoV-2 vaccine course. The PwMS were mostly vaccinated with the Pfizer-BioNTech (COMIRNATY) vaccine (72.97%; n = 27). The mean time from the first dose (after Pfizer, AstraZeneca, Moderna and Johnson&Johnson vaccines) was 56.59 days (SD ± 39.79) and from the second dose (after Pfizer, AstraZeneca, and Moderna vaccines) was 28 days (SD ± 27.84). Detailed data on the characteristics of the study group during visit 2 is set out in Table 1. On visit 2, IgG-S were the most common in PwMS (71.3%, n = 62). IgA-S were present in 64.7% (n = 55), and PwMS and IgG-N in 18.4% (n = 16). The distribution of antibodies did not differ between males and females and between patients treated with individual DMTs. Detailed data on the analysis of anti-SARS-CoV-2 antibodies is set out in Table 2.

On visit 2, PwMS were divided into four subgroups: 1) COVID-19 (-) and not vaccinated with any dose of vaccine; 2) COVID-19 (-) and vaccinated with the full course of vaccination; 3) COVID-19 (+) and not vaccinated with any dose of vaccine; and 4) COVID-19 (+) and vaccinated with a full course of vaccination. Detailed data on the presence of antibodies in subgroups on visit 2 is set out in Table 3.

The vaccine increased the chance of detection of IgG-S and IgA-S, while no stat istical difference was indicated for the detection of IgG-N (IgA-S, $p < 0.0001$; IgG-S, $p < 0.0001$; IgG-N $p = 0.91$) (Fig. 1). Moreover, no effect of the individual vaccine type on the presence of antibodies was demonstrated. In addition, no variation in vaccine effect in relation to the used DMT was detected (IgA-S, $p = 0.28$; IgG-S, $p = 0.26$; IgG-N, $p = 0.99$).

In the logistic regression model, no statistical difference between the results of any immunoglobulin tests could be identified between the COVID-19 (+) and COVID-19 (-) groups. There was no DMT effect on antibody production against SARS-CoV-2. There was no association between EDSS and antibody results (IgA-S, $p = 0.80$; IgG-S, $p = 0.50$; IgG-N, $p = 0.41$). The history of COVID-19 was not associated with any of the used DMTs. No effect of the MS duration on the synthesis of antibodies against SARS-CoV-2 was demonstrated.

Both visits

Sixty-eight patients participated in both visits, of whom 60.29% (n = 41) were women. The mean age of the patients was 42 years (± 11.5). The mean disease duration was 8.1 years

Table 4. Analysis of anti-SARS-CoV-2 antibodies in subgroups (patients attended both visits 1 and 2)

Level	IgA-S	IgG-N	IgG-S
COVID-19 (-) and vaccine (-) n = 31	42.9% (n = 12)	17.9% (n = 5)	50.0% (n = 14)
COVID-19 (-) and vaccine (+) n = 49	92.0% (n = 23)	7.7% (n = 2)	92.3% (n = 24)
COVID-19 (+) and vaccine (-) n = 7	75.0% (n = 3)	25.0% (n = 1)	75.0% (n = 3)
COVID-19 (+) and vaccine (+) n = 22	100.0% (n = 9)	44.4% (n = 4)	100.0% (n = 9)

IgG-S — IgG antibodies against S protein; IgA-S — antibodies against S protein; IgG-N — IgG antibodies against N protein; COVID-19 (-) — no positive antigen/PCR test in past; COVID-19 (+) — positive history of COVID-19; vaccine (-) — patients who were not vaccinated against COVID-19 with any dose of vaccine; vaccine (+) — patients who were vaccinated with full course against COVID-19

(± 6.59). In this group of patients, the most common DMT was INF (38.23%; n = 26), then DMF (33.82%; n = 23), and then GA (22.06%; n = 15). Detailed data on the characteristics of the study group that participated in both visits 1 and 2 is set out in Table 1.

On visits 1 and 2, 2.9% (n = 2) and 76.47% (n = 52) PwMS were IgG-S positive, respectively. On visit 1, 2.9% (n = 2) PwMS were IgA-S positive, compared to 72.06% (n = 49) on visit 2. On visit 1, none of the PwMS detected IgG-N; on visit 2, these antibodies were present in 17.65% (n = 12). Detailed data on the analysis of anti-SARS-CoV-2 antibodies is set out in Table 2.

