



Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.

Contents lists available at [ScienceDirect](https://www.sciencedirect.com)

Multiple Sclerosis and Related Disorders

journal homepage: www.elsevier.com/locate/msard

Original article



Six-month humoral response to mRNA SARS-CoV-2 vaccination in patients with multiple sclerosis treated with ocrelizumab and fingolimod

Rocco Capuano^a, Alvino Biseco^a, Miriana Conte^a, Giovanna Donnarumma^b, Manuela Altieri^a, Elena Grimaldi^b, Gianluigi Franci^c, Annalisa Chianese^b, Massimiliano Galdiero^b, Nicola Coppola^d, Gioacchino Tedeschi^a, Antonio Gallo^{a,*}

^a Department of Advanced Medical and Surgical Sciences, University of Campania "Luigi Vanvitelli", Naples, Italy

^b Department of Experimental Medicine, University of Campania "Luigi Vanvitelli", Naples, Italy

^c Department of Medicine, Surgery and Dentistry "Scuola Medica Salernitana", University of Salerno, 84081 Baronissi, Italy

^d Department of Mental Health and Public Medicine, University of Campania "Luigi Vanvitelli", Naples, Italy

ARTICLE INFO

Keywords:

Multiple sclerosis
Covid-19
SARS-CoV-2 mRNA vaccine
Ocrelizumab
Fingolimod
Long term humoral response

ABSTRACT

Introduction: Real-world clinical data suggest an attenuated short-term humoral response to SARS-CoV-2 vaccines in patients with multiple sclerosis (pwMS) receiving high efficacy (HE) disease modifying therapies (DMTs) such as Ocrelizumab (OCR) and Fingolimod (FNG). Long-term humoral response in pwMS treated with these HE-DMTs has been poorly investigated. The aim of our study was to explore: i) the humoral response up to six months after a full cycle of the BNT162b2 mRNA Covid-19 vaccine in pwMS treated with OCR and FNG and to compare it to age- and sex-matched healthy controls (HCs); ii) the relationship between humoral response and clinical and immunological characteristics of the studied population.

Methods: Serum samples were collected from HCs and pwMS treated with OCR or FNG at the following time points: before BNT162b2 mRNA Covid-19 vaccine (T0), and 4 (T1), 8 (T2), 16 (T3) and 24 (T4) weeks after the first dose.

Sera were stored at -20°C and tested for the quantitative detection of IgG antibodies to SARS-CoV-2 trimeric spike protein (Anti-TSP IgG) expressed in binding antibody units (BAU). At T1 neutralizing antibodies (Nabs) titres were assessed. The relationship between Anti-TSP IgG at each time-point and clinical and laboratoristic analyses were analysed by the Spearman correlation coefficient.

Results: 47 HCs and 50 pwMS (28 on OCR and 22 on FNG) were included in the study. All HCs mounted a positive humoral response at T1 and preserved it up to six months. At T1 only 57.1% pwMS on OCR ($p < 0.001$ compared with HCs) and 40.9% on FNG ($p < 0.001$) had a positive humoral response at T1, with only 39.3% and 27.3% maintaining a positive response at sixth months (T4), respectively. A strong positive correlation was observed between Nabs titres and Anti-TSP IgG at T1 ($\rho = 0.87$, $p < 0.0001$) with Nabs titres significantly higher in HCs compared with pwMS on OCR and FNG ($p < 0.0001$). We also found a strong positive correlation between time-window since last OCR infusion and anti-TSP IgG titres at all time-points (T1 $\rho = 0.58$, $p = 0.001$; T2 $\rho = 0.59$, $p = 0.001$; T3 $\rho = 0.53$, $p = 0.004$; T4 $\rho = 0.47$, $p = 0.01$). In the FNG group we observed a significant correlation between the humoral response measured from T1 to T4 and: i) treatment duration (T1: $\rho = -0.65$, $p = 0.001$; T2: $\rho = -0.8$, $p < 0.001$; T3: $\rho = -0.72$, $p < 0.001$; T4: $\rho = -0.67$, $p < 0.001$), ii) disease duration (T1: $\rho = -0.5$, $p = 0.017$; T2: $\rho = -0.6$, $p = 0.003$; T3: $\rho = -0.58$, $p = 0.005$; T4: $\rho = -0.57$, $p = 0.006$), and iii) baseline total lymphocyte count (T1: $\rho = 0.37$, $p = 0.08$; T2: $\rho = 0.45$, $p = 0.03$; T3: $\rho = 0.43$, $p = 0.04$; T4: $\rho = 0.45$, $p = 0.03$).

