




Original research

Anti-CD20 therapies decrease humoral immune response to SARS-CoV-2 in patients with multiple sclerosis or neuromyelitis optica spectrum disorders

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ABSTRACT

Background SARS-CoV-2 seroconversion rate after COVID-19 may be influenced by disease-modifying therapies (DMTs) in patients with multiple sclerosis (MS) or neuromyelitis optica spectrum disorders (NMO-SD).

Objective To investigate the seroprevalence and the quantity of SARS-CoV-2 antibodies in a cohort of patients with MS or NMO-SD.

Methods Blood samples were collected in patients diagnosed with COVID-19 between 19 February 2020 and 26 February 2021. SARS-CoV-2 antibody positivity rates and Ig levels (anti-S IgG titre, anti-S IgA index, anti-N IgG index) were compared between DMTs groups. Multivariate logistic and linear regression models were used to estimate the influence of DMTs and other confounding variables on SARS-CoV-2 serological outcomes.

Results 119 patients (115 MS, 4 NMO, mean age: 43.0 years) were analysed. Overall, seroconversion rate was 80.6% within 5.0 (SD 3.4) months after infection. 20/21 (95.2%) patients without DMT and 66/77 (85.7%) patients on DMTs other than anti-CD20 had at least one SARS-CoV-2 Ig positivity, while this rate decreased to only 10/21 (47.6%) for patients on anti-CD20 ($p < 0.001$). Being on anti-CD20 was associated with a decreased odd of positive serology (OR, 0.07 (95% CI 0.01 to 0.69), $p = 0.02$) independently from time to COVID-19, total IgG level, age, sex and COVID-19 severity. Time between last anti-CD20 infusion and COVID-19 was longer (mean (SD), 3.7 (2.0) months) in seropositive patients compared with seronegative patients (mean (SD), 1.9 (1.5) months, $p = 0.04$).

Conclusions SARS-CoV-2 antibody response was decreased in patients with MS or NMO-SD treated with anti-CD20 therapies. Monitoring long-term risk of reinfection and specific vaccination strategies in this population may be warranted.

Trial registration number NCT04568707.

INTRODUCTION

Since the outbreak of COVID-19, national and international studies have analysed risk factors for COVID-19 severity in patients with multiple sclerosis (MS).^{1–3} Depending on the countries, around 70% of patients with MS receive disease-modifying

therapies (DMTs).^{4, 5} French COVISEP registry¹ identified that neurological disability, age and obesity were risk factors for COVID-19 severity, and most critically ill patients were not receiving any DMT. Italian MUSC² and North American COVIMS³ registries have identified that DMTs targeting B lymphocytes as well as corticosteroids are associated with an increased risk of severe COVID-19. Although the early stages of the immune response against SARS-CoV-2 primarily involve innate immunity followed by specific T and B cell adaptive immunity,^{6, 7} it is likely that B cells' immune response defect in patients treated with anti-CD20 results in impaired clearance of SARS-CoV-2, hence, a higher risk of severe and/or prolonged symptomatology.

As with any viral infection, the cellular and humoral immune responses are expected to be protective against reinfection. However, there is still very little data on the risk of COVID-19 recurrence, which seems rare but more common in elderly and immunocompromised patients.^{8, 9} A recent study performed in Wuhan demonstrated that more than 70% of patients who recovered from COVID-19 display antibodies recognising the SARS-CoV-2 receptor binding domain (RBD) of the spike (S) or the nucleocapsid (N) protein over a 6-month period follow-up.¹⁰ Moreover, IgG-S titres correlate with the capacity to neutralise SARS-CoV-2, the presence of neutralising antibodies being likely to help protect against a new infection. However, the level, functionality and waning of neutralising antibodies vary greatly among individuals,¹¹ which may prone some patients to become more vulnerable to reinfection in subsequent waves of COVID-19 outbreak.

Several case series have suggested that patients with inflammatory CNS disease on anti-CD20 had a decreased seroconversion rate compared with patients on other DMTs.^{12, 13} However, there is currently no systematic evaluation of seroconversion after COVID-19 in patients with inflammatory CNS disease treated with immunosuppressants and generally in immunocompromised population. In order to improve our knowledge of the impact of different DMTs on the immune response to SARS-COV2, we conducted a prospective study evaluating the seroprevalence and the

level of anti-S IgG, anti-S IgA and anti-N IgG in a cohort of patients with MS or neuromyelitis optica spectrum disorders (NMO-SD), receiving or not immunomodulatory or immunosuppressive therapy.

METHODS

Data collection

We conducted a monocentric prospective study to collect serum samples of patients with MS or NMO-SD who were included in the COVISEP registry (ClinicalTrials.gov NCT04355611), which aimed to determine the characteristics of COVID-19 in MS or NMO-SD.

All patients from the COVISEP registry and living in Paris area were contacted to perform a blood sampling for this study. Patients were informed about the objective of the study and gave written informed consent prior to their participation.

Serum sampling was scheduled as soon as possible but at least 21 days after onset of COVID-19 (inclusion), at 6 months and at 12 months follow-up. We present here the results of the cross-sectional analysis of all serum samples collected at inclusion.

The study protocol is registered on ClinicalTrials.gov.

All study data were deidentified and collected using an electronic Clinical Record Form on RedCap (<https://www.projectredcap.org/>). The study followed the Strengthening Reporting of Observational Studies in Epidemiology guidelines.

