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COVID-19

Hypogammaglobulinemia is associated with reduced antibody response after anti-SARS-CoV-2 vaccination in MS patients treated with antiCD20 therapies

Angelo Bellinvia[1] · Maria Grazia Aprea[1] · Emilio Portaccio[1] · Luisa Pastò[1] · Lorenzo Razzolini[1] · Mattia Fonderico[1] · Ilaria Addazio[1] · Matteo Betti[1] · Maria Pia Amato[1,2]

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Abstract

Background COVID-19 vaccination is highly recommended to multiple sclerosis (MS) patients. Little is known about the role of patients' clinical and demographic characteristics in determining antibody response.

Methods We evaluated safety and efficacy of anti-SARS-CoV-2 vaccines on 143 included MS patients. Then, we analyzed antibody titer in a subgroup, assessing clinical and demographic variables associated with protection and antibody titer.

Results After completing the vaccination cycle, the rate of local adverse events was similar after the first and second dose. A higher proportion of systemic AEs was reported after the second dose (65.7% vs 24.5% after the first dose). Antibody response was evaluated in 97 patients. Higher EDSS (OR 0.6, 95% CI 0.4–0.9, $p = 0.006$) and treatment with antiCD20 (OR 0.02, 95% CI 0.003–0.098, p 0.001) were associated with a lower chance of having an efficacious response. Higher weight was associated with higher Ab titer ($\beta = 15.2$, 95% CI 2.8–27.6, $p = 0.017$), while treatment with antiCD20 with lower titers ($\beta = -1092.3$, 95% CI −1477.4 to −702.2, $p < 0.001$). In patients treated with antiCD20, hypogammaglobulinemia (β −543, 95% CI −1047.6 to −39.1, $p = 0.036$) and treatment duration (β −182, 95% CI −341.4 to −24.3, $p = 0.027$) were associated with lower Ab titer.

Conclusion Our study confirms that COVID-19 vaccination in MS patient is safe and effective in preventing symptomatic COVID-19 and should be recommended to all patients. Moreover, we suggest a possible role of hypogammaglobulinemia in reducing Ab response in patients treated with antiCD20 therapies.

Keywords COVID-19 · Vaccination · Multiple sclerosis · AntiCD20 · Hypogammaglobulinemia

Background

Vaccination against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the most promising strategy to overcome coronavirus disease 2019 (COVID-19) pandemic [1]. Indeed, the National Multiple Sclerosis (MS) Society and other expert organizations recommended vaccination against SARS-CoV-2 to all patients with MS [2–4]. So far, no safety concerns have emerged related to the administration of the currently approved COVID-19 vaccines in people with MS (pwMS). In a recent study involving 555 pwMS who underwent vaccination with BNT162b2 vaccine, no increase of relapse activity was detected [3]. As further confirmation, a recent prospective study including 324 patients with MS who received BNT162b2 vaccine did not disclose an increased risk of clinical relapses during the 2-month follow-up after the first vaccine dose [5]. Regarding efficacy, several studies have been conducted so far [6–18]. On the whole, these studies suggest a less robust immune response in patients treated with antiCD20 therapies [6, 8, 10, 13, 17–19] and possibly fingolimod [19, 20], while other DMTs, such as natalizumab [21] and cladribine [12], seemed to have no effect on vaccination response. As for antiCD20, factors found to be associated with seroconversion in patients with MS were time since last antiCD20 infusion and total time

Angelo Bellinvia and MG Aprea contributed equally to this work.

✉ Angelo Bellinvia
angelo.bellinvia@unifi.it

[1] NEUROFARBA Department, University of Florence, Largo Palagi, 1, 50139 Florence, Italy

[2] IRCCS Don Carlo Gnocchi, Florence, Italy

on treatment [22], whereas the effects of antiCD20 mAb-related hypogammaglobulinemia on prior immunization are not known [23]. The only study which assessed immunoglobulin G (IgG) levels as a possible predictor of response [11] found no significant results. With this background, the objectives of the present study were the following: (a) to evaluate safety of COVID-19 vaccines on MS patients in a real-world Italian cohort; (b) to assess the efficacy of these vaccines on a 6-month follow-up; and (c) to assess efficacy on a subset of MS patients treated with antiCD20 therapies.