Conclusions: Our long-term data show a weakened and short-lasting humoral response to SARS-CoV-2 mRNA vaccine in pwMS treated with OCR and FNG when compared with HCs. MS neurologists should take into account the time elapsed since the last infusion for pwMS on OCR, and the lymphocyte count as well as the disease and

Abbreviations: PWMS, Patients with multiple sclerosis; HE-DMTS, High efficacy disease modifying therapies; OCR, Ocrelizumab; FNG, Fingolimod; Anti-TSP IgG, IgG antibodies to SARS-CoV-2 spike protein; NAb, neutralizing antibodies.

* Corresponding author.

E-mail addresses: rcapu@hotmail.it (R. Capuano), alvino.biseco@unicampania.it (A. Biseco), giovanna.donnarumma@unicampania.it (G. Donnarumma), elena.grimaldi@unicampania.it (E. Grimaldi), gfranci@unisa.it (G. Franci), massimiliano.galdiero@unicampania.it (M. Galdiero), nicola.coppola@unicampania.it (N. Coppola), gioacchino.tedeschi@unicampania.it (G. Tedeschi), antonio.gallo@unicampania.it (A. Gallo).

<https://doi.org/10.1016/j.msard.2022.103724>

Received 13 January 2022; Received in revised form 23 February 2022; Accepted 3 March 2022

Available online 4 March 2022

2211-0348/© 2022 Elsevier B.V. All rights reserved.

treatment duration for those on FNG when called to counsel such pwMS regarding the vaccination with the SARS-CoV-2 mRNA vaccine.

1. Introduction

The two approved Covid-19 mRNA vaccines have proven a 95% efficacy in preventing moderate-to-severe Covid-19 in healthy subjects. (Polack et al., 2020; Baden et al., 2020) However, immunocompromised patients have not been included in clinical trials and real-world clinical data pointed to an attenuated immune response to SARS-CoV-2 vaccines in patients with multiple sclerosis (pwMS) receiving some immunosuppressive disease modifying therapies (DMTs). (Brill et al., 2021; Apostolidis et al., 2021; Disanto et al., 2021; A Achiron et al., 2021; Gallo et al., Sep; Capuano et al., 2021)

PwMS treated with platform (i.e. Glatiramer acetate, Interferons, Teriflunomide, Dimethyl fumarate) as well as high-efficacy (HE) DMTs, such as Alemtuzumab, Cladribine and Natalizumab, seem to preserve the humoral response to SARS-CoV-2 mRNA vaccines. (Capuano et al., 2021; Sormani et al., 2021).

On the contrary, a blunted humoral response to these vaccines has been reported in pwMS treated with two HE-DMTs such as Ocrelizumab (OCR), a B-cell-depleting anti-CD20 monoclonal antibodies, and Fingolimod (FNG), a sphingosine 1-phosphate receptor modulator. (A Achiron et al., 2021; Gallo et al., Sep; Sormani et al., 2021; A Achiron et al., 2021; Guerrieri et al., 2021) These latter results are in line with previous evidences showing an attenuated humoral response to other vaccines in pwMS treated with OCR and FNG. (Bar-Or et al., 2020; Kappos et al., 2015) The mechanism of action of these drugs – i.e. B-cell depletion (OCR) and inhibition of germinal center reaction (FNG) – has been proposed to explain these findings.

As regards the SARS-CoV-2 mRNA vaccines, studies conducted so far (A Achiron et al., 2021; Gallo et al., Sep; Guerrieri et al., 2021) have just focused on the evaluation of the short-term (up to one month after the second vaccine dose) humoral response, while the long-term humoral response has not been yet investigated in OCR/FNG-treated pwMS. (A Achiron et al., 2021) The evaluation of the long-term humoral response in pwMS treated with OCR and FNG might be very useful to inform clinicians on how to counsel about mRNA Covid-19 vaccine timing and booster doses.

On this background, we therefore aimed to investigate: i) the longitudinal humoral response to the BNT162b2 mRNA Covid-19 vaccine up to six months from the first dose in pwMS on OCR or FNG, comparing it with age- and sex-matched healthy controls (HCs), and ii) the relationship between longitudinal humoral response and routine clinical and immunological data in the studied population.

2. Methods

We conducted an observational prospective study at the Multiple Sclerosis Center of the I Neurologic Clinic of the University of Campania “Luigi Vanvitelli”.

