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Humoral and Cellular Immunity after Vaccination against SARS-CoV-2 in Relapsing-Remitting Multiple Sclerosis Patients Treated with Interferon Beta and Dimethyl Fumarate

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Abstract: The impact of vaccines against SARS-CoV-2 on immunity of patients with multiple sclerosis (PwMS) is still not fully known. Further clarification could help address medical concerns related to the use of immunosuppressive and immunomodulatory medications, known as disease-modifying therapies (DMTs), in PwMS, as well as ensure adequate protection against severe outcomes of COVID-19.. Therefore, the aim of our study was to evaluate the humoral and cellular immune response in PwMS treated with DMTs. The concentrations of IgG Spike (S) anti-SARS-CoV-2 antibodies, and IgG Nucleocapsid (N) anti-SARS-CoV-2 antibodies as well as Interferon gamma *(IFN-γ)* titers were analyzed in PwMS groups treated with dimethyl fumarate (DMF), interferon beta (IFN), and healthy control group. Almost 100% of PwMS experienced seroconversion, which resulted from either vaccination and/or prior infection. Additionally, there were no significant differences between the study and control groups in terms of IgG (S) and (N) anti-SARS-CoV-2 antibody levels. However, interferon gamma titers were lower in both PwMS groups which may indicate adequate humoral and decreased cellular response of examined PwMS. Additionally, after division of the whole study group into two subgroups according to the time since last vaccination, IgG (S) anti-SARS-CoV-2 and *IFN-γ* concentrations were significantly lower in case of patients who were immunized more than 200 days before sample collection. No differences were observed in case of subgroups in which sample collection was less than 200 days after vaccination when compared to the control group. This could indicate a time-related and decreased immunity in PwMS treated with DMTs.

Keywords: COVID-19; SARS-CoV-2; Vaccine; Multiple Sclerosis; Humoral; Cellular; Immunity

1. Introduction

In 2019, the severe respiratory syndrome coronavirus 2 (SARS-CoV-2) was found in Wuhan, China. Infection with this virus causes a disease, which affects respiratory tract system, known as COVID 19. It spread worldwide and on March 11, 2020, when nearly 118 000 confirmed cases were detected, World Health Organization (WHO) announced COVID-19 as a pandemic [1,2]. Until April 13, 2024, almost 705 million confirmed cases had been reported, resulting in almost 7 million deaths [3]. The pandemic status continued until May 5, 2023 [4]. COVID-19 has a variety of clinical courses, ranging from asymptomatic, through symptoms like fatigue, fever, diarrhea, cough, dyspnea, malaise, myalgia, rhinitis, headache, diarrhea, chest pain [5], to severe outcomes such as acute

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respiratory distress syndrome (ARDS) or even death [6]. The majority of clinical cases result in asymptomatic or mild outcomes [5]. COVID-19 infection initiates, when spike protein of SARS-CoV-2, one from the viruses four structural proteins (spike, nucleocapsid, envelope, and membrane), binds to receptors on the host cell surface (angiotensin-converting enzyme 2 [ACE2] or Neuropilin-1 [NRP-1] receptors) [7,8]. The SARS-CoV-2 (N) protein which is connected with viral RNA seems to be responsible for RNA transcription and replication in host cells. The reverse transcription polymerase chain reaction tests (PCR-RT) as well as antigen or serological tests play an important role in a diagnostics procedures used for COVID-19 diagnosis [9].

PCR-RT and antigen methods are useful for confirming the ongoing SARS-CoV-2 infection [10]. The serological method detects antibodies produced when a patient is exposed to the virus. This immune response is known as the humoral response [11]. In 2020, the first vaccines against COVID-19 were introduced. Various vaccine platforms were developed, including mRNA vaccines, where mRNA coding Spike protein is implemented (BNT162b2, mRNA-1273) [12]; protein subunit vaccines, where spike protein is used for immunization (NVX-CoV2373) [13]; and vector vaccines, which involve prepared adenoviruses transferring genetic material to host cells (ChAdOx1 nCoV-19, Ad26.COV2.S)[14]. Two types of immunization can be distinguished: active immunization, in which the patient is exposed to SARS-CoV-2 infection, and passive immunization, which occurs through vaccination. Despite the presence of five classes of antibodies, only three of them (IgM, IgA, and IgG) are relevant for determining the type of immunity in patients [11]. Presence of antibodies in IgM and IgA classes detected in human serum or plasma are present early, about 6-8 days after infection [15] and after 49 – 56 days after immunization onset they become negative. IgG class antibodies can be detected 9-13 days after infection and may last up to 80-105 days or even longer [16-18]. Anti-SARS-CoV-2 N antibodies play a significant role in humoral immunity obtained after infection. However, those antibodies are absent in humoral immunity reached after vaccination, where anti-SARS-CoV-2 S antibodies are present [19]. Additionally, after immunization, SARS-CoV-2 antigens activate naive lymphocytes T, causing them to release a substantial amount of interferon gamma. What is more, after immunization, naive lymphocytes T are activated, resulting in the release of a substantial amount of interferon gamma. Some studies indicate that cellular immunity lasts longer than humoral, even for years [20-22]. Fact of obtaining humoral and cellular immunity may be linked with lower risk of severe infection outcomes [23].