Population of interest

Inclusion criteria were: age ≥ 18 years, diagnosis of COVID-19 with at least one of the following criteria: (1) biologically confirmed COVID-19 diagnosis based on SARS-CoV-2 PCR positivity in nasopharyngeal swab, (2) typical thoracic CT abnormalities (ground glass opacities), (3) typical symptoms (asthenia, dyspnoea, cough, fever, anosmia/ageusia of sudden onset) in the context of a COVID-19 pandemic.

Definition of clinical endpoints

Demographics and clinical endpoint collection were previously described.¹ Briefly, beside MS or NMO-SD characteristics, we collected COVID-19 symptoms, diagnosis data and severity on an ordinal severity score ranging from 1 to 7¹⁴.

Biological analysis

Blood samples were centrifugated at 4°C, then 2 mL of serum were divided into 4 aliquots of 500 μ L and stored at -80°C until further testing of all samples collected for the study.

One aliquot was used for total immunoglobulin quantification (total IgG, IgA and IgM) using a Roche immunoassay kit (Ref 03507343190) on Cobas 8000 c502 analyser. The reference ranges for the three immunoglobulins are IgG (7–16 g/L), IgA (0.7–4 g/L), IgM (0.4–2.3 g/L).

A second aliquot was used for SARS-CoV-2 antibody assays. Serum IgG was measured using the Abbott Alinity instrument with the Abbott SARS-CoV-2 IgG assay. The assay is a chemiluminescent microparticle immunoassay for semiquantitative detection of IgG against nucleoprotein (N) and quantitative detection of IgG against spike (S) protein. Serum IgA against the S1 domain of the S protein was measured using enzyme-linked immunosorbent assays (anti-SARS-CoV-2 ELISA, EuroImmun). All immunoassays were used and interpreted according to the manufacturer's instructions. Positivity of anti-S IgG is defined by a titre $\geq 1.7 \log$ AU/mL, an anti-N IgG index ≥ 0.8 indicates a positive serology and an anti-S IgA ratio ≥ 1.1 indicates a positive serology.

Statistical analysis

Data analyses were performed in Python using Pandas package V.1.0.3, Scipy V.1.4.1 and Statsmodels V.0.11.1.

Descriptive statistics were performed on demographic and clinical variables. Given the mechanism of action targeting the same epitope (CD20), we grouped anti-CD20 therapies (ocrelizumab, rituximab and ofatumumab) for statistical analyses.

Seropositivity for the three tested anti-SARS-CoV-2 Igs was compared between groups of patients with several DMTs using χ^2 tests. Index of anti-S IgA, anti-N IgG, titre of anti-S IgG and levels of total IgG, IgA and IgM were compared between groups of patients with several DMTs using one-way analysis of variance (ANOVA) and subsequent t-test for one by one group comparisons if needed. Two-sided $p < 0.05$ was considered statistically significant.

In order to assess the interaction between anti-S IgG titre and delay from COVID-19 onset, we performed linear regression models using IgG titre as dependent variable and time as independent variable within groups of patients with different DMTs and a linear mixed-effect model with a 'time by DMT group' main effect.

A multivariate logistic regression model was performed to investigate the association between anti-SARS-CoV-2 serology positivity as defined by at least one positive immunoassay among the three tested Igs, and the following metrics as independent variables: DMT defined by a three-level variable (no DMT, DMT other than anti-CD20 and CD20), total IgG, age, sex, obesity (as defined by body mass index (BMI) $> 30 \text{ kg/m}^2$), time from COVID-19 onset and COVID-19 severity as defined by a two-level variable (no hospitalisation vs hospitalisation due to COVID-19).

Only anti-S IgG titre provides a quantitative measure of SARS-CoV-2 immune response as opposed to anti-N IgG and anti-S IgA index, which are semiquantitative measures. Therefore, we performed a multivariate linear regression model to quantify the association between anti-S IgG titre and the same above metrics as independent variables.

RESULTS

Study population

Among 334 patients from the COVISEP registry living in Paris area, 119 patients with MS ($n=115$) or NMO-SD ($n=4$) were included in this study. Patients were diagnosed with COVID-19 between 19 February 2020 and 26 February 2021, and blood samples were collected between 6 November 2020 and 1 April 2021.

All patients met the criteria for COVID-19 infection. When performed, SARS-CoV-2 PCR on nasopharyngeal swab was positive in 85/90 patients (94.4%) and negative in 5/90 patients (5.6%). The five patients with initial negative SARS-CoV-2 PCR had a positive SARS-CoV-2 serology performed as standard-of-care by their treating neurologist. All patients without initial SARS-CoV-2 PCR had typical COVID-19 symptoms (mainly anosmia in 23/29 patients).

Demographics and clinical characteristics

Demographics and clinical characteristics of the cohort are presented in [table 1](#). Most patients (112/119, 94.1%) had a benign course of COVID-19. Among the seven patients who needed hospitalisation, three needed supplemental oxygen and one patient needed high-flow oxygen device. Patients requiring hospitalisation for COVID-19 received the following DMTs: ocrelizumab (2), rituximab (1), dimethylfumarate (2), teriflunomide (1), no DMT (1).