Serum samples were collected from HCs and pwMS at the following scheduled time-points with respect to vaccination with BNT162b2 mRNA Covid-19 vaccine: baseline (T0; before the first dose), and 4 (T1), 8 (T2), 16 (T3), 24 (T4) weeks after the first dose.

Exclusion criteria were: i) age <18 years; ii) history of Covid-19 before the vaccine or Covid-19 infection during the follow-up, iii) positive SARS-CoV-2 IgG antibodies at T0, iv) steroid administration within the month before the first dose of vaccine, v) relevant comorbidity.

Sera were stored at -20°C and tested at the virology lab of our University Hospital, for the detection of i) IgG titers against SARS-CoV-2 spike protein (Anti-TSP IgG), using the LIAISON® SARS-CoV-2 Trimeric-IgG assay (DiaSorin-S.p.A.), at all time-points (T0-T4), (Bonelli et al., 2021) and ii) neutralizing antibodies (NAbs) titers at T1, using a

96-well cell culture microplates as previously reported. (Stelitano et al., 2021)

The IgG titres were expressed as binding antibody units (BAU), with 33.8 BAU/mL as negative/positive cut-off value. (Kristiansen et al., 2021) Anti-TSP IgG titres above 2080 BAU/mL (maximum allowed limit) were 1:10 diluted to obtain a value in the detectable range. The local Ethics Committee (named “Comitato Etico Università degli Studi della Campania Luigi Vanvitelli – Azienda Ospedaliera Universitaria Luigi Vanvitelli – AORN Ospedali dei Colli) approved the study that was performed in accordance with the principles of Helsinki Declaration (approval code: 0,015,914).

All analyses were performed using STATA v14. Absolute numbers and percentage were used to describe categorical variables, means and standard deviations (SD) and medians and interquartile ranges (IQR) were used for continuous variables. Data distribution was assessed using the Shapiro-Wilk test, and non parametric tests were chosen. The sociodemographic and clinical variables were compared between groups using the Kruskal-Wallis or Fisher Exact Test as appropriate. Correlations between Anti-TSP IgG titers and clinical and laboratory data at each time-point were analysed by the Spearman correlation coefficient. A sensitivity analysis was performed by removing pwMS with a progressive form of MS.

3. Results

Among 139 pwMS treated at our MS Center and under serological anti-SARS-CoV-2 monitoring, we selected those on OCR ($n = 32$) and FNG ($n = 27$). As control group, we used 52 age- and sex-matched HCs enrolled in a surveillance program at our Clinic.

Forty-seven HCs, 28 pwMS on OCR and 22 on FNG were included in the final analysis: 6 were excluded because of Anti-TSP IgG positivity (1 HCs, 2 OCR and 3 FNG) attributable to asymptomatic Covid-19 infection and 6 were excluded due to Covid-19 infection during the follow-up (4 HCs, 2 OCR and 2 FNG).

All subjects received two vaccine doses of BNT162b2. All pwMS on FNG were relapsing-remitting (RR) MS; as regards the sample of pwMS on OCR, 18 were RRMS and 10 were progressive MS.

Socio-demographic and clinical characteristics of HCs and pwMS are reported in Table 1.

There were no differences between OCR and FNG groups regarding the age, sex, disease duration, and disability measured by the Expanded Disability Status Scale (EDSS), Anti-TSP IgG (BAU/mL) at T0. The only difference found at T0 was the longer treatment duration of pwMS on FNG ($p = 0.003$).

As shown in Table 2, all HCs mounted a positive (>33.8 BAU/mL) humoral response at T1 and preserved it up to six-month. On the other hand, only 16 (57.1%) pwMS on OCR ($p < 0.001$ compared with HCs) and 9 (40.9%) on FNG ($p < 0.001$ compared with HCs) reached positivity at T1 and only 11 (39.3%) and 6 (27.3%), respectively, maintained it at sixth-month follow-up (T4). Notably, 2 pwMS on OCR and 2 on FNG that had a negative Anti-TSP IgG titre at T1, became (weakly) positive at T2. As regards the comparison between pwMS on OCR and those on FNG, no significant differences were observed in the positivity rate at any time-point (Table 2).

Quantitative analysis of Anti-TSP IgG titers showed significant higher titres in HCs compared with those of pwMS on OCR and on FNG at all time-points (Table 3). No differences were found at all time-points between pwMS on OCR and those on FNG (Table 3). At T1 NAbs titres were significantly higher in HCs compared with pwMS on OCR and FNG, while no difference was found between pwMS on OCR and those on FNG. Moreover, when we tested the association between Anti-TSP IgG

Table 1
Socio-demographic and clinical characteristics of HCs and pwMS.