The PwMS who participated in both visits were divided into four subgroups: 1) COVID (-) and not vaccinated with any dose of vaccine; 2) COVID (-) and vaccinated with a full course of vaccine; 3) COVID (+) and not vaccinated with any dose of vaccine; and 4) COVID (+) and vaccinated with a full course of vaccine. In the COVID (+) and vaccinated PwMS (n = 9), 100% were positive for both IgG-S and IgA-S. When analysing the entire group of vaccinated PwMS (n = 35), regardless of the earlier SARS-CoV-2 infection history, the presence of IgG-S was demonstrated in 94.3% (n = 33), and IgA-S in 94.1% (n = 32). Detailed data on the analysis of anti-SARS-CoV-2 antibodies in subgroups is set out in Table 4.

Discussion

This study presents the serological status of PwMS treated with DMTs in a single MS centre in north-eastern Poland. Antibodies were assessed for two SARS-COV-2 proteins (S and N) in two immune classes (IgA and IgG). The study group included PwMS vaccinated with a full course against COVID-19 and unvaccinated, with and without a history of SARS-CoV-2 infection. The age of the study group, the percentage of each sex, and the average duration of the disease corresponded with the entire population of PwMS in Poland [11]. Although it is known that PwMS are at higher risk of death from some infections including respiratory failure, recent reports have shown that PwMS do not suffer from COVID-19 more severely than the general population, and there is no significantly higher mortality in this group of patients [15, 16, 13].

However, the serological response to SARS-CoV-2 infection and vaccination is still being investigated [17]. Serological tests determine who has been exposed to the virus and are an essential tool for assessing vaccine immunity. In our study, PwMS were examined for the ability to produce antibodies against SARS-CoV-2, both after infection and vaccination. For this reason, our study determined antibodies against the N and S proteins. Available vaccines against SARS-CoV-2 induce the production of antibodies against the spike (S) protein only [18]. After infection, antibodies to various coronavirus proteins may be synthesised, e.g. against S and N protein [19]. The clinically most important difference between these antibodies is that only antibodies to the S protein have neutralising properties, which means they have protective properties against subsequent infection [5].

During visit 1, approximately 3–4 months after the beginning of the pandemic, only a few patients had antibodies against the S protein; no antibodies against the N protein were detected in any of the participants. This proves the low prevalence of SARS-CoV-2 infection among PwMS at that time. This was probably due to the extensive restrictions in Poland and the self-isolation of PwMS in the first months of the pandemic. No epidemiological studies conducted in Poland in 2020 have been published. Therefore, the data from visit 1 cannot be compared. The OBSER-CO seroepidemiological study conducted in Poland in May–June 2021 calculated the prevalence of SARS-CoV-2 in the 20–59 year-old age group (Podlaskie Voivodeship, Poland) to be 46.8-52.5% [20]. Our research shows that PwMS had lower exposure to the SARS-CoV-2 virus compared to the general population of a similar age. Similar conclusions were observed by Morales et al. in Spain, who assessed the incidence of COVID-19 in the local MS population as being lower than that in the Spanish general population [21].

This is probably, as mentioned earlier, due to the high fear of COVID-19 and the self-isolation of PwMS, especially among those treated with DMT. Neurologists treated PwMS

from the first months of the pandemic, appealed for epidemiological vigilance, and called for an improved sanitary regime due to the suspected more severe course of the disease. The data from visit 2 clearly showed the dynamics of the pandemic development, including the introduction of vaccination against COVID-19. The most common antibodies detected during visit 2 were IgG-S (71.6%), then IgA-S (65.1%), and the least common among PwMS were IgG-N (18.2%). In one study of 546 (74.2% taking DMT) PwMS, 11.7% (n = 64) of them had antibodies to SARS-CoV-2 [22]. Unfortunately, this study did not mention either the class of antibodies or the protein against which these antibodies were directed.

In another multicentre study by Sormani et al., conducted on 423 PwMS in Italy, Turkey, and Brazil, who were taking DMTs, the prevalence of IgG antibodies against SARS-CoV-2 was 76.8% [23]. Another study (n = 28) in PwMS with confirmed COVID-19 reported IgG seroprevalence in 83.3% (n = 20) of patients [23].

The results of the abovementioned studies seem to be in line with our observations. In our cohort, the mean time from COVID-19 positive antigen/PCR result to antibodies determination was 151 days (SD ± 66.75), and the mean time from the first dose of vaccine was 56.59 days (SD ± 39.79) and from the second dose was 28 days (SD ± 27.84). Recent data from a general Norwegian population shows that neutralising antibodies produced after the disease are maintained in 95% 10–12 months after COVID-19 onset [24]. Antibodies to S protein, produced after vaccination, are also detected one year after immunisation [25]. Therefore, in the study group, the time from vaccination and infection to sample collection was selected so that humoral immunity had time to develop and not disappear. Our work presents an analysis of antibodies in the IgG and IgA class and against individual SARS-CoV-2 proteins (S and N). It should be noted that no commercial IgA-N tests were available at the time of our study.