Methods

We conducted a prospective, monocentric, observational study on MS patients consecutively referring to the MS Center of the Neurological Rehabilitation Unit (Careggi University Hospital, Florence). Patients were recruited between April and September 2021. Eligible patients were men and women aged at least 18 years, diagnosed with MS, and which received at least one dose of a mRNA COVID-19 vaccine (Pfizer BNT162b2 or Moderna mRNA-1273 Tx Inc.). The following data were acquired at baseline through a telephone call or a brief in-person interview: age, sex, weight, height, comorbidities, history of allergies, history of vaccination-related adverse events (AEs), disease duration, Expanded Disability Status Scale (EDSS) [24] score at last visit, currently prescribed DMT, other medical conditions and therapies, and type of COVID-19 vaccine administered. The safety of vaccination was assessed in the whole sample (patients who received at least one dose of COVID-19 vaccine). An AE was considered as related to vaccination in case of occurrence within 21 days after vaccination [25]. AEs after each dose were categorized as follows: local (i.e., pain and swelling in the injection site) and systemic (i.e., fatigue, fever, chills, myalgias, and arthralgias). Severity of AEs was classified as follows: mild, moderate, or severe according to the Common Terminology Criteria of Adverse Events (CTCAE) v 5.0 [26]. Serious AEs were defined as events related to the vaccination resulting in death, hospitalization, disability, or life-threatening events.

As for vaccine-related risk of disease reactivation, relapse activity over a 6-month period before vaccination was compared with relapse and/or MRI activity (new/enlarging T2 lesions and/or gadolinium-enhancing lesions) observed over a 6-month period after.

The efficacy of vaccination was assessed in the subgroup of patients who completed the vaccination cycle (two doses of COVID-19 vaccine) through measurement of serum antibody (Ab) titer, performed at least 4 weeks after completion of the vaccination cycle. Ab titer was determined using high-throughput chemiluminescent immunoassay (CLIA) platforms, chemiluminescent microparticle immunoassay (CMIA), or enzyme-linked immunosorbent assay tests (ELISA). All immunogenicity results were reported according to World Health Organization International Standard as binding antibody units (BAU) per mL. A titer $\geq$ 40 BAU/mL was considered as protective [27]. Patients receiving antiCD20 therapies were treated using the following schedule: for rituximab, they initially received two 1000 mg infusions with a 2-week interval between infusions, and then one 1000 mg infusion every 6 months. Similarly, patients treated with ocrelizumab received two 300 mg infusions with a 2-week interval, and then one 600 mg infusion every 6 months. In patients receiving antiCD20, Ig levels were regularly assessed at 1, 3, and 5 months after infusion, as part of monitoring protocol adopted in our clinic. Hypogammaglobulinemia was defined as an IgG, IgA, or IgM count below the respective cut-off (7 g/L; 0.7 g/L; 0.4 g/L) at the last available assessment before the first vaccine dose. All the patients signed an informed consent. The study was approved by the ethical committee of the University of Florence.

Statistical analysis

Continuous variables were described as mean and standard deviation (SD) or median and interquartile range (IQR) when appropriate, while categorical variables were described as frequency and percentage. Intra-group comparisons were performed using the Wilcoxon signed-rank test and the McNemar test, while inter-group comparisons were performed using the Student's *t*-test, the Mann–Whitney *U*-test, or the chi-squared test, when appropriate.

Safety of vaccination was defined as rate of AEs and sAEs following vaccination, and disease activity in the 6 months before and after vaccination. Predictors of safety outcomes were assessed using multivariable linear regression models, including as covariates the following variables: sex; age; weight; disease duration; treatment with DMTs (yes/no); current EDSS; allergies (yes/no); comorbidities (yes/no); disease course (RR/SP/PP); and follow-up duration (months).

As for efficacy of vaccination, we adopted two different definitions: first, we considered protection as a dichotomous (yes/no) variable, defining protection as a titer $\geq$ 40 BAU/mL; then we evaluated predictors of Ab titer considered as a continuous variable. Therefore, different multivariable analyses (binomial logistic regression and linear regression analyses) were performed, including as covariate the following variables: sex; age; weight; disease duration; treatment with DMTs (yes/no); current EDSS; allergies (yes/no); comorbidities (yes/no); disease course (RR/SP/PP); and follow-up duration (months). Treatment with DMTs was further classified as treatment with antiCD20, other DMTs, and no treatment.

Finally, we repeated efficacy analysis focusing on patients treated with antiCD20 therapies. In this subgroup, hypogammaglobulinemia, latency between last infusion and first vaccine (in months), latency between hypogammaglobulinemia detection and first vaccine (in months), and treatment duration (in years) were added in the models as possible covariates.

Results

Over the study period, 143 patients (108 females, mean age 45 ± 12.1 years, median EDSS score 2.0, IQR 1.5–2.5) were available for the safety analyses. One hundred twenty-one patients (84.6%) received the mRNA-1273 vaccine, while 22 (15.4%) the BNT162b2 vaccine. Ninety-seven (67.8%) agreed to undergo a blood test to assess antibody levels. One hundred thirty-seven (95.8%) patients completed the vaccination cycle. The efficacy of vaccination was assessed only in patients who completed the two-dose vaccination cycle. Table 1 depicts the main clinical and demographic characteristics of the whole sample.