Multiple sclerosis (MS) is a chronic autoimmune demyelinating disease, which affects 2.8 million people worldwide [24,25] and almost 45.000 individuals in Poland [25]. Typical patient diagnosed with MS is an approximately 32 years old female. Many patients with multiple sclerosis (PwMS) are treated with disease-modifying treatments (DMTs), which are immunomodulating and immunosuppressive medications aimed at reducing progressive disability and radiological progression of the disease [26,27]. This approach is referred to as NEDA 3 (no evidence of disease activity), indicating the absence of clinical and radiological signs of disease activity [28]. Those patients with autoimmune disease and specific treatment applied, constitute a group with unclear immunity status.

It remains unknown what was their response after vaccination against SARS-CoV-2. Available scientific reports on the influence of Disease-Modifying Therapies (DMTs) on humoral and cellular immunity against SARS-CoV-2 primarily focus on medications used in High Efficacy Treatments (HETs), such as natalizumab, fingolimod, alemtuzumab, ocrelizumab, and cladribine [29]. Data regarding other drugs, such as interferon beta (IFN) and dimethyl fumarate (DMF) or glatiramer acetate, remains limited, particularly for those who received their last dose of SARS-CoV-2 vaccination more than 200 days prior. In available literature, immunomodulatory therapies such as DMF (dimethyl fumarate) and INF (interferon) are mostly described as safe, due to their mechanisms of action. DMF induces the depletion of memory T cells, reduces the count of activated T cells, and supports the expansion of naïve T cells [30]. However, some scientists have reported that people with multiple sclerosis (PwMS) treated with DMF could experience low levels of lymphocytes in peripheral blood, which may decrease the development of immune responses [31]. Interferon beta,

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in general, suppresses inflammation by decreasing the activation of T cells and inducing the differentiation of oligodendrocytes. The direct mechanism of action of interferon beta is still unknown [32]. It is important to note that interferon and dimethyl fumarate are commonly used as primary DMT choices [33, 34]. Therefore, the aim of our study was to evaluate the humoral and cellular immune response in PwMS treated with dimethyl fumarate and interferon beta.

2. Materials and Methods

2.1. Patients

The intended PwMS group being treated with dimethyl fumarate (DMF) and interferon (INF) consisted of approximately 100 patients (65 and 35 individuals, respectively). This calculation was based on the number of patients receiving treatment with the selected drugs at the clinic. Some patients were excluded from the study based on the exclusion criteria and deficiencies in the tests conducted. Finally, the study group consisted of 72 PwMS, who were treated with dimethyl fumarate (n= 48) or interferon beta (n= 24) in a national drug funding program conducted by the Department of Neurology, Medical University of Bialystok. Every case of MS was diagnosed using McDonald criteria (2017) [35] and strictly monitored in accordance with the polish national drug program guidelines. All patients were treated with current DMT at least 12 months before first dose of anti-SARS-CoV-2 vaccination and with the Expanded Disability Status Scale (EDSS) [36] below 4.5. 72.2% of PwMS studied in investigation were women. Mean age was 40.4 years in subgroup treated by DMF, and 48.63 years in subgroup treated by IFN. The managed control group, which was comparable to both study groups, consisted initially of 40 participants. Due to patients refusals during data collection, and only qualitative results in case of few patients, the control group ultimately comprised 28 physically healthy individuals without a diagnosis of MS. This group closely resembled the MS group in terms of age and sex, and did not have any significant comorbidities; most participants were free from medical conditions, with only a small number reporting hypertension, diabetes managed with oral medication, or hypothyroidism (Table 1). All participants received at least two doses of the mRNA vaccine or at least one dose of Ad26.COV2.S.