Table 1 Demographic and disease characteristics of patients with multiple sclerosis or neuromyelitis optica spectrum disorder

Diagnosis	MS	NMO-SD
Number (n)	115	4
Demographics		
Age, mean (SD)	43.2 (11.4)	37.7 (13.5)
Sex ratio (F/M)	85/30	3/1
Disease duration, mean (SD)	12.5 (9.7)	7.4 (10.8)
Disease course (n)	98 RRMS/13 SPMS/4 PPMS	
EDSS, median (range)	2.0 (0.0–8.0)	2.75 (2.0–6.5)
DMTs		
No DMT	21 (18.3%)	–
Interferon beta	4 (3.5%)	–
Glatiramer	10 (8.7%)	–
Teriflunomide	17 (14.8%)	–
Dimethylfumarate	23 (20.0%)	–
Natalizumab	14 (12.2%)	–
Fingolimod	6 (5.2%)	–
Ocrelizumab	14 (12.2%)	–
Rituximab	4 (3.5%)	2 (50%)
Ofatumumab	–	1 (25%)
Other*	2 (1.7%)	1 (25%)
Comorbid conditions		
Cardiovascular disease	3 (2.6%)	0 (0.0%)
Pulmonary disease	4 (3.5%)	0 (0.0%)
Diabetes	2 (1.7%)	0 (0.0%)
Obesity (BMI >30 kg/m ²)	6 (5.2%)	0 (0.0%)
Current smoker	8 (7.0%)	0 (0.0%)
COVID-19 symptoms		
Asthenia, n (%)	79 (68.7%)	3 (75.0%)
Fever, n (%)	64 (55.7%)	3 (75.0%)
Cough, n (%)	51 (44.3%)	1 (25.0%)
Anosmia/agueusia, n (%)	61 (53.0%)	2 (50.0%)
Headache, n (%)	54 (47.0%)	1 (25.0%)
Dyspnoea, n (%)	21 (18.3%)	0 (0.0%)
Digestive disorders, n (%)	26 (22.6%)	1 (25.0%)
COVID-19 severity		
Ground glass opacity on thoracic CT scan, n/performed (%)	15/23 (65.2%)	0/1 (0.0%)
No hospitalisation, n (%)	109 (94.8%)	3 (75.0%)
Hospitalised, not requiring supplemental oxygen, n (%)	3 (2.6%)	1 (25.0%)
Hospitalised, requiring supplemental oxygen, n (%)	3 (2.6%)	0 (0.0%)

*Other treatments for patients with multiple sclerosis: methotrexate (n=1); azathioprine (n=1); and for patients with NMO-SD: mycophenolate mofetil (n=1). DMT, disease-modifying therapy; NMO-SD, neuromyelitis optica spectrum disorder; PP MS, primary progressive multiple sclerosis; RR MS, relapsing remitting multiple sclerosis; SP MS, secondary progressive multiple sclerosis.

COVID-19 symptoms were similar to previously reported in the general population, the most common symptoms being asthenia (82/119, 68.9%), fever (67/119, 56.3%) and anosmia (63/119, 52.9%).

SARS-CoV-2 serological results

SARS-CoV-2 serological results and total Ig quantification per DMTs groups are presented in table 2. Mean (SD) delay between COVID-19 onset and blood sampling was 5.0 (3.4) months and was not statistically different among groups of patients.

Table 2 SARS-CoV-2 serological results and total immunoglobulin levels by disease-modifying therapies

	No DMT	Anti-CD20	Interferon beta	Glatiramer	Teriflunomide	Dimethyl-fumarate	Natalizumab	Fingolimod	Other*
Number	21	21	4	10	17	23	14	6	3
Delay from COVID-19 (months), mean (SD)	5.4 (3.7)	4.2 (3.1)	7.6 (2.7)	5.4 (4.0)	5.4 (3.6)	5.3 (3.4)	5.0 (3.5)	3.4 (2.3)	1.9 (0.2)
SARS-CoV2 serology									
Anti-S IgG positivity, n (%)	20 (95.2%)	8 (38.1%)	4 (100%)	9 (90%)	13 (76.5%)	19 (82.6%)	12 (92.8%)	4 (66.7%)	3 (100%)
Anti-S IgG titre (log AU/mL), mean (SD)	2.7 (0.8)	1.4 (1.6)	2.9 (0.9)	2.5 (0.8)	2.2 (1.1)	2.3 (1.3)	2.5 (1.1)	2.1 (0.9)	3.0 (0.3)
Anti-S IgA positivity, n (%)	15 (71.4%)	7 (33.3%)	3 (75%)	7 (70%)	11 (64.7%)	12 (52.2%)	12 (85.7%)	3 (50%)	3 (100%)
Anti-S IgA index, mean (SD)	2.4 (3.2)	1.1 (0.5)	1.8 (0.9)	1.9 (1.5)	1.5 (1.2)	1.4 (0.8)	2.1 (1.0)	3.8 (6.5)	2.2 (0.7)
Anti-N IgG positivity, n (%)	17 (80.9%)	3 (14.3%)	2 (50%)	8 (80%)	11 (64.7%)	18 (78.3%)	10 (71.4%)	4 (66.7%)	3 (100%)
Anti-N IgG index, mean (SD)	2.7 (2.5)	0.5 (1.0)	1.1 (1.0)	3.2 (2.8)	2.3 (2.8)	2.3 (2.0)	1.3 (1.2)	2.0 (1.8)	4.1 (2.3)
Total IgG (g/L), mean (SD)	10.4 (2.7)	9.1 (2.0)	14.2 (2.1)	12.4 (2.4)	9.5 (2.3)	10.7 (2.5)	12.0 (2.7)	9.0 (3.4)	11.3 (2.3)
Total IgA (g/L), mean (SD)	2.2 (0.8)	1.9 (1.0)	1.4 (0.4)	2.6 (0.7)	1.4 (0.7)	1.9 (0.7)	2.2 (0.9)	1.5 (0.7)	1.6 (0.9)
Total IgM (g/L), mean (SD)	1.3 (0.8)	0.8 (0.4)	2.1 (1.9)	1.7 (0.8)	0.9 (0.5)	1.2 (0.5)	0.8 (0.9)	0.9 (0.3)	1.4 (1.1)

For numerical variables, results are marked in bold when different (p<0.05) from other groups by ANOVA (for numerical dependent variables). Independent variable is 'DMT group' (9-level variable), and p values are calculated for a DMT group vs the intercept of the model.