Safety

After the first dose, 53 (37.1%) patients experienced local AEs, more commonly pain in the injection site, while 35 (24.5%) had systemic AEs, more commonly fatigue. All local and systemic AEs were reported as mild. One (0.7%) patient had a sAE, a Varicella Zoster Virus encephalitis, requiring hospitalization and prolonged treatment with antiviral agents to resolve. After the second dose of the vaccine, the rate of patients reporting local adverse events was no different from the first dose ($n = 49$, 34.5%, $p = 0.626$), while 94 (65.7%) experienced systemic AEs, more than double compared with the first dose ($p < 0.001$), more commonly fever and fatigue (Table 2). All local and systemic AEs were reported as mild. No patients reported sAEs after the second dose. There were no significant differences comparing the two administered vaccines. The multivariable analyses did not find any demographic or clinical predictor of vaccine-related AE/sAE.

Disease activity

Ten (6.9%) patients among the 143 included experienced clinical and/or MRI disease activity during follow-up. The

Table 2 Safety data

Adverse events	First dose	Second dose	*p*
Local, *n* (%)	53 (37.1)	49 (34.5)	0.626
Systemic, *n* (%)	35 (24.5)	94 (65.7)	< 0.001
sAEs, *n* (%)	1 (0.7)	0 (0)	*ns*

Legend: *sAEs*, serious adverse events

Table 1 Demographic and clinical characteristics of the included patients

	Whole sample ($n = 143$)	Included in Ab titer analysis ($n = 97$)	Excluded from Ab titer analysis ($n = 45$)	*p*
F, *n* (%)	108 (75.5)	74 (76.3)	34 (73.9)	0.836
Age, mean ± SD	45 ± 12.1	44.4 ± 12.1	46.3 ± 12.1	0.388
Weight, kg ± SD	68.1 ± 15.3	66.7 ± 13.8	71.1 ± 17.9	0.127
Any allergy	28 (20)	20 (21.3)	8 (18.2)	0.821
Any comorbidity	40 (29)	24 (25.5)	16 (36.4)	0.228
Disease duration, mean ± SD	12.8 ± 9.8	12.4 ± 9.3	13.8 ± 10.8	0.398
Disease course RR, *n* (%)	131 (91.6)	92 (94.8)	39 (86.7)	0.111
Relapses in the past year, mean ± SD	0.3 ± 0.6	0.3 ± 0.6	0.3 ± 0.5	0.636
EDSS, median (IQR)	2.0 (1.5–2.0)	1.5 (1.5–2.5)	2.0 (1.5–3.0)	0.390
Treated with DMTs, *n* (%)	125 (88.8)	90 (93.8)	35 (76.1)	0.002
High-efficacy DMTs, *n* (%)	67 (54.9)	55 (61.1)	12 (37.5)	0.021
AntiCD20, *n* (%)	26 (18.2)	21 (21.6)	5 (15.6)	0.346
Duration of treatment with antiCD20, years, mean ± SD	2.3 ± 1.6	2.3 ± 1.7	2.2 ± 1.4	0.865
Responders, *n* (%)	81 (83.5)*	81 (83.5)*	*n/a*	*n/a*
Follow-up (months), mean ± SD	5.9 ± 1	6.1 ± 0.9	5.5 ± 1.2	0.001

*Calculated on 97 patients

Legend: *F*, females; *SD*, standard deviation; *BMI*, body mass index; *RR*, relapsing-remitting; *EDSS*, Expanded Disability Status Scale; *DMTs*, disease-modifying therapies

proportion of patients with disease activity after vaccination was comparable to that in the 6 months before (5.6% vs 6.9%, $p=0.999$). No predictors of disease activity were found in the multivariable analysis.