Table 1. Characteristics of the study and control groups.

Group	Count	Mean Age	Female	Male
Study group (DMF+IFN)	72	43.14 ± 2.79 (95%) [1]	72.22 % (n= 52)	27.78% (n= 20)
DMF	48 (66.67%)	40.4 ± 3.13 (95%) [1]	70.83% (n= 34)	29.19% (n= 14)
IFN	24 (33.33%)	48.63 ± 4.88 (95%) [1]	75% (n= 18)	25% (n= 6)
Control Group	28	44.57 ± 4.3 (95%) [1]	85.71 % (n= 24)	14.29 % (n= 4)

[1] Mean (Confidence Interval); DMT- dimethyl fumarate; IFN- interferon beta.

2.2. Patients Vaccination Status

Mean time from the last vaccination against SARS-CoV-2 to blood sample collection was 320.56 days in subgroup of PwMS treated with DMF, 320.5 days in subgroup of PwMS treated with IFN and 320.54 days in the whole study group as well as 316.29 in the control group (Table 2). The shortest interval between last dose of vaccination against SARS-CoV-2 and sample collection was 84 days. In the whole study and control groups most frequent vaccine used in all doses was BNT162b2 (Table 3). The longest mean intervals between the first and the second dose of vaccination was in PwMS treated with DMF 47.35 days (vs. INF 43.04 days; vs. Control 40.43 days). Although mean intervals between the second and third doses of immunization against SARS-CoV-2 were evaluated, the control group

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had the longest mean – 286.86 days (vs. INF 190.73 days; vs. DMF 224.61 days) and 312.95 days (vs. INF 231.6 days; vs. DMF 261.18 days), respectively.

Third dose of vaccination against SARS-CoV-2 was chosen more frequent by the individuals from the control group rather than the study group (Table 3).

Table 2. Time from last vaccination to blood sample collection in the study and control groups.

Group	Time from last vaccination to blood sample collection (days)
Study group (DMF+IFN)	320.54 (321; 180.5; 436.5) [1]
DMF	320.56 (282; 180.5; 466.5)[1]
IFN	320.5 (344.5; 197.5; 385.5)[1]
Control Group	316.29 (262; 184; 440)[1]

[1] Mean (Median; Q1; Q3); DMT- dimethyl fumarate; IFN- interferon beta.

Table 3. Detailed characteristics of the studied groups vaccination status.

Vaccination		Study Group		Control Group
		DMF (n=48)	IFN (n=24)	(n=28)
1st dose	BNT162b2	79.17% (n= 38)	79.17% (n= 19)	96.43% (n= 27)
	mRNA-1273	12.5% (n= 6)	20.83% (n= 5)	3.57% (n= 1)
	Ad26.COV2.S	4.17% (n= 2)	-	-
	NVX-CoV2373	2.08% (n= 1)	-	-
	ChAdOx1 nCoV-19	2.08% (n= 1)	-	-
2nd dose	BNT162b2	79.17% (n= 38)	79.17% (n= 19)	96.43% (n= 27)
	mRNA-1273	12.5% (n= 6)	20.83% (n= 5)	3.57% (n= 1)
	NVX-CoV2373	2.08% (n= 1)	-	-
	ChAdOx1 nCoV-19	2.08% (n= 1)	-	-
3rd dose	BNT162b2	60.42% (n= 29)	58.33% (n= 14)	74.07 % (n= 20)
	mRNA-1273	-	3.6% (n= 1)	3.57% (n= 1)

2.3. Biochemical Analyses

Blood samples were collected during routine control visits with participants diagnosed with multiple sclerosis enrolled in a pharmacological treatment program, spanning from May 30, 2022, to March 16, 2023. Analysis of humoral immunity against SARS-CoV-2 was performed by chemiluminescent microparticle immunoassay (CMIA). The concentrations of IgG antibodies against the receptor binding domain of S1 protein [AU/ml] and against N protein were assessed using the automatic Alinity system (Abbott, USA). Results ≥50 AU/ml for IgG (S) anti-SARS-CoV-2 and ≥1.4 IgG (N) anti-SARS-CoV-2 were considered as positive. The concentrations of Interferon gamma were determined using Euroimmun Quan-T-Cell ELISA kit to assess the cellular immune response also in patients without detectable antibodies. Results ≥200 mIU/ml were marked as positive.