	HCs (47)	pwMS on OCR (28)	pwMS on FNG (22)	p
Age [years] mean (SD)	41.4 (13.2)	41 (10.4)	43.8 (13.4)	NS
Female sex- n (%)	28 (59.6)	13 (46.4)	10 (45.5)	NS
Disease duration [months] – mean, median (SD; IQR)	–	123.1, 130.7 (72.3; 60–176)	147.25, 122 (102.4;76.4–190)	NS
EDSS – median (IQR)	–	4 (1.5–5.5)	2.5 (1–4)	NS
Treatment duration [months] – mean, median (SD; IQR)	–	21.9, 24 (9.9; 15.5–28.3)	54.8, 64.1 (37.8; 15.7–81.9)	p = 0.003
Time elapsed between last OCR infusion and vaccination [months] – mean, median (SD; IQR)	–	6, 5.6 (2.5; 3.5–8.3)	–	–
Total CD20 lymphocyte within 30 days before vaccination [cells/ mL] – mean, median (SD; IQR)	–	27.1, 8 (69; 0–15.5)**	–	–
Serum IgG [mg/dL] – mean, median (SD; IQR)	–	873.4, 845*** (207; 690–1074)	–	–
Total lymphocyte within 30 days before vaccination [cells/mL] – mean, median (SD; IQR)	–	–	833.5, 767.5 (468.7; 560–1177)	–

Abbreviations: HCs: healthy controls; pwMS: people with Multiple Sclerosis; OCR: ocrelizumab; FNG: fingolimod; SD: standard deviation; IQR: interquartile range; EDSS: Expanded Disability Status Scores; Anti-TSP IgG: anti-trimeric spike protein specific immunoglobulin G; BAU/mL: Binding Arbitrary Unit per ml; NS = not significant.

** Normal range values 90–660 cell/mL.

*** Normal range values 700–1600 mg/dL.

titres and NABs at T1, we observed a strong and consistent positive correlation ($\rho = 0.87$, $p < 0.0001$ for all subjects; $\rho = 0.8$, $p < 0.001$ for HCs; $\rho = 0.65$, $p < 0.001$ for pwMS on OCR; $\rho = 0.62$, $p = 0.002$ for pwMS on FNG).

At all time-points, we observed a positive correlation between Anti-TSP IgG titres and the time elapsed since last OCR infusion at baseline (T1 $\rho = 0.58$, $p = 0.001$; T2 $\rho = 0.59$, $p = 0.001$; T3 $\rho = 0.53$, $p = 0.004$; T4 $\rho = 0.47$, $p = 0.01$). In the same group of patients, we did not find any association with serum IgG levels and CD20 B-cell counts at baseline, as well as with disease duration, disability and treatment duration.

For pwMS on FNG, we observed a significant and consistent negative correlation between Anti-TSP IgG titres and disease duration (T1: $\rho = -0.5$, $p = 0.017$; T2: $\rho = -0.6$, $p = 0.003$; T3: $\rho = -0.58$, $p = 0.005$; T4: $\rho = -0.57$, $p = 0.006$), and treatment duration (T1: $\rho = -0.65$, $p = 0.001$; T2: $\rho = -0.8$, $p < 0.001$; T3: $\rho = -0.72$, $p < 0.001$; T4: $\rho = -0.67$, $p < 0.001$). A positive correlation between Anti-TSP IgG titres and total lymphocyte count measured within 30 days from baseline (T1: $\rho = 0.37$, $p = 0.08$; T2: $\rho = 0.45$, $p = 0.03$; T3: $\rho = 0.43$, $p = 0.04$; T4: $\rho = 0.45$, $p = 0.03$) was also found in the FNG group.

The sensitivity analysis applied did not show different results for pwMS with relapsing or progressive form of MS.

Mild to moderate vaccine adverse reactions were commonly reported in HCs and pwMS, with rates expected in the general population; (Polack et al., 2020) no serious or unexpected local and/or systemic side effects were observed in both groups.

Table 2
Anti-TSP IgG > 33.8 BAU/mL at different time-points.