Efficacy

Three patients (2.1%) of 143 receiving at least one dose of either vaccine reported to have symptomatic COVID-19 during follow-up. Efficacy data in terms of Ab response were available for 97 patients out of 143 included. Patients included in this efficacy analysis were more frequently treated with DMTs (93.8 vs 76.1, $p=0.002$) and with high-efficacy DMTs (61.1 vs 37.5, $p=0.021$), and had a longer follow-up time (6.1 ± 0.9 vs 5.5 ± 1.2 months, $p=0.001$) compared with the excluded patients. Eighty-one patients (83.5%) were immunized (Ab titer ≥ 40 BAU/mL), with a mean titer of 1129 ± 122 BAU/mL. Non-responders ($n=16$, 16.5%) had a mean titer of 2.6 ± 9.9 BAU/mL ($p<0.001$) (Table 3). Non-responders were more frequently males (44.7 vs 19.8, $p=0.039$), had a longer disease duration (16.5 ± 7.9 vs 11.5 ± 9.3 year, $p=0.035$), had a higher disability burden (median EDSS 3.0, IQR 2.5–3.5 vs 1.5, IQR 1.0–2.0, $p=0.002$), and were treated more frequently with high-efficacy DMTs (93.8% vs 50.6%, $p=0.003$) compared to responders. The time elapsed between the second vaccine dose and Ab testing was not significantly different between responders and non-responders (2.5 ± 1.3 vs 1.8 ± 0.8, $p=0.109$). Among non-responders, 14 patients (87.5%) were treated with antiCD20 therapies, four with ocrelizumab and 10 with rituximab. This rate was significantly higher compared with responders (9.6%, $p<0.001$). The other two non-responders were treated with dimethyl fumarate (DMF) and natalizumab (NTZ). There was a 100% response rate in untreated patients ($n=7$), and patients treated with teriflunomide ($n=1$), azathioprine ($n=2$), cladribine ($n=4$), glatiramer acetate ($n=4$), fingolimod ($n=1$), and interferon-beta ($n=5$). The response rate was 95.7% in patients treated with DMF ($n=22$), and 96.6% in patients treated with NTZ ($n=28$).

We conducted different multivariable analyses looking for factors affecting efficacy of the vaccination, expressed as a categorical (yes/no) variable (Ab titer ≥ 40 BAU/mL) and using Ab titer as a continuous variable. In the first model, the binomial regression analysis showed that higher disability (OR 0.6, 95% CI 0.4–0.9, $p=0.006$) and treatment with antiCD20 (OR 0.02, 95% CI 0.003–0.1, $p<0.001$) were associated with a lower response rate. Looking at the variables influencing Ab titer, weight (β 15.2, 95% CI 2.8–27.6, $p=0.017$) was associated with an increase in Ab titer, while treatment with antiCD20 was associated with lower Ab titers ($\beta-1092.3$, 95% CI -1477.4 to -707.2, $p<0.001$). Results of the multivariable analyses are reported in Tables 4 and 5.

AntiCD20

Focusing on patients treated with antiCD20 therapies with Ab titer available ($n=21$), the response rate was 33.3% ($n=7$), which was significantly different compared with untreated patients and patients treated with other DMTs

Table 3 Demographic and clinical characteristics and Ab titer of responders and non-responders to vaccination based on Ab titer

	Responders ($n=81$)	Non-responders ($n=16$)	p
F, *n* (%)	65 (80.2)	9 (56.3)	**0.039***
Age, mean ± SD	44.5 ± 12.5	43.6 ± 10.1	0.787
Weight, kg ± SD	66.3 ± 13.1	68.6 ± 17.4	0.556
Any allergy	17 (21.5)	3 (20)	0.895
Any comorbidity	23 (29.1)	1 (6.7)	0.064
Disease duration, mean ± SD	11.5 ± 9.3	16.5 ± 7.9	**0.035***
RR, *n* (%)	76 (93.8)	16 (100)	0.594
Relapses in the past year, mean ± SD	0.5 ± 0.7	0.4 ± 0.8	0.639
EDSS, median (IQR)	1.5 (1.0–2.0)	3.0 (2.5–3.5)	**0.002***
Treated with DMTs, *n* (%)	75 (92.6)	16 (100)	0.258
High-efficacy DMTs, *n* (%)	41 (50.6)	15 (93.8)	**0.003***
AntiCD20, *n* (%)	7 (9.6)	14 (87.5)	**< 0.001***
Duration of treatment with antiCD20, years, mean ± SD	0.8 ± 0.7	3.3 ± 1.3	**< 0.001***
Follow-up (months), mean ± SD	6.1 ± 0.8	6.0 ± 0.9	0.962
Total Abs, mean ± SD	1129 ± 122	2.6 ± 9.9	**< 0.001***
Lag between second dose and test, mean ± SD	2.5 ± 1.3	1.8 ± 0.8	0.109

Legend: *F*, females; *SD*, standard deviation; *BMI*, body mass index; *RR*, relapsing-remitting; *EDSS*, Expanded Disability Status Scale; *DMTs*, disease-modifying therapies. * = statistically significant

Table 4 Demographic and clinical factors associated with immunization in the whole sample

Model 1	OR (95% CI)	*p*
Higher EDSS	0.6 (0.4–0.9)	0.006
Model 2		
AntiCD20 treatment	0.02 (0.003–0.098)	< 0.001

Model 1 included as a covariate treatment with DMTs (yes/no); model 2 treatment with antiCD20 vs other DMTs/no treatment

Legend: *OR*, odds ratio; *CI*, confidence interval; *DMTs*, disease-modifying therapies.