2.4. Surveys

Simultaneously to the blood samples collection the data about duration of the disease, DMT drug usage, details of vaccination against SARS-CoV-2 (date of vaccination, type of vaccine, possible adverse effects after vaccination, date of possible SARS-CoV-2 infection, neurological condition, date of last relapse) were collected from patients. To minimize the potential for errors during questionnaire completion, a physician was present to provide assistance when necessary.

2.5. Statistical Analysis

The data obtained from the questionnaires and investigations performed on blood samples were statistically analyzed. The analysis used statistical tests with a significance level of $\alpha = 0.05$. The Shapiro-Wilk test was used to determine whether the variable distributions were normal. As all results were not normally distributed, Kruskal-Wallis rank sum test was used to examine differences

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between all groups. Statistically significant differences were defined as comparisons resulting in $p < 0.05$. All statistical analyses were performed using Statistica 13.1.

3. Results

3.1. Analysis of Humoral Immunity Against SARS-CoV-2

All of the examined groups presented nearly 100% positive results of IgG (S) anti-SARS-CoV-2 antibodies. Only two patients from subgroup of PwMS treated with DMF did not reached the concentration of antibodies higher than 50 AU/ml in serum. The mean concentration of IgG (S) anti-SARS-CoV-2 antibodies in serum of PwMS treated with DMF was 15506.18 AU/ml, treated with IFN was 22450.1 AU/ml, and in the control group was 21369.09 AU/ml. Performed statistical analysis revealed that there were no significant differences between concentrations of IgG (S) anti-SARS-CoV-2 antibodies (DMF vs. control group, p= 0.08; IFN vs. control group, p= 0.09; DMF+IFN vs. control group, p= 0.051). This may indicate that humoral response of all tested groups was generally similar. Several studies found that after 6 months from vaccination, its effectiveness evaluated as concentration of IgG (S) antibodies against SARS-CoV-2 was much lower than after 7-60 days from immunization, and that it continued to decrease at a different rate [37-42]. According to this fact, we have divided all tested groups in two subgroups: individuals, whose blood sample have been taken less than 200 days and those whose blood has been drawn more than 200 days after last dose of vaccination. Obtained mean values in all studied groups were as follows: 29840.93 AU/ml in PwMS treated with DMF if blood collection was performed less than 200 days, and 8990.37 AU/ml if later than 200 days after vaccination; 61966.05 AU/ml in subgroup of PwMS treated with IFN if blood collection was less than 200 days and 9278.12 AU/ml for patients treated with IFN if blood collection was more than 200 days since last dose of vaccination. Control group reached 32029.68 AU/ml and 16319.34 AU/ml, respectively. Obtained concentrations were significantly higher in the study subgroups, where the time from vaccination to sample collection was less than 200 days, when compared to those where the time from vaccination to collection of samples was more than 200 days (DMF, p= 0.0013; IFN, p= 0.0027; control group, p= 0.2281). The mean concentration of IgG (S) anti-SARS-CoV-2 antibodies was highest in PwMS subgroup treated by IFN, collected in less than 200 days after last dose of vaccine against SARS-CoV-2 (details were presented in Table 4), indicating no statistically significant differences (DMF vs. control, p= 0.7; IFN vs. control, p= 0.35; DMF+IFN vs. control, p= 0.95). After 200 days, IgG (S) anti-SARS-CoV-2 levels in study subgroups were lower than in control group, which was statistically significant (DMF vs. control, p= 0.037; IFN vs. control, p= 0.015; DMF+IFN vs. control, p= 0.012). This confirms the previous assumptions about similar humoral response in the first months after vaccination in the studied groups and its earlier decrease with time. Due to the fact that after division into subgroups, the number of patients in some cases was less than 20, this is a limitation of our study, and the results should be interpreted with caution. However, it is worth noting that both of the patient groups as well as the control group were strictly selected, which on the other hand may indicate a high reliability of the presented results.

Table 4. Mean and median concentrations of antibodies IgG (S) anti-SARS-CoV-2 in study and control group divided to <200 and >200 days after last dose of SARS-CoV-2 vaccination.