	HCs (47)	pwMS on OCR (28)	pwMS on FNG (22)	p
T1 (4 weeks after first vaccine dose) Number (%)	47 (100)	16 (57.1)	9 (40.9)	* $p <$ 0.001 ** $p <$ 0.001 *** $p =$ 0.2
T2 (8 weeks after first vaccine dose) Number (%)	47 (100)	18 (64.3)	11 (50)	* $p <$ 0.001 ** $p <$ 0.001 *** $p =$ 0.23
T3 (16 weeks after first vaccine dose) Number (%)	47 (100)	15 (53.6)	10 (45.4)	* $p <$ 0.001 ** $p <$ 0.001 *** $p =$ 0.39
T4 (24 weeks after first vaccine dose) Number (%)	47 (100)	11 (39.3)	6 (27.3)	* $p <$ 0.001 ** $p <$ 0.001 *** $p =$ 0.27

Abbreviations: HCs: healthy controls; pwMS: people with Multiple Sclerosis; OCR: ocrelizumab; FNG: fingolimod; Anti-TSP IgG: anti-trimeric spike protein specific immunoglobulin G; BAU/mL: Binding Arbitrary Unit per mL; Comparisons were performed by means of the Fisher Exact Test. Significant values are reported in bold.

* Comparison between HCs and pwMS on OCR;

** Comparison between HCs and pwMS on FNG;

*** Comparison between pwMS on OCR and pwMS on FNG.

4. Discussion

In the present study, we corroborated and extended previous findings on the humoral response to BNT162b2 mRNA Covid-19 vaccine in pwMS treated with HE-DMTs. In particular, we explored i) the longitudinal humoral response up to six months after the first vaccine dose in pwMS treated with OCR and FNG and compared it to age- and sex-matched HCs, and ii) the clinical and demographic factors predicting/influencing the short and the long-term humoral response.

As regards the first objective, our data showed that, while in all HCs Anti-TSP IgG titres converted to positivity at T1 and remained so during the six-month follow-up, only 57.1% of pwMS on OCR and 40.9% of those on FNG became positive at T1. Moreover, a subsequent further decline was observed in pwMS on OCR and FNG, such that only 39.3% and 27.3%, respectively, remained positive after 6 months.

These results, beyond confirming a blunted short-term humoral response to BNT162b2 mRNA Covid-19 vaccine in pwMS treated with OCR and FNG (Disanto et al., 2021; A Achiron et al., 2021; Sormani et al., 2021; Guerrieri et al., 2021), show that a higher rate of Anti-TSP IgG titres negativization occurs in these pwMS.

Interestingly, we observed that 2 pwMS treated with OCR and 2 with FNG that were negative at T1 became positive at T2 (no pwMS became positive after T2). These findings show that the rise of humoral response can be slower, more than just reduced, at least in some OCR- and FTY-treated pwMS.

As regard the quantitative analysis, the anti-TSP IgG titers of OCR- and FNG-treated pwMS were significantly lower at all time-points when compared with HCs; contrariwise, we did not find any differences - at any time-point - between pwMS on OCR and those on FNG.

To summarize, our short-term results are in line with recent studies showing a weakened humoral response in pwMS treated with anti-CD20 and S1P receptor-modulator drugs. (Disanto et al., 2021; A Achiron et al., 2021; Sormani et al., 2021; Guerrieri et al., 2021)

Moving to the long-term humoral response after BNT162b2 mRNA Covid-19 vaccine, this is the first study - to the best of our knowledge - that investigated a cohort of pwMS longitudinally, with multiple samples collected at different time-points, up to six months. A previous cross-sectional study highlighted a reduced humoral response in pwMS treated with OCR and FNG after a median time of 70 (IQR 80.5–94.3) and 76 days (75.7–100.4), respectively (A Achiron et al., 2021). Our results, therefore, extend and complement this study.

As regards the second objective of our study, we found a positive association between time since last OCR infusion and anti-TSP IgG titres at all time-points. This finding, in agreement with previous evidences, (Sormani et al., 2021; A Achiron et al., 2021) suggests that a stronger humoral response can be achieved distancing the vaccine as far as possible from last OCR infusion. This consistent observation translates in clinical practice into a complex trade-off between the risk of MS reactivation, associated with a postponement of OCR administration, and the risk of Covid-19 associated with a postponement of the vaccination.