Table 5 Demographic and clinical factors associated with Ab titer in the whole sample and in patients treated with rituximab or ocrelizumab

Whole sample	Beta (95% CI)	*p*
Weight	15.2 (2.8–27.6)	0.017
Treatment with antiCD20	− 1092.3 (− 1477.4; − 707.2)	< 0.001
AntiCD20	Beta (95% CI)	*p*
Hypogammaglobulinemia	− 543.3 (− 1047.6 to − 39.1)	0.036
Treatment duration	− 182.8 (− 341.4 to − 24.3)	0.027

Model 2 treatment with antiCD20 (yes/no); model 3 excluded treatment; model 4 included only patients treated with antiCD20 DMTs (rituximab or ocrelizumab). Legend: *AEs*, adverse events; *CI*, confidence interval; *DMTs*, disease-modifying therapies

(97.4% $p < 0.001$). In the multivariable analysis, hypogammaglobulinemia ($\beta - 543$, 95% CI − 1047.6 to − 39.1, $p = 0.036$) and duration of treatment with antiCD20 ($\beta - 182$, 95% CI − 341.4 to − 24.3, $p = 0.027$) were associated with a lower Ab titer (Table 5). No predictors of response were found in the binomial logistic regression analysis.

Discussion

Anti-SARS-CoV-2 vaccines have proven to be safe and efficacious in preventing symptomatic COVID-19 in the general population [28, 29], and the pwMS as well [6, 9–11, 14, 15, 17, 18]. In our study, we confirmed safety and efficacy of COVID-19 vaccination in the MS population, with the exception of patients receiving antiCD20, for whom the efficacy was significantly reduced. Our findings further explored predictors of vaccination efficacy in this subgroup of patients, highlighting the negative impact of hypogammaglobulinemia and treatment duration on Ab titers. We found that COVID-19 vaccination in MS was overall safe. Local and systemic AEs were reported in up to 37% of cases after the first dose, and up to 65.7% after the second dose. All of these AEs were classified as "mild" using the CTCAE [26]. The only sAE was a Varicella Zoster encephalitis, occurring after the first dose and resolving after hospitalization and prolonged antiviral intravenous treatment. These safety findings were similar to those reported in the literature (Table 6). In the study by Achiron et al., the largest on the topic so far, the reported rate of AEs was 29.7% after the first dose and 40.2% after the second. In our study, we obtained similar data, and we further analyzed the reported AEs identifying a more than doubled rate of systemic AEs after the second dose, consistent with that reported in the RCTs [28, 29]. In another recent study on 130 patients treated with ocrelizumab or fingolimod, AEs were observed in 63.4% MS patients treated with antiCD20 and 37.9% patients treated with fingolimod. The only variable associated with AEs was a higher lymphocyte count (1410 vs 1183, $p = 0.003$) [6], data that was not available in our study.

The most common reported systemic AEs in our cohort was fatigue. Fatigue is a specific MS symptom that expresses the subjective inability to perform daily life activities due to a lack of energy [30]. Fatigue is present in up to 90% of MS patients, can be the only symptom of a relapse, and can have primary or secondary causes [31]. Our results indicated an increased prevalence of fatigue after vaccination compared with the general population, while the rates of the other reported AEs were comparable with that reported in the phase III RCTs of COVID-19 vaccines. Consistently, fatigue is one of the most frequent AEs after vaccination in MS patients reported in the literature [3, 6, 8, 9, 15, 18].

As for disease activity after vaccination, in our cohort, 10 patients (6.9%) experienced a relapse and/or subclinical MRI activity after vaccination. However, there was no increase in relapse rate over the 6 months after vaccination as compared with the 6 months preceding vaccination ($p = 0.999$). This finding is in line with those of other Italian cohorts [4, 5, 8], focused only on clinical disease activity, confirming that mRNA vaccine administration is not associated with a short-term increase in relapse rate, nor with pseudo-relapses.

A few studies so far have explored clinical and demographic predictors of response to the vaccine administration [3, 11, 14]. The first of these studies showed an association between older age, smoking and male sex, and lower antibody titers [14], while the other two studies did not find any significant predictors [3, 11]. In our study, the efficacy of vaccination was of 97.9% in preventing symptomatic COVID-19 during the short-term follow-up, consistently with previous studies on MS patients [4, 5, 8] and the phase III RCTs of the two vaccines [28, 29]. The rate of responders among the subgroup of 97 patients with Ab titer available was of 83.5%, which raised to 97.4% after excluding patients treated with antiCD20 therapies. In the univariate analysis, non-responders were more frequently males, had a higher disability, a longer disease duration, and were more frequently treated with high-efficacy DMTs. In the multivariable analyses, higher EDSS and antiCD20 therapies were associated with lower response rate, while weight and