It is worth emphasising that most patients in the study group were treated with DMF, GA, or INF, and that DMT is most used in Poland. It has been demonstrated that PwMS treated with DMTs (particularly DMF, GA, INF) are immunocompetent in producing antibodies to SARS-CoV-2. It has also been demonstrated that vaccination against SARS-CoV-2 significantly induces the production of antibodies to the S protein ($p < 0.0001$), which has neutralising properties. Recent studies and meta analyses have confirmed the efficacy of COVID-19 vaccines among PwMS treated with DMF, INF, and GA [26]. The presented study showed that more vaccinated PwMS with no history of COVID-19 (92.6%; n = 25) produce neutralising antibodies than do those who suffered from COVID-19 but were not vaccinated (75.0%; n = 6). This indicates that vaccination is more effective than infection in generating immunity against SARS-CoV-2.

Moreover, in the group of PwMS who were infected with COVID-19 and vaccinated, the presence of neutralising antibodies was 100%. On visit 2, among both unvaccinated patients and those who had no history of COVID-19, neutralising antibodies were found in 50% (n = 21), which shows that there is a group of PwMS who have contracted SARS-CoV-2 asymptomatically, producing immune antibodies. The presented study did not show any relationship between the ability to produce antibodies against the S protein concerning any of the tested DMTs.

This study has some limitations. Notably, the proportion of PwMS treated with individual DMTs was uneven. Most patients were treated with DMF, INF, or GA. The results of the study refer only to these medications. It should be noted that the study did not include PwMS treated with ocrelizumab or another anti-CD-20 DMT. A study conducted on 473 PwMS has shown that anti-CD-20 treatment generates a lower antibody response after COVID-19 and after vaccination ($p > 0.001$) [27]. Other studies and reviews conducted so far confirm these conclusions [28, 29]. Soromani et al., in a study conducted on 780 PwMS, indicated that patients treated with fingolimod did not produce neutralising antibodies effectively after vaccination, possibly related to the leukopenia present with fingolimod treatment [26, 30]. Although our study group included patients treated with fingolimod, cladribine, and natalizumab, these groups of patients were too small for a reliable statistical analysis. In the presented study, it was impossible to demonstrate the vaccine's effect on the production of antibodies, as a majority of the participants were vaccinated with the Pfizer vaccine.

As expected, the analysis of responses against the N protein was inconsistent. Statistical analyses showed that IgG-N is not associated with infection of COVID-19. A study carried out among 683 healthcare workers at one hospital in Tokyo showed that IgG-N antibodies are the most reliable test for assessing past SARS-CoV-2 infections, including asymptomatic infections [31]. Our research does not confirm that conclusion, but the different study groups are worth noting. It seems that assessment of past infection on the basis of the N protein test is ineffective in the group of PwMS treated with DMT. The relationship between IgG-N and time from COVID-19 was also analysed, which confirmed statistical insignificance. So far, no other studies have been published analysing the anti-N protein response in PwMS. Investigating this problem in a larger group of patients with multiple sclerosis should be considered in the future. To sum up, it is worth emphasising that analyses were carried out on a population not-examined (so far) in terms of humoral response to SARS-CoV-2. Multiple sclerosis patients are a diverse population that, in addition to genetic factors, are also influenced by environmental factors. The research we present is novel because analysis of antibodies against the SARS-CoV-2 protein has not previously been carried out in north-eastern Poland. Moreover, the current literature does not provide data on the presence of antibodies against N and S proteins in other multiple sclerosis populations.

Clinical implications/future research

We have here demonstrated that vaccination against SARS-CoV-2 significantly induces the production of antibodies against the S protein, while no difference between vaccinated and unvaccinated patients was shown in the detection of the N protein. The PwMS population requires further research in terms of serology after SARS-CoV-2 infection. Moreover, the present study did not show any relationship between the ability to produce antibodies against the S protein concerning any of the used DMTs (particularly DMF, GA, and INF). Our research showed that PwMS treated with a DMT used in the study group, especially DMF, GA and INF, are immunocompetent in producing antibodies against the SARS-CoV-2 virus, and that the prevalence of SARS-CoV-2 is lower than in the general population.

Conflicts of interest: *None.*
Funding: *None.*

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