For categorical variables (eg, Ig positivity), a X² test was performed with the 9-level 'DMT group' as independent variable. For this group-level analysis, all p values were <0.05. Using a post-hoc X² test, we found a significantly decreased positivity rate (marked in bold) for all Ig in anti-CD20 group vs pooled other DMT groups.

*Other treatments: methotrexate (n=1); azathioprine (n=1); mycophenolate mofetil (n=1). ANOVA, analysis of variance; DMT, disease-modifying therapy.

Regarding total Ig levels, we found that patients on interferon and glatiramer acetate had higher IgG levels relative to other groups ($p=0.005$ and $p=0.03$, respectively), patients on teriflunomide had lower IgA levels relative to other groups ($p=0.003$), and patients on anti-CD20 and natalizumab had lower IgM levels relative to other groups ($p=0.01$ and $p=0.04$, respectively).

Overall, 96/119 (80.6%) patients had at least one positive SARS-CoV-2 Ig serological testing. Relative to all other DMT groups, patients on anti-CD20 had lower anti-S IgG positivity ($p<0.001$), lower anti-S IgG titre ($p<0.001$), a lower anti-S IgA positivity ($p=0.05$) and a lower anti-N IgG positivity ($p<0.001$). No differences were found among other DMT subgroups.

The percentage and number of patients with a positive and negative serological testing grouped by three levels of DMTs (anti-CD20, DMTs other than CD20 and no DMT) are presented in figure 1 and in online supplemental table (including demographic characteristics for each DMT group). Only 10/21 patients (47.6%) on anti-CD20 had at least one SARS-CoV-2 Ig positivity, while this was the case for 66/77 (85.7%) patients on DMTs other than anti-CD20 and for 20/21 (95.2%) patient without DMT ($p<0.001$). Patients on anti-CD20 had lower seroconversion rate compared with other groups for all three anti-S/anti-N Ig testing (figure 1C).

Among the 119 patients included in this serological study, 6 patients had a BMI >30 , all of them had at least one positive SARS-CoV-2 Ig serological testing. DMTs were dimethylfumarate (1), teriflunomide (1), fingolimod (1), ocrelizumab (1) and no DMT (2).

Anti-S IgG titre

The titre of anti-S IgG among the three DMT groups is presented in figure 2A. Patients on anti-CD20 had a lower anti-S IgG titre (mean (SD), 1.4 (1.6)) relative to patients on other DMTs (2.4 (1.1)) or no DMT (2.7 (0.8) ($p<0.001$ by ANOVA)).

At the entire cohort level in this cross-sectional analysis, we did not find a correlation between time from COVID-19 and anti-S IgG titre ($p=0.82$). To test whether this finding holds at the level of each DMT group, we performed linear regressions between anti-S IgG titre and time from COVID-19 in each DMT group (figure 2B). This figure shows that patients on anti-CD20 exhibited a higher rate of anti-S IgG decrease over time. By adding the variable ‘time by DMT group’ in the linear regression model, we found a trend for an interaction between time and DMT group for patients on anti-CD20 compared with the two other groups ($p=0.06$).

Multivariate regression models of SARS-CoV-2 serological results

Among independent variables including DMT group, time from COVID-19, total IgG level, age, sex, obesity and COVID-19 severity, only anti-CD20 group was associated with a decreased odd of positive serology (OR, 0.07 (95% CI 0.01 to 0.69, $p=0.02$)) (figure 3A).

Using the same independent variables, variables associated with anti-S IgG titre were anti-CD20, total IgG level and hospitalisation. Being on anti-CD20 was associated with a decreased anti-S IgG titre (estimate, -1.06 (95% CI -1.79 to -0.34), $p=0.004$). Hospitalisation for COVID-19 was also associated with a decreased anti-S IgG titre (estimate, -0.97 (95% CI -1.86 to -0.08), $p=0.03$). Total IgG level was positively associated with anti-S IgG titre (estimate, 0.16 (95% CI 0 to 0.32), $p=0.05$). There was a positive association between obesity and