Table 6 Studies exploring safety and efficacy of vaccination against COVID-19 in MS patients

Authors, year, country	Study design	Included patients	Outcomes	Safety results	Efficacy results
Konig et al., 2021, Norway [6]	Prospective, observational, multicenter	130 MS patients	Antibody response after third dose of COVID-19 vaccines in antiCD20 and fingolimod-treated patients	Adverse effects were observed in 63.4% MS patients treated with antiCD20 and 37.9% patients treated with fingolimod. Patients who reported AEs had higher lymphocyte counts compared to those who did not (1410 vs 1183, $p=0.003$)	Increasing in Ab titer after third dose in both antiCD20 and fingolimod groups (8.9 vs 49.4 in antiCD20 and 9.2 vs 25.1 in the fingolimod group, $p<0.001$)
Achtnichts et al., 2021, Switzerland [7]	Cross-sectional, observational, monocentric	16 MS patients	Seroconversion after the third mRNA SARS-CoV-2 vaccine in B cell-depleted pwMS with limited or no IgG response after the standard immunization	Not assessed	After the third vaccination, one out of 16 patients showed an IgG titer deemed clinically relevant. Only the seroconverted patient had measurable B-cell counts at the time of the third vaccination
Giossi R. et al., 2021, Italy [8]	Case–control, prospective, multicenter	39 MS patients, 273 healthcare workers (HCWs)	Serological response in MS patients compared to the one from a control population of HCWs; response in patients receiving treatments associated with possible reduced immune response vs other treatments	Solicited AEs were all mild to moderate in severity, generally reported in the first days after vaccination, and resolved in the following days. Two MS patients reported a clinical relapse after the second vaccine dose	Median anti-Spike levels were similar between patients (1471.0 BAU/mL; IQR 779.7 to 2357.0) and controls (1479.0 BAU/mL; IQR 813.1 to 2528.0) ($p=0.53$). Patients included in the under scrutiny treatments group showed reduced anti-Spike levels (156.4 BAU/mL; IQR 33.4 to 559.1) compared to those receiving other treatments (1582.4 BAU/mL; IQR 1296.5 to 2219.0) ($p=0.001$)
Ciampi E. et al., 2022, Chile [9]	Prospective observational, multicenter	178 MS patients	Safety and humoral response of inactivated virus and mRNA vaccines against SARS-CoV-2 in patients with MS	The most frequent AE was local pain (14%), with 4 (2.2%) patients experiencing mild-moderate relapses within 8 weeks of first vaccination compared to 11 relapses (6.2%) within the 8 weeks before vaccination (chi-squared 3.41, $p=0.06$)	Overall humoral response was observed in 66.9% (62.6% inactivated vs 78.4% mRNA, $p=0.04$). In the multivariate analysis including antiCD20 patients, the predictors for a positive humoral response were receiving the mRNA vaccine (OR 8.11 (1.79–36.8), $p=0.007$) and a lower number of total infusions (OR 0.44 (0.27–0.74) $p=0.002$)

Table 6 (continued)

Authors, year, country	Study design	Included patients	Outcomes	Safety results	Efficacy results
Conte W.L., USA [10]	Case–control, observational, single-center	67 MS patients	Antibody response in patients treated with AntiCD20 and S1p modulators vs other treatments	Not assessed	Patients who received OCR or OFA had decreased odds of forming antibodies (OR 0.031, $p < 0.001$, 95% CI (0.003–0.268)). Patients who received S1P modulators did not have decreased odds of forming antibodies (OR 0.413, $p = 0.413$, 95% CI (0.28–21.7)). However, when analyzing the antibody response as a continuous variable, patients on S1P modulators showed lower absolute levels of antibodies ($p = 0.024$)
Katz J.D. et al., 2021, USA [11]	Prospective observational, single-center	48 MS patients	Humoral response to SARS-CoV-2 vaccines in adult MS patients treated with OCR, using NTZ as a real-world comparator. T-cell response for those OCR-treated patients who did not produce detectable antibodies	Not assessed	Eighteen percent of ocrelizumab and 100% of natalizumab patients had a positive antibody response. In ocrelizumab-treated patients, there was no correlation between age, sex, BMI, total number of infusions, immunoglobulin G, CD19, or absolute lymphocyte count and antibody response. There was a trend suggesting that a longer interval between the last infusion and vaccination increased the likelihood of producing antibodies ($p = 0.062$). All ocrelizumab patients with negative antibody responses had positive T-cell responses

Table 6 (continued)