In the FNG-treated group, instead, we observed - for the first time - a strong and consistent negative association between humoral response and treatment and disease duration. Phenotypical immune changes similar to immunosenescence seen in older adults (e.g. reduction in absolute count of CD4 with a CD4/CD8 ratio reversal of 1:2 and decrease in the recent thymic emigrant T cells) have been demonstrated in pwMS with longer disease duration and in those with long-term exposition to FNG. (Dema et al., 2021; Schwanzitz et al., 2016) These major changes of the immune system might explain the inverse correlation found between humoral response to BNT162b2 mRNA Covid-19 vaccine and longer disease duration as well as longer exposition to FNG. We also found a moderate positive association between total lymphocyte count before vaccination and anti-TSP IgG titres at different time-points in FNG-treated pwMS. These data, which confirm and expand the results of a previous study (A Achiron et al., 2021), deserve further verification in order to be implemented in clinical decisions regarding the use of vaccines in pwMS treated with FNG. Considering the blunted humoral and cellular response (A Achiron et al., 2021) to BNT162b2 mRNA Covid-19

vaccine in FNG-treated pwMS, a booster vaccine dose should be strongly recommended in these patients, particularly in those with longer disease duration, longer drug exposition and lower absolute lymphocyte count.

Given the assessment of Nabs titers, we could also explore the relationship between Anti-TSP IgG and Nab titres at T1, finding a strong correlation in the whole studied population as well as in each subgroup (HCs, FNG-pwMS, OCR-pwMS). This results further supports the use of validated Anti-TSP IgG commercial tests (<https://www.fda.gov>) - when it becomes necessary to evaluate the humoral response to Covid-19 vaccines in clinical practice - in place of dosing NAb titers that require dedicated and highly-equipped labs, as well as costly and time-consuming procedures.

In conclusion, our results confirm and expand the growing evidence that pwMS on treatment with OCR and FNG mount a weakened and transient humoral response to mRNA vaccine against Covid-19. Clinicians should differently counsel pwMS regarding the mRNA Covid-19 vaccination based on which HE-DMT they are assuming between OCR and FNG. For pwMS on OCR, they should primarily take into account time elapsed since the last OCR infusion. For pwMS on FNG, they should consider lymphocyte count, disease duration and treatment exposition. Bearing in mind the available data on a booster dose in immunosuppressed solid-organ transplant recipients, (Kamar et al., 2021) our results strongly support the recommendation of a booster dose in pwMS on OCR and FNG, as already stated by current Italian National Health System Covid-19 vaccination guidelines since October 2021.

Finally, since we still do not know if a blunted humoral response is associated with a lower protection against SARS-CoV-2 infection or severe Covid-19, the MS community should urgently gather data on SARS-CoV-2 infection and Covid-19 outcomes in vaccinated pwMS on OCR and FNG.

This study is not exempt from limitations. Firstly, since the Italian National Health System (NHS) guidelines gave indication that MS patients had to be vaccinated with mRNA vaccines, this study has no data on other SARS-CoV-2 vaccines. Secondly, we did not assess the B and T cell response to mRNA vaccine. The cellular immune response is a key

Table 3
Anti-TSP IgG BAU/mL at different time-points and neutralizing antibodies at T2.

	HC (47)	pwMS on OCR (28)	pwMS on FNG (22)	p
Serum Anti-TSP IgG (BAU/mL) titre before vaccination – mean, median (SD; IQR)	4.93, 4.81 (0.7; 4.81–4.81)	5.6, 4.81 (3.5; 4.81–4.81)	4.81, 4.81 (0; 4.81–4.81)	NS
Serum Anti-TSP IgG (BAU/mL) titre 4 weeks after first vaccine dose (T1) – mean, median (SD; IQR)	3627, 1810 (3790; 1130–5560)	412, 45.75 (681; 4.81–598)	110, 22.15 (196; 5.5–116)	[^] p < 0.001 [^] p < 0.001 ^{^^} p = 0.7
Serum NAbs titre 4 weeks after first vaccine dose (T2)– median (1–25–75–99 percentiles)	1:40 (0–1:40–1:160–1:640)	0 (0–0–1:10–1:640)	0 (0–0–0–1:10)	[^] p < 0.001 [^] p < 0.001 ^{^^} p = 0.3
Serum Anti-TSP IgG (BAU/mL) titer 8 weeks after first vaccine dose (T2)– mean, median (SD; IQR)	3044, 2460 (2201; 1480–4300)	381.7, 141.5 (565.6; 5.3–469)	65.5, 31.75 (81.8; 14–84)	[^] p < 0.001 [^] p < 0.001 ^{^^} p = 0.2
Serum Anti-TSP IgG (BAU/mL) titer 16 weeks after first vaccine dose (T3) – mean, median (SD; IQR)	1162, 1050 (711; 614–1670)	162.2, 46.4 (258.6; 4.81–238)	45.4, 22.1 (59; 14–47.4)	[^] p < 0.001 [^] p < 0.001 ^{^^} p = 0.5
Serum Anti-TSP IgG (BAU/mL) titre 24 weeks after first vaccine dose (T4) – mean, median (SD; IQR)	684, 568 (446; 331–986)	95.6, 19.1 (181.3; 5.43–100)	34.3, 17.45 (43.1; 9–37.9)	[^] p < 0.001 [^] p < 0.001 ^{^^} p = 0.7