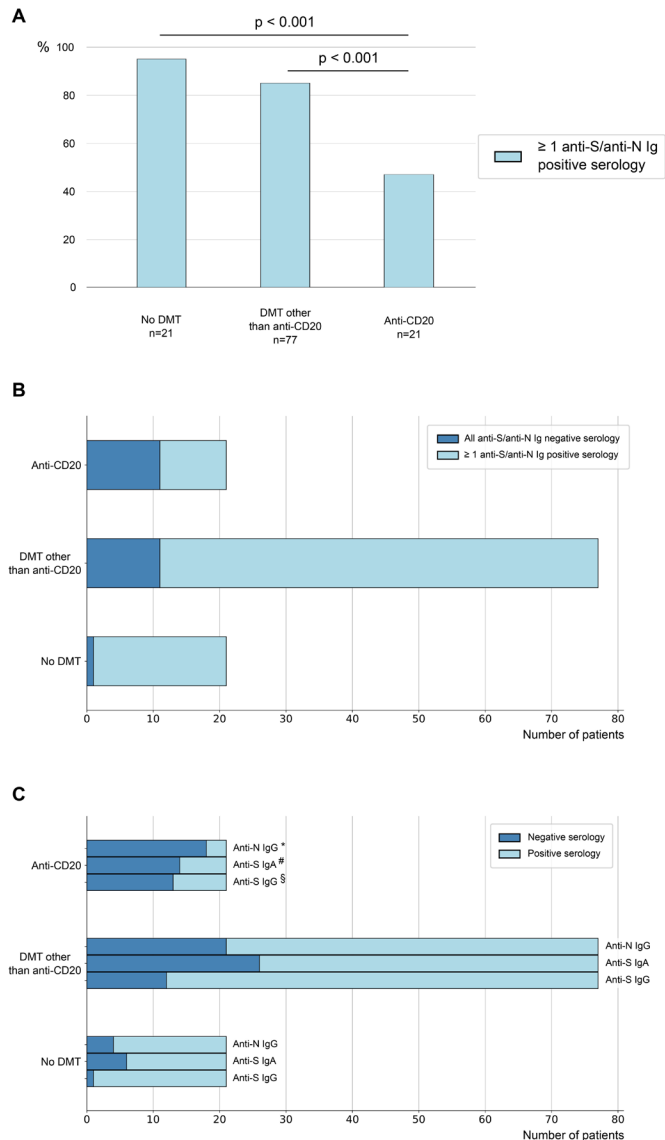


Figure 1 SARS-CoV-2 serology per DMT group in patients with multiple sclerosis or neuromyelitis optica spectrum disorder. (A) Percentage of patients with at least one positive serology among anti-S IgG, anti-S IgA and anti-N IgG testing. (B) Number of patients with at least one positive serology among anti-S IgG, anti-S IgA and anti-N IgG testing. (C) Number of patients with positive serology for each Ig testing. * $p<0.001$ for group differences for anti-N IgG positivity between anti-CD20 and both no DMT and DMT other than anti-CD20; # $p=0.03$ for group differences for anti-S IgA between anti-CD20 and no DMT, $p=0.01$ for group differences for anti-S IgA between anti-CD20 and DMT other than anti-CD20; § $p<0.001$ for group differences for anti-S IgG positivity between anti-CD20 and both no DMT and DMT other than anti-CD20. Group comparisons were performed by χ^2 tests. DMT, disease-modifying therapy.

anti-S-IgG titre, although not significant (estimate: 0.89, 95% CI -0.09 to 1.81, $p=0.08$) (figure 3B).

Seroconversion for patients on anti-CD20

We investigated whether demographic or clinical characteristics could differentiate seropositive ($n=10$) versus seronegative ($n=11$) patients on anti-CD20. Time between last anti-CD20 infusion and COVID-19 was longer (mean (SD), 3.7 (2.0) months) in seropositive patients compared with seronegative patients (mean (SD), 1.9 (1.5) months, $p=0.04$).

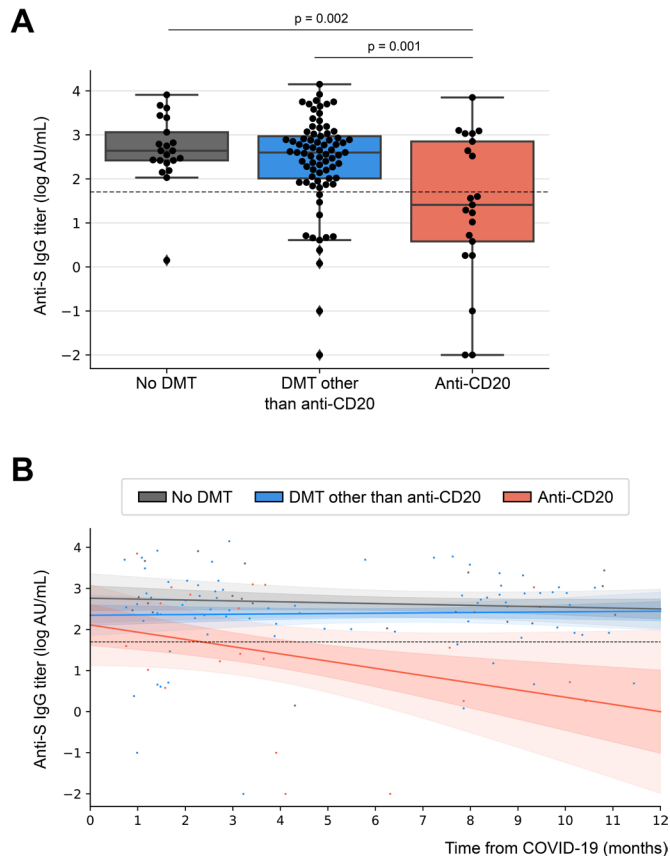


Figure 2 Anti-S IgG titre among DMT groups of patients with multiple sclerosis or neuromyelitis optica spectrum disorder. (A) Boxplot of anti-S IgG titre per DMT group. (B) Linear regression (with 1 and 2 SD confidence band) of anti-S IgG titre over time from COVID-19 onset per DMT group. Each point represents a patient of the study. Positivity of anti-S IgG is defined by a titre ≥ 1.7 log AU/mL and is shown by a dashed line in A and B. DMT, disease-modifying therapy.

(figure 4), excluding the only patient on ofatumumab. Both groups were not statistically different regarding age, sex, IgG level, time from COVID-19 onset, COVID-19 severity and duration of anti-CD20 therapy. All three patients on anti-CD20 who were hospitalised for COVID-19 were eventually seronegative.