Authors, year, country	Study design	Included patients	Outcomes	Safety results	Efficacy results
Brill L. et al., 2021, Israel [12]	Prospective observational, single-center	67 MS patients, 30 HCs	Antibody response in patients treated with cladribine vs HCs	Not assessed	MS patients treated with cladribine ($n=32$) had 100% positive serology response against the SARS-CoV-2 spike protein following the second vaccine dose (mean S1/S2-IgG and RBD-IgG: 284.5 ± 104.9, 13,041 ± 9411 AU/mL and 226.3 ± 121.4, 10,554 ± 11,405 AU/mL, respectively
Mariottini A. et al., 2021, Italy [18]	Retrospective, observational, single-center	120 MS patients	Antibody response after vaccination for SARS-CoV-2	Adverse effects were observed in roughly $^1/_3$ of patients. 2 patients experienced a clinical relapse	Anti-Spike IgG antibodies were detectable in 85%, being the proportion lower in patients receiving antiCD20 antibodies (45%) compared to non-depletive treatments (99%), $p<0.0001$
Pitzalis M. et al., 2021, Italy [14]	Prospective, observational, single-center	912 MS patients and 63 healthy controls	Antibody response 30 days after the second dose of BNT162b2 vaccine	Not analyzed	Absence of significant difference between untreated MS patients and healthy control in anti-S antibodies response ($p=0.51$); significantly lower level of anti-S antibodies production in MS patients treated with teriflunomide (IRR = 0.51, $p=1.44$E-05), azathioprine (IRR = 0.37, $p=3.26$E-06), natalizumab (IRR = 0.72, $p=0.034$), fingolimod (IRR = 0.13, $p=1.89$E-39), ocrelizumab (IRR = 0.10, $p=4.88$E-38), and rituximab (IRR = 0.22, $p=8.36$E-07) compared to untreated patients; reduced levels of antibodies in older (IRR = 0.98, $p=3.85$E-03), male individuals (IRR = 0.83, $p=0.021$) or reduced EDDS (IRR = 0,93, $p=1.31$E-04)

Table 6 (continued)

Authors, year, country	Study design	Included patients	Outcomes	Safety results	Efficacy results
Räuber S. et al., 2021, Germany [15]	Retrospective, observational, single-center	59 OCR-treated patients with MS	Anti-SARS-CoV-2-antibody titers and SARS-CoV-2-specific T-cell response	92.7% reported mild side effects of vaccination	Anti-SARS-CoV-2(S) antibodies were found in 27.1% and SARS-CoV-2-specific T-cell response in 92.7% of cases
Achiron A. et al., 2021, Israel [3]	Observational, single-center	555 MS patients who received the first dose of BNT162b2 vaccine and 435 who received the second dose	Characterize safety and occurrence of immediate relapses following COVID-19 vaccination	29.7% reported side effects after the first dose and 40.2% after the second, 16% and 14.2% had local AEs after the first and second dose, respectively, fever and flu-like symptoms were reported by 2% and 11.9% of patients	Not analyzed
Achiron A. et al., 2021, Israel [16]	Observational, single-center	125 MS patients	Antibody response 30 days after the second dose of BNT162b2 vaccine	Not analyzed	Protective humoral immunity was observed in 97.9% of healthy subjects, 100% of untreated MS patients, 100% of MS patients treated with cladribine, 22.7% of patients treated with ocrelizumab, and 3.8% of those treated with fingolimod
Sokratis A. et al., 2021, USA [17]	Observational, single-center	20 patients with MS on antiCD20 antibody monotherapy and 10 healthy controls	Antigen-specific antibody, B cell, and T-cell responses	Not analyzed	Eighty-nine percent of patients developed detectable anti-Spike IgG and only 50% mounted detectable anti-RBD IgG responses by T5. All patients generated antigen-specific CD4 and CD8 T-cell responses after vaccination

Table 6 (continued)

Authors, year, country	Study design	Included patients	Outcomes	Safety results	Efficacy results
Katz et al., 2022, USA [35]	Prospective, observational, single-center	48 patients with MS treated with OCR ($n=33$) or NTZ ($n=15$)	Antigen-specific antibodies, T-cell responses	Not analyzed	Eighteen percent of ocrelizumab and 100% of natalizumab patients had a positive antibody response. In ocrelizumab-treated patients, there was no correlation between age, sex, BMI, total number of infusions, immunoglobulin G, CD19, or absolute lymphocyte count and antibody response. There was a trend suggesting that a longer interval between the last infusion and vaccination increased the likelihood of producing antibodies ($p=0.062$)

antiCD20 therapies with lower Ab titer. As for weight, other studies exploring possible predictors of vaccine response in patients with MS did not report a similar association [3, 11, 14]. Conflicting results have been reported in the general population. Indeed, a higher BMI has been associated with reduced response rate [32] or was reported to have no effect on humoral response [33] after COVID-19 vaccine. The association between higher EDSS and reduced response rate could be explained, at least in part, to the higher disability burden in patients treated with antiCD20 therapies, which indeed was the strongest predictor of reduced vaccine response. This finding is consistent with the available evidence [6, 7, 11, 13, 15].