Group	Time from last vaccination to sample collection (days)	Number of patients	Mean [AU/ml]	Median [AU/ml]	p-Value[1]
DMF	<200	15	29840.93	22624.6 (6525.9; 37955.5) [2]	p= 0.0013
	>200	33	8990.37	3886.9 (1224.5; 12550.25) [2]	
IFN	<200	6	61966.05	29769.2 (12005.2; 76249.6) [2]	p= 0.0027
	>200	18	9278.12	3203.8 (981.7; 10146.2) [2]	
Control	<200	9	32029.68	27298 (8043.6; 62862.6) [2]	p= 0.2281
	>200	19	16319.34	9544.9 (3145.7; 31571.1) [2]	

[1] Kruskal-Wallis Test, [2] Median (Q1, Q3); DMF- dimethyl fumarate, IFN- interferon beta.

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Furthermore, the concentration of IgG (N) SARS-CoV-2 antibodies in all groups were measured. In subgroups of PwMS treated with DMF, IFN and in the control group, (50%, 66.67%, and 57.14%, respectively) were negative which indicates that only part of examined individuals had direct contact with virus due to infection. Mean concentrations of IgG (N) anti-SARS-CoV-2 antibodies in serum PwMS treated with DMF, IFN, in the whole study group and in the control group were as follows: 2.46, 2.17, 2.36, and 1.82, respectively. Details were described in Table 5. The acquired data was compared, and there were neither statistically significant differences between the overall study group vs. control group (p= 0.8478), nor between the separated subgroups vs. control group (DMF p= 0.58, IFN p= 0.63), which also confirms similar humoral response of all patients. Due to the fact that precise determination of last SARS-CoV-2 infection is impossible, IgG (N) antibodies were only assessed as a one group, without the additional subgroup division.

Table 5. Characteristics of IgG (N) anti-SARS-CoV-2 in the study and control groups.

Groups studied.	Percentage of negative results	Mean	Median
DMF	50% (n= 24)	2.46	1.1 (0.13; 4.0)
IFN	66.67 % (n= 16)	2.17	0.64 (0.1; 2.88)
DMF+IFN	55.56% (n= 40)	2.36	0.87 (0.12; 3.68)
Control	57.14% (n= 16)	1.82	0.74 (0.17; 2.74)

DMF- dimethyl fumarate; IFN- interferon beta.

3.2. Analysis of Cellular Immunity Against SARS-CoV-2

As data presented so far revealed that the decrease in IgG (S) anti-SARS-CoV-2 concentration may be compensated by cellular immunity, which is characterized as interferon gamma concentration [43], it was measured to assess the cellular response in tested groups. In the study subgroups: PwMS treated with DMF, PwMS treated with IFN, and in control group negative results were obtained (20.84%, 12.5%, 3.57%, respectively). Mean concentrations of interferon gamma in tested groups were respectively 1659.41 mIU/ml, 1613.81 mIU/ml, and 2904.46 mIU/ml. Performed statistical analysis revealed, that titers obtained by PwMS treated with DMF and IFN were lower than those obtained in the control group, what was statistically significant (p= 0.001). Interferon gamma concentration in serum measured in both groups separately was also lower, although only in case of group treated with DMF statistical significance has been proved (p= 0.0008). Obtained data draws attention to the fact, that quick and violent response of T-cells is a crucial factor of mild COVID-19 outcome [44]. Taking above into consideration, decision about conducting a more detailed analysis of the obtained results was made.

Participants in the investigation were divided into two groups, as described before. One group took into consideration individuals, which time between last vaccination against SARS-CoV-2 and the blood drawn was less than 200 days, and the second one, where that period lasted more than 200 days. When considering <200 days groups, the highest mean of interferon gamma concentration was obtained in group of PwMS treated with interferon beta, although when we considered groups with >200 days period, the control group reached the highest concentration. The study compared single subgroups and the whole study group to the control group. When compared the <200 days groups, mean differences were not statistically significant (DMF vs. control, p= 0.61; IFN vs. control, p=0.2; DMF+IFN vs. control p= 0.92). However, when the time between the last passive immunization by SARS-CoV-2 and the collection of a blood sample was more than 200 days, the highest mean was reached by the control group (3004.94 mIU/ml vs. 1374.61 mIU/ml (DMF) vs. 1520.30 mIU/ml (IFN), respectively), what was statistically significant (DMF vs. control, p= 0.00045; IFN vs. control, p= 0.004; DMF+IFN vs. control, p= 0.0002).