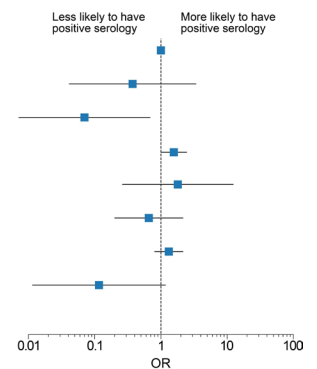
DISCUSSION

Our results showed that the seroconversion rate in patients with MS or NMO-SD after COVID-19 was high, with $\sim 80\%$ of patients having a positive serology within an average of 5 months after infection. However, this serological response was heterogeneous. Patients treated with anti-CD20 had a seroconversion rate two times as low as untreated patients or patients receiving DMT other than anti-CD20. Interestingly, patients on anti-CD20 who remained seronegative differed from seropositive patients only by the time between last anti-CD20 infusion and COVID-19.

Given the mechanism of action of anti-CD20, it is not surprising to observe a decrease in the production of anti-SARS-CoV-2 antibodies in patients on anti-CD20, independently of other factors that may influence anti-SARS-CoV-2 antibody level such as age, gender, time to infection or severity of COVID-19.¹⁵ Anti-CD20 are B-cell depleting therapies, highly effective in reducing

A Positive anti-S/anti-N serology

Group	OR (95% CI)
No DMT	NA
DMT other than anti-CD20	0.37 (0.04-3.42)
Anti-CD20	0.07 (0.01-0.69)
Total IgG (per 2 g/L)	1.57 (0.99-2.48)
Time from COVID-19 (per 1 year)	1.80 (0.26-12.48)
Male	0.66 (0.20-2.17)
Age (per 10 years)	1.32 (0.80-2.17)
Hospitalization for COVID-19	0.12 (0.01-1.18)
Obesity (BMI > 30 kg/m ²)	NQ (0.00-inf)



B Anti-S IgG titer

Group	Estimate (95% CI)
No DMT	NA
DMT other than anti-CD20	-0.25 (-0.83, 0.34)
Anti-CD20	-1.06 (-1.79, -0.34)
Total IgG (per 2 g/L)	0.16 (0.00, 0.32)
Time from COVID-19 (per 1 year)	-0.34 (-1.07, 0.40)
Male	-0.27 (-0.75, 0.21)
Age (per 10 years)	0.13 (-0.06, 0.32)
Hospitalization for COVID-19	-0.97 (-1.86, -0.08)
Obesity (BMI > 30 kg/m ²)	-0.86 (-0.09, 1.81)

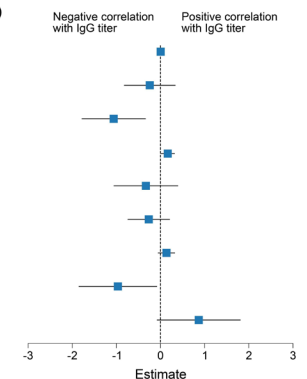


Figure 3 Determinants of SARS-CoV-2 humoral response in patients with multiple sclerosis or neuromyelitis optica spectrum disorder. (A) Multivariate logistic regression model evaluating the odds ratio (95% CI) of the variables associated with SARS-CoV-2 positive serology (anti-S and/or anti-N Ig). (B) Multivariate linear regression model evaluating the estimate (95% CI) of the variables associated with anti-S IgG titre. BMI, body mass index; DMT, disease-modifying therapy; NQ, not quantifiable, as the model did not converge for obesity variable.

inflammatory activity in MS^{16 17} and NMO-SD.^{18 19} Anti-CD20 therapies act by resetting the pool not only of circulating B lymphocytes but also of CD20+ memory B cells.²⁰ However, tissue-resident B cells are relatively spared as well as antibody-producing plasma cells that do not express

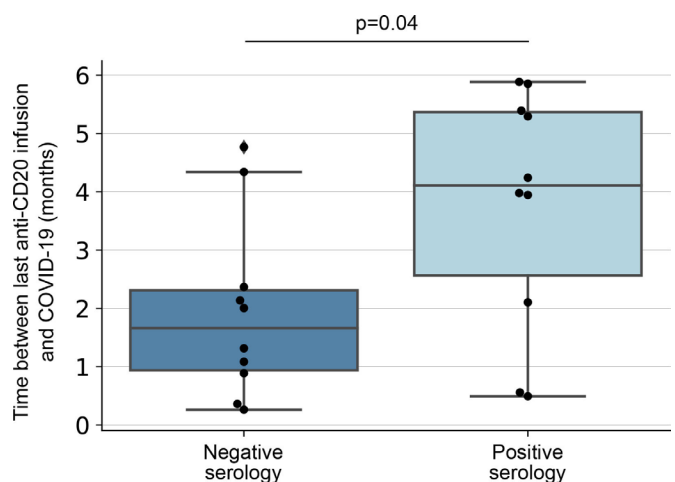


Figure 4 Time between the last anti-CD20 infusion and COVID-19 patients in receiving anti-CD20 therapy, according to SARS-CoV-2 serological status.