Abbreviations: Anti-TSP IgG: anti-trimeric spike protein specific immunoglobulin G; BAU/mL: Binding Arbitrary Unit per ml; Nabs: neutralizing antibodies; SD: standard deviation; IQR interquartile range.

[^] Comparison between HCs and pwMS on OCR; ^{^^} Comparison between HCs and pwMS on FNG; ^{^^^} Comparison between pwMS on OCR and pwMS on FNG. Comparisons were performed by means of the Kruskal-Wallis with post-hoc analysis. Significant values are reported in bold.

player in the active protection from severe Covid-19; in recent studies on pwMS with a blunted humoral response to SARS-CoV-2 mRNA vaccines due to anti-CD20 and FNG therapy, the authors were able to show an adequate (comparable with HCs) T-cell response only in pwMS treated with OCR (Brill et al., 2021; Apostolidis et al., 2021), but not in those treated with FNG. (A Achiron et al., 2021) Finally, we tested NAb titers only at T1 because of budget limitations; a follow-up assessment of NAb would have helped to further characterize the humoral response in OCR- and FNG-treated pwMS.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Institutional review board statement

The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Local Ethics Committee (approval code: 0,015,914).

Informed consent statement

Informed consent was obtained from all subjects involved in the study.

Author disclosures

Rocco Capuano, Miriana Conte, Giovanna Donnarumma, Manuela Altieri, Elena Grimaldi, Gianluigi Franci, Annalisa Chianese, Nicola Coppola, Massimiliano Galdiero have no disclosures; Alvino Bisecco, Gioacchino Tedeschi and Antonio Gallo– received speaker's honoraria and/or compensation for consulting service and/or speaking activities from Biogen, Roche, Merck, Novartis, Celgene and Genzyme

CRedit authorship contribution statement

Rocco Capuano: Formal analysis, Investigation, Methodology, Visualization, Writing – original draft, Validation, Writing – review & editing. **Alvino Bisecco:** Investigation, Writing – review & editing, Validation. **Miriana Conte:** Investigation, Writing – review & editing, Validation. **Giovanna Donnarumma:** Methodology, Validation, Writing – review & editing. **Manuela Altieri:** Writing – review & editing, Validation. **Elena Grimaldi:** Conceptualization, Validation. **Gianluigi Franci:** Methodology, Validation, Writing – review & editing. **Annalisa Chianese:** Conceptualization, Validation. **Massimiliano Galdiero:** Methodology, Validation, Writing – review & editing. **Nicola Coppola:** Methodology, Validation, Writing – review & editing. **Gioacchino Tedeschi:** Conceptualization, Project administration, Writing – review & editing, Validation. **Antonio Gallo:** Conceptualization, Project administration, Writing – review & editing, Validation.

Declaration of Competing Interest

Rocco Capuano, Miriana Conte, Giovanna Donnarumma, Manuela Altieri, Elena Grimaldi, Gianluigi Franci, Annalisa Chianese, Nicola

Coppola, Massimiliano Galdiero have no disclosures; Alvino Bisecco, Gioacchino Tedeschi and Antonio Gallo– received speaker's honoraria and/or compensation for consulting service and/or speaking activities from Biogen, Roche, Merck, Novartis, Celgene and Genzyme

Acknowledgements

Gabriella Andreone, Federica Giuliano, Salvatore Abbadessa, Carolina Vitulano, Federica Matrone, Mario Risi, Riccardo Borgo, Pasquale Sozio for helping in data acquisition and analysis.