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3.3. Questionnaire Analysis

Individuals in the completed survey study reported similar percentages of troubling symptoms in the time between their last SARS-CoV-2 immunization (DMF 22.92% vs. IFN 20.83%). Contact with infected person was observed more frequently in PwMS group treated with dimethyl fumarate than other groups (83.33% for DMS vs. 66.67% for IFN vs. 35.71% for control group).

Interestingly, patients treated with IFN beta were more likely to suspect that they are suffering from COVID-19 (IFN 50% vs. DMF 37.5% vs. control group 35.71%). What is more, when participants were asked about attendant symptoms, they provided interesting information. PwMS treated with DMF and IFN complained about the cough most frequently (27.08%; 33.33%, respectively), followed by fever (16.67%; 25%, respectively) and dyspnea (10.42%; 20.83%, respectively). Different results were obtained in control group, where most frequent symptoms were fever and cough (both 28.57%), followed by dyspnea 21.43%.

Additional data was also obtained regarding adverse occurrences after vaccination against SARS-CoV-2. In the study subgroup treated with dimethyl fumarate after first dose of vaccination, the most frequent symptoms were: local pain 37.5% (n= 18), fever and fatigue 16.67% (n= 8) as well as headache 10.42% (n= 5). Following the second dose of vaccination, most prevalent were local pain 26.09% (n= 12), fever and fatigue 19.57% (n= 9), and muscle pain 17.39% (n= 8). Survey in the subgroup treated with IFN revealed that after first dose of treatment most common adverse events were local pain 41.67% (n= 10), fever and fatigue 16.67% (n= 4). After second dose of vaccination most common were local pain 45.83% (n= 11), fever 20.83% (n= 5) and fatigue 16.67% (n= 4). Third dose of vaccination have similar impact on PwMS IFN group except lower incidents of local pain 29.17% (n= 7). Data obtained from the control group revealed that after first dose most frequent adverse events were local pain 21.42% (n= 6), fever and headache 17.86% (n= 5), as well as muscle pain 10.71% (n= 3). After second dose of vaccination participants complained about local pain and headache 14.29% (n= 4) as well as fever 7.14% (n= 2). Mild symptoms with short duration (approximately 1-2 days) were noticed in all groups and after all doses.

4. Discussion

Our study examined the immunity status and adverse events occurring after SARS-CoV-2 vaccination in a subgroup of individuals with multiple sclerosis, who had previously contracted SARS-CoV-2 and were being treated with dimethyl fumarate and interferon beta. When vaccines aimed at preventing severe cases of COVID-19 were developed, their effects on this specific group of patients, especially those treated with immunosuppressive and immunomodulating drugs were largely unknown. Over time, initial scientific reports indicated that vaccinations against SARS-CoV-2 are generally safe for PwMS, even for those undergoing disease-modifying therapy (DMT) [45,46]. However, there have also been reports suggesting that several DMTs may reduce humoral immunity (e.g., ocrelizumab, fingolimod, siponimod) [47-50] and cellular immunity following vaccination (e.g., ocrelizumab, cladribine, interferon beta, fingolimod) [51-53]. In case of anti-CD20 drug it is suggested that it may be an effect of B-cell depletion, what involve lower B-cell activity and lower humoral and cellular immunity level [47]. Decrease of involved humoral and cellular immunity in PwMS treated with sphingosine-1-phosphate receptor modulator (S1P e.g. siponimod, fingolimod) is probably caused by lower expression of S1P receptors on lymphocytes and reduce their count, what may decrease obtained humoral and cellular immunity levels [50]. Cladribine may have an influence on decreased cellular immunity level in a fact of causing reconstitution of immunity [53]. However, it is important to emphasize that the data on one of the most frequently used disease-modifying therapies (DMTs)[33,34], such as dimethyl fumarate and interferon beta, are limited. Furthermore, it has been observed that adverse events following vaccination are mostly mild and with short duration, similar to those in the general population. This observation aligns with the findings of Czarnowska et al. and Ciampi et al., although there is a discrepancy in the percentage distribution of individual symptoms [45,46].