CD20. This action is exerted from the first 2 weeks after treatment onset,²¹ and the time to repopulation can be variable, from 4 months to much longer in some patients. This variability may reflect the heterogeneity of the humoral response to SARS-CoV-2 in patients on anti-CD20, associated with the time between infusion and COVID-19 in our cohort. Conversely, we did not observe a link between seroconversion and the duration of exposure to anti-CD20, but this could be due to the low number of patients as well as the relatively recent availability of ocrelizumab in France (2019). The therapeutic strategy of extending anti-CD20 dosing interval might be considered in the perspective to allow a better humoral immune response in the context of COVID-19 pandemic, but only if the control of the neurological disease allows it, as suggested in MS,²² but not in NMO-SD where it is not recommended.

Several studies have shown that patients with MS or NMO-SD taking anti-CD20 have an increased risk of infection,²³ possibly related to hypogammaglobulinemia.²⁴ In our cohort, total IgG level tended to be associated with anti-S IgG titre and anti-SARS-CoV-2 Ig positivity. The postinfectious immune response has generally been poorly studied in patients with MS, contrarily to the postvaccination humoral response. VELOCE study has compared the humoral response to several vaccines in 68 patients with MS on ocrelizumab versus 34 patients either untreated or on interferon beta.²⁵ IgG response to tetanus toxoid-containing vaccine, pneumococcal vaccine and influenza vaccine was halved in patients on ocrelizumab compared with the control group. Similarly, humoral response to vaccine was found to be severely reduced in patients with rheumatoid arthritis treated by rituximab.^{26 27}

In our cohort, the seroconversion rate with teriflunomide and fingolimod was slightly lower than that of the other groups, but this difference was not statistically significant, probably due to the small number of patients (6 patients on fingolimod and 17 patients on teriflunomide). It is possible that for fingolimod, the decrease in circulating B cells may impair the humoral response to SARS-CoV-2, but this hypothesis should be investigated in a larger cohort.

There is very little data on seroconversion after COVID-19 in immunocompromised populations. In a cohort of 42 patients with kidney transplantation, 71.4% of patients had positive anti-N IgG serology 2 months after COVID-19, but only 36.4% still had positive serology 6 months after infection.²⁸ In our study, using the same detection kit for anti-N IgG, 14.3% of patients on anti-CD20 were seropositive for anti-N IgG within an average of 4.2 months after COVID-19.

Other factors can influence seroconversion rates, including obesity,²⁹ COVID-19 severity or IgG level, as assessed in our cohort.

In the general population, several studies have found a high seroconversion rate after COVID-19. In Iceland, out of a population of 1215 patients with a positive SARS-CoV-2 PCR, 91.1% of patients had a positive serology up to 4 months after diagnosis.³⁰ These results are in line with the positivity level observed in our population (95.2% in untreated patients and 85.7% in patients on DMTs outside anti-CD20).

However, it is important to keep in mind that the detection of antibodies against the SARS-CoV-2 proteins does not reflect the neutralising activity on the virus, assessed by anti-RBD antibody level, which decreases with an estimated half-life of 36 days.^{31 32} However, it is probable that patients on anti-CD20 also have a reduced neutralising activity, similar to the reduced production of anti-S antibodies.

A crucial point when comparing SARS-CoV-2 seropositivity rate between studies concerns the sensitivity of serological tests, which is heterogeneous depending on the assay kits. However, most of the assay methods have a sensitivity over 98% and a specificity close to 100%.³³ False positives are rare and may relate to cross-seropositivity with other coronaviruses or infectious agents or the presence of rheumatoid factor.³⁴ In our study, the possibility of centralised analysis of the samples is a strength to allow good reproducibility in the detection and quantification of anti-SARS-CoV-2 antibodies and an asset for the longitudinal follow-up, which is planned until 1 year.

Finally, it is important to remember that the T cell response against SARS-CoV-2 is crucial at different times of infection, and specific T cell response was identified several months after COVID-19, even in patients without detectable circulating antibodies.³⁵ This cellular response should be at least partially preserved in patients treated with anti-CD20 and could help protect against severe forms of COVID-19 and limit the risk of reinfection. Preliminary data showed that T-cell response to SARS-CoV-2 evaluated by Elispot could be detected up to 9 months after COVID-19, even in patients on ocrelizumab.³⁶

Limitations

Our study was designed to provide uniform immunological data with a centralised analysis. A limitation of the study is the relatively small number of patients per DMT group, which may have hampered the evaluation of the humoral immune response for some DMT groups. Sphingosine-1-phosphate receptor modulators might also decrease humoral response to SARS-CoV-2, but a larger sample size will be needed to investigate this hypothesis.

The heterogeneous delay between blood sampling and COVID-19 onset may also be a limitation, however, the vast majority of patients displayed an elevated antibody titre beyond 6 months from COVID-19, apart from patients on anti-CD20.

CONCLUSION

This study demonstrates a weaker serological response against SARS-CoV-2 in patients with MS or NMO-SD treated with anti-CD20, while the seropositivity rate of patients not treated with anti-CD20 is similar to what has been observed in the general population. The long-term clinical consequences of this decrease in the humoral response to SARS-CoV-2 remain unknown and should be carefully monitored.

Postvaccination serological follow-up studies will be necessary to investigate the potential effect of anti-CD20 and other DMTs on humoral response to SARS-CoV-2 vaccine and eventually to adapt the vaccine strategy. Importantly, the association between the time from last anti-CD20 infusion and the seroconversion may lead to recommend anti-COVID-19 vaccination timing as far as possible from anti-CD20 infusion, for example, starting from 4 months after infusion.