Other studies have indicated the possibility of reduced response after fingolimod as well [16, 20, 34], while patients treated with cladribine were reported to have a good antibody response [12]. Unfortunately, in our study, only one patient was treated with fingolimod and four with cladribine, preventing us to obtain reliable inferences about these therapies.

Focusing to the subgroup of patients treated with antiCD20, in our sample, 7 (33.3%) showed a sufficient response to the vaccine in terms of Ab production, consistently with the 18–45% response rate reported in the literature [6, 9–11, 14, 15, 17, 18]. Looking more closely at factors associated with vaccine response among patients treated with antiCD20, in the univariate analysis disease duration, age, hypogammaglobulinemia, and treatment duration were possibly associated with Ab response. However, in the multivariable analysis, only hypogammaglobulinemia and longer treatment duration were retained as significant predictors of reduced response to vaccination. The role of hypogammaglobulinemia as a factor influencing vaccination efficacy has not been fully elucidated. In previous studies on anti-SARS-CoV-2 vaccine response in MS patients receiving antiCD20 (ocrelizumab or ofatumumab), no association between Ig levels and ability to mount a humoral response was found [10, 11, 15, 35]. Differences in size and characteristics of study samples could account for these inconsistencies. Moreover, it has to be noted that, in our study, 10 out of the 14 non-responder patients received rituximab. Further studies are needed in order to confirm the influence of Ig levels on response to vaccines and on potential different effects on vaccinations between ocrelizumab and rituximab.

As for treatment duration, a few studies have previously reported an association between longer treatment duration and lower Ab titer or lower probability of achieving a protective titer in MS patients. In a multicenter study on 912 MS patients treated with any DMT [14], treatment duration had a minor effect on Ab response at the multivariate analysis. In another study, focusing on 99 patients treated with antiCD20 therapies, a higher number of total infusions was associated with a lower probability of having an efficacious

response at a binomial logistic regression analysis [9]. Other recent studies reported a negative correlation between treatment duration and lower Ab titer in patients treated with antiCD20 [15, 18], without adjusting for other clinical and demographic factors. Finally, one study did not find any correlation between treatment duration and antibody response in MS patients treated with ocrelizumab [6]. In line with previous evidence, in our sample, we confirmed the association between longer treatment duration and lower Ab titer after anti-SARS-CoV-2 vaccine in MS patients treated with antiCD20, independently of other relevant demographic and clinical confounders.

In interpreting the study findings, a few limitations should be taken into account. The study was monocentric and sample size, particularly for the antiCD20 subgroup, was relatively small. Moreover, Ab titer was available only in 67.8% of patients and was measured at different time points. However, the time elapsed from the vaccination and Ab measurement was included as a possible confounder in the multivariable models. Besides, different methods were used to measure Ab titer (CLIA, CMIA, ELISA). This could represent a source of variability; however, previous studies [36, 37] and one meta-analysis [38] demonstrated moderate to perfect agreement, excellent sensitivity, and high specificity in detecting serological response against SARS-CoV-2 after infection for the abovementioned IgG assays. However, all immunogenicity results were reported according to World Health Organization International Standard [27]. Conversely, one study on a small number of SARS-CoV-2-naive individuals found good correlation, but not interchangeability, between five different anti-SARS-CoV-2 antibody assays after vaccination, even when converted to BAU/mL [39]. Finally, data on T-cell response and lymphocyte count were not available.

Despite the abovementioned limitations, our study confirms the efficacy and safety of anti-SARS-CoV-2 vaccines in MS patients in a real-world observational cohort. Moreover, it is the first suggesting a possible association between hypogammaglobulinemia and response to the COVID-19 vaccines in patients treated with either rituximab or ocrelizumab. Future multicenter studies with larger cohorts are advisable, in order to detect, on the one hand, factors associated with Ab response in this subgroup of patients and in other at-risk populations (such as lymphopenic patients treated with other DMT) and, on the other hand, to clarify the actual protective role of T-cell response. This information can help the clinician in identifying patients who could benefit from booster/adjunctive vaccine and close monitoring during the ongoing COVID-19 pandemic.

Author contribution All authors contributed to the study conception and design. Material preparation and data collection were performed by AB and MGA. The first draft of the manuscript was written by AB and MGA, and all authors commented on previous versions of the manuscript. Statistical analysis was performed by AB. All authors read and approved the final manuscript.

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Declarations

Ethical approval The study has been approved by the Ethical committe of Area Vasta Centro. All the patients signed an informed consent.

Conflict of interest None.

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