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Taking the above into consideration, we decided to conduct our investigation with groups of PwMS treated with interferon beta and dimethyl fumarate. It was evident that humoral immunity would decrease over time; thus, in addition to analyzing IgG (S) anti-SARS-CoV-2 concentrations, we measured interferon gamma titers as a marker of cellular immunity against SARS-CoV-2. This approach complements humoral immunity and may help maintain a protective role against severe outcomes of COVID-19 [43,44]. In our investigation, we selected two populations of PwMS treated with dimethyl fumarate and interferon beta, as well as a group of healthy individuals as a control group. Every effort was made to select participants who corresponded to the typical profile of patients suffering from multiple sclerosis (described in the Methods section). Individuals from both the study and control groups were vaccinated against SARS-CoV-2, largely receiving two doses of BNT162b2.

In previous studies, seroconversion has been documented following both SARS-CoV-2 infection and vaccination against the virus. Bsteh et al. reported that 76% of patients with multiple sclerosis (PwMS) who had undergone SARS-CoV-2 infection, confirmed via RT-PCR testing approximately 5.2 months post-infection, exhibited detectable levels of IgG (S) antibodies against SARS-CoV-2. Furthermore, this study revealed that PwMS receiving immunomodulatory treatment were more likely to develop antibodies compared to those treated with immunosuppressive agents, although both groups demonstrated lower seroconversion rates than the general population [54]. In alignment with these findings, Sormani et al. examined a cohort of 423 patients with confirmed positive RT-PCR results or symptomatic COVID-19 and found that 76% exhibited positive IgG anti-SARS-CoV-2 levels, with a slightly lower rate of 73.5% observed among PwMS with solely positive RT-PCR results. Blood samples were collected at a median of 75 days following symptom onset. Notably, Sormani et al. also reported a decline in anti-SARS-CoV-2 IgG titers over a 90-day period post-infection, a finding that may aid in determining whether seroconversion among our study participants occurred as a result of SARS-CoV-2 infection or vaccination [55]. In another investigation involving PwMS with confirmed SARS-CoV-2 infection (n=187) and clinically suspected COVID-19 (n=56), but who had not been vaccinated, it was found that 83.44% exhibited positive IgG antibody results. This study indicated that antibodies could be detected up to 13.1 months following COVID-19 diagnosis, which contrasts with the findings of Sormani et al. Additionally, the proportion of anti-SARS-CoV-2 IgG (N) antibodies was found to be higher than that of anti-SARS-CoV-2 IgG (S) antibodies, particularly among PwMS treated with interferon [56], which could be related to the type of seroconversion. Ciampi et al. reported that 66.9% of a cohort of 178 PwMS displayed positive titers of anti-SARS-CoV-2 S1 antibodies four weeks after vaccination against SARS-CoV-2, including six PwMS who presented with clinical symptoms. Notably, a larger cohort of PwMS received an inactivated vaccine, while those vaccinated with an mRNA vaccine demonstrated a higher positivity rate of 78.4%. Interestingly, nearly 100% of positive antibody results were observed in PwMS treated with immunomodulatory disease-modifying therapies (DMT) [46].

As we reported, seroconversion approached nearly 100% in both study groups, aligning with findings from Bsteh et al., who collected blood samples three months after a single vaccine dose, and Sormani et al., who measured IgG (S) anti-SARS-CoV-2 levels two weeks post-second dose of vaccination [47,57]. Milo et al. reported a slightly lower seroconversion rate of 75% following the second and third doses of the anti-SARS-CoV-2 vaccination. Notably, among patients with multiple sclerosis (PwMS) treated with ocrelizumab and fingolimod, a significant proportion (218 out of 522 individuals) exhibited a decrease in the levels of anti-IgG antibodies against SARS-CoV-2. This observation suggests a potential impact of these treatments on the immune response to vaccination [58].

This indicates that the seropositive status of PwMS vaccinated against SARS-CoV-2 remains stable over time. Initial results showed no statistical significance in mean titers of IgG (S) anti-SARS-CoV-2 antibodies; hence, we decided to categorize our patients' blood sample analysis into two groups: those who obtained samples less than 200 days and more than 200 days after their last vaccination. Our findings revealed that during the first 200 days post-vaccination, the humoral

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immunity levels in our PwMS subgroups were relatively similar to those of the control group. These results are consistent with those of Sormani et al., Lambrianides et al., who measured serum antibodies three months post-vaccination, and Krajnc et al., who reported results five months after the second dose [47,59,60]. Unexpectedly, over 200 days post-vaccination, both PwMS subgroups exhibited significantly lower mean concentrations of IgG (S) anti-SARS-CoV-2 antibodies compared to the control group.