References

- Achiron, A., Mandel, M., Dreyer-Alster, S., et al., 2021a. Humoral immune response to COVID-19 mRNA vaccine in patients with multiple sclerosis treated with high-efficacy disease-modifying therapies. *Ther. Adv. Neurol. Disord.* 14, 175628642110128.
- Achiron, A., Mandel, M., Dreyer-Alster, S., et al., 2021b. Humoral immune response in multiple sclerosis patients following PfizerBNT162b2 COVID19 vaccination: up to 6 months cross-sectional study. *J. Neuroimmunol.* 361, 577746.
- Apostolidis, S.A., Kakara, M., Painter, M.M., et al., 2021. Cellular and humoral immune responses following SARS-CoV-2 mRNA vaccination in patients with multiple sclerosis on anti-CD20 therapy. *Nat. Med.* 2021, 1–12.
- Baden L.R., Sahly HM El, Essink B., et al. Efficacy and Safety of the mRNA-1273 SARS-CoV-2 Vaccine. <https://doi.org/10.1056/NEJMoa2035389> 2020; 384: 403–416.
- Bar-Or, A., Calkwood, J.C., Chognot, C., et al., 2020. Effect of ocrelizumab on vaccine responses in patients with multiple sclerosis: the VELOCE study. *Neurology* 95, e1999–e2008.
- Bonelli, F., Blocki, F.A., Bunnell, T., et al., 2021. Evaluation of the automated LIAISON® SARS-CoV-2 Trimeric IgG assay for the detection of circulating antibodies. *Clin. Chem. Lab. Med.* <https://doi.org/10.1515/cclm-2021-0023>.
- Brill, L., Rechtman, A., Zveik, O., et al., 2021. Humoral and T-cell response to SARS-CoV-2 vaccination in patients with multiple sclerosis treated with ocrelizumab. *JAMA Neurol.* <https://doi.org/10.1001/JAMANEURO.2021.3599>.
- Capuano R., Donnarumma G., Bisecco A., et al. Humoral response to SARS-CoV-2 mRNA vaccine in patients with multiple sclerosis treated with natalizumab. Epub ahead of print 13 August 2021. DOI: [10.1177/17562864211038111](https://doi.org/10.1177/17562864211038111).
- Dema, M., Eixarch, H., Villar, L.M., et al., 2021. Immunosenescence in multiple sclerosis: the identification of new therapeutic targets. *Autoimmun. Rev.* 20 <https://doi.org/10.1016/J.AUTREV.2021.102893>.
- Disanto, G., Sacco, r., bernasconi, e., et al., 2021. association of Disease-Modifying Treatment and Anti-CD20 infusion timing with humoral response to 2 SARS-CoV-2 vaccines in patients with multiple sclerosis. *JAMA Neurol.* <https://doi.org/10.1001/JAMANEURO.2021.3609>.
- Gallo, A., Capuano, R., Donnarumma, G., et al., 2021. Preliminary evidence of blunted humoral response to SARS-CoV-2 mRNA vaccine in multiple sclerosis patients treated with ocrelizumab. *Neurol Sci* 42 (9), 3523–3526. <https://doi.org/10.1007/s10072-021-05397-7>.
- Guerrieri, Lazzarin, Zanetta, et al., 2021. Serological response to SARS-CoV-2 vaccination in multiple sclerosis patients treated with fingolimod or ocrelizumab: an initial real-life experience. *J. Neurol.* <https://doi.org/10.1007/S00415-021-10663-X>.
- Kamar, N., Abravanel, F., Marion, O., et al., 2021. Three doses of an mRNA Covid-19 vaccine in solid-organ transplant recipients. *N. Engl. J. Med.* 385, 661–662.
- Kappos, L., Mehling, M., Arroyo, R., et al., 2015. Randomized trial of vaccination in fingolimod-treated patients with multiple sclerosis. *Neurology* 84, 872–879.
- Kristiansen, P.A., Page, M., Bernasconi, V., et al., 2021. WHO international standard for anti-SARS-CoV-2 immunoglobulin. *Lancet* 397, 1347–1348.
- Polack, F.P., Thomas, S.J., Kitchin, N., et al., 2020. Safety and efficacy of the BNT162b2 mRNA Covid-19 vaccine. *N. Engl. J. Med.* 383, 2603–2615.
- Schwanitz, N., Boldt, A., Stoppe, M., et al., 2016. Longterm fingolimod treatment of multiple sclerosis induces phenotypical immunosenescence. *Neurology* 86 (16 Supplement).
- Sormani, M.P., Inglese, M., Schiavetti, I., et al., 2021. Effect of SARS-CoV-2 mRNA vaccination in MS patients treated with disease modifying therapies. *EBioMedicine* 0, 103581.
- Stelitano, D., Weisberg, S.P., Goldklang, M.P., et al., 2021. Rapid and flexible platform to assess anti-SARS-CoV-2 antibody neutralization and spike protein-specific antivirals. *mSphere* 6. <https://doi.org/10.1128/MSPHERE.00571-21>.