Investigating the features concerning PwMS treated with dimethyl fumarate (DMF) or interferon beta (IFN), particularly in relation to the estimated concentration of IgG (S) anti-SARS-CoV-2 antibodies, is crucial. Available data presented by Milo et al. described PwMS treated with DMF and IFN six months after their last SARS-CoV-2 vaccination. The findings revealed that the mean concentration of IgG (S) anti-SARS-CoV-2 antibodies was slightly lower in both groups of PwMS than in the control group ($p < 0.001$), which aligns with our results [58]. In contrast, Maglione et al. conducted a similar study but did not find any significant differences in IgG (S) concentrations between PwMS treated with DMF or IFN and the control group [61]. This suggests that PwMS may have an appropriate immune response, but their antibody levels decline more rapidly than those in the control group, indicating that booster vaccinations may be beneficial. To draw definitive conclusions, further investigations with larger sample sizes are necessary.

Furthermore, data on the concentration of IgG (N) anti-SARS-CoV-2 antibodies were collected. It was unexpected that a number of PwMS in the study group (DMF 50%, IFN 66.67%) and the control group (57.14%) exhibited low levels of antibodies. This finding was attributed to a small percentage of participants who had previously undergone SARS-CoV-2 infection across all investigated groups. The IgG (N) anti-SARS-CoV-2 antibodies displayed similar concentrations, with no statistically significant differences, and should not interfere with the accurate assessment of humoral immunity following vaccination.

What is more, we also tested cellular immunity after vaccination against SARS-CoV-2 based on interferon gamma secretion by activated T lymphocytes [62]. Our study shows that the mean concentration of interferon gamma in PwMS subgroups is significantly lower than in the control group. In contrast, data obtained by Trümpelmann et al. show that PwMS treated with IFN beta have cellular immunity levels comparable to those of the general population. Although there were some differences, Trümpelmann et al. conducted their research with samples taken six weeks after the second dose of vaccination, and most of the PwMS were vaccinated with the mRNA-1273 vaccine [63]. A similar observation was made by Maglione et al., who discovered that one and six months after immunization against SARS-CoV-2, the concentration of interferon gamma was comparable in groups of patients with MS treated with dimethyl fumarate (DMF), IFN beta, and the general population [61]. Additionally, an interesting finding by Tortorella et al. indicated that PwMS treated with IFN beta had significantly lower interferon gamma titers than those measured in healthcare workers (blood collection was performed 2-4 weeks after vaccination) [51]. To verify this thesis, we decided to divide the groups similarly to our analysis of IgG (S) antibodies. In the category with a time duration between vaccination and blood sample collection of less than 200 days, the interferon gamma titers in the study subgroups were lower than those in the controls, but statistical significance was absent. However, assessment of samples collected from groups more than 200 days after vaccination against SARS-CoV-2 revealed that mean interferon gamma titers in the study subgroups were significantly lower than in the control group. The obtained results may suggest that PwMS have fewer activated T lymphocytes or that interferon gamma has a shorter duration of activity.

5. Conclusions

To sum up, our study shows that humoral immunity following vaccination against SARS-CoV-2 in PwMS treated with dimethyl fumarate (DMF) and interferon beta (IFN) is adequate during the first 200 days, comparable to that of the general population. After this period, immunity decreases and is lower than in individuals without multiple sclerosis, although it remains present. Furthermore, individuals with multiple sclerosis receiving DMF or IFN beta treatment exhibited a similar SARS-

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CoV-2 infection rate and comparable concentrations of IgG (N) anti-SARS-CoV-2 antibodies relative to the general population. Importantly, PwMS treated with DMF and IFN beta had lower concentrations of interferon gamma compared to the general population. Protective titers of antibodies and interferon gamma against severe COVID-19 outcomes have not been established [64]. This finding may suggest an earlier loss of post-vaccination immunity in PwMS treated with selected disease-modifying therapies (DMTs). Our investigation has several limitations, including a small sample size and the specific markers measured. Additionally, the lack of data concerning relapses or steroid usage in the study group was due to incomplete information in the surveys. Moreover, the surveys were conducted simultaneously with blood collection during a specific time period post-vaccination, which may have decreased the accuracy of the collected data. Therefore, further studies and follow-ups are necessary.

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