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REFERENCES

- Louapre C, Collongues N, Stankoff B, *et al.* Clinical characteristics and outcomes in patients with coronavirus disease 2019 and multiple sclerosis. *JAMA Neurol* 2020;77:1079–88.
- Sormani MP, De Rossi N, Schiavetti I, *et al.* Disease-modifying therapies and coronavirus disease 2019 severity in multiple sclerosis. *Ann Neurol* 2021;89:780–9.
- Salter A, Fox RJ, Newsome SD, *et al.* Outcomes and risk factors associated with SARS-CoV-2 infection in a North American registry of patients with multiple sclerosis. *JAMA Neurol* 2021;78:699.
- Winkelmann A, Loebermann M, Reisinger EC, *et al.* Disease-modifying therapies and infectious risks in multiple sclerosis. *Nat Rev Neurol* 2016;12:217–33.
- Moisset X, Fouchard A-A, Pereira B, *et al.* Untreated patients with multiple sclerosis: a study of French expert centers. *Eur J Neurol* 2021;28:2026–36.
- Sette A, Crotty S. Adaptive immunity to SARS-CoV-2 and COVID-19. *Cell* 2021;184:861–80.
- Rydzynski Moderbacher C, Ramirez SI, Dan JM, *et al.* Antigen-specific adaptive immunity to SARS-CoV-2 in acute COVID-19 and associations with age and disease severity. *Cell* 2020;183:996–1012.
- Hansen CH, Michlmayr D, Gubbels SM, *et al.* Assessment of protection against reinfection with SARS-CoV-2 among 4 million PCR-tested individuals in Denmark in 2020: a population-level observational study. *Lancet* 2021;397:1204–12.
- Yang HS, Costa V, Racine-Brzostek SE, *et al.* Association of age with SARS-CoV-2 antibody response. *JAMA Netw Open* 2021;4:e214302.
- Wu J, Liang B, Chen C, *et al.* SARS-CoV-2 infection induces sustained humoral immune responses in convalescent patients following symptomatic COVID-19. *Nat Commun* 2021;12:1813.
- Chia WN, Zhu F, Ong SWX, *et al.* Dynamics of SARS-CoV-2 neutralising antibody responses and duration of immunity: a longitudinal study. *Lancet Microbe* 2021;2:e240–9.
- Maillart E, Papeix C, Lubetzki C, *et al.* Beyond COVID-19: do MS/NMO-SD patients treated with anti-CD20 therapies develop SARS-CoV2 antibodies? *Mult Scler Relat Disord* 2020;46:102482.
- Zabalza A, Cárdenas-Robledo S, Tagliani P, *et al.* COVID-19 in multiple sclerosis patients: susceptibility, severity risk factors and serological response. *Eur J Neurol* 2020 doi:10.1111/ene.14690
- Cao B, Wang Y, Wen D, *et al.* A trial of lopinavir-ritonavir in adults hospitalized with severe Covid-19. *N Engl J Med* 2020;382:1787–99.
- Noh JY, Kwak J-E, Yang J-S, *et al.* Longitudinal assessment of anti-SARS-CoV-2 immune responses for six months based on the clinical severity of COVID-19. *J Infect Dis* 2021 doi:10.1093/infdis/jiab124
- Hauser SL, Bar-Or A, Cohen JA, *et al.* Ofatumumab versus teriflunomide in multiple sclerosis. *N Engl J Med* 2020;383:546–57.
- Hauser SL, Bar-Or A, Comi G, *et al.* Ocrelizumab versus interferon beta-1a in relapsing multiple sclerosis. *N Engl J Med* 2017;376:221–34.
- Kim S-H, Huh S-Y, Lee SJ, *et al.* A 5-year follow-up of rituximab treatment in patients with neuromyelitis optica spectrum disorder. *JAMA Neurol* 2013;70:1110–7.
- Cabre P, Mejdoubi M, Jeannin S, *et al.* Treatment of neuromyelitis optica with rituximab: a 2-year prospective multicenter study. *J Neurol* 2018;265:917–25.
- Lee DSW, Rojas OL, Gommerman JL. B cell depletion therapies in autoimmune disease: advances and mechanistic insights. *Nat Rev Drug Discov* 2021;20:179–99.
- Bar-Or A, Calabresi PAJ, Arnold D, *et al.* Rituximab in relapsing-remitting multiple sclerosis: a 72-week, open-label, phase I trial. *Ann Neurol* 2008;63:395–400.
- Maarouf A, Rico A, Boutiere C, *et al.* Extending rituximab dosing intervals in patients with MS during the COVID-19 pandemic and beyond? *Neurol Neuroimmunol Neuroinflamm* 2020;7:e825. doi:10.1212/NXI.0000000000000825
- Luna G, Alping P, Burman J, *et al.* Infection risks among patients with multiple sclerosis treated with fingolimod, natalizumab, rituximab, and injectable therapies. *JAMA Neurol* 2020;77:184–91.
- Avouac A, Maarouf A, Stellmann J-P, *et al.* Rituximab-Induced hypogammaglobulinemia and infections in AQP4 and MOG antibody-associated diseases. *Neurol Neuroimmunol Neuroinflamm* 2021;8:e977. doi:10.1212/NXI.0000000000000977
- Bar-Or A, Calkwood JC, Chognot C, *et al.* Effect of ocrelizumab on vaccine responses in patients with multiple sclerosis: the VELOCE study. *Neurology* 2020;95:e1999–2008.

- 26 van Assen S, Holvast A, Benne CA, *et al.* Humoral responses after influenza vaccination are severely reduced in patients with rheumatoid arthritis treated with rituximab. *Arthritis Rheum* 2010;62:75–81.
- 27 Bingham CO, Looney RJ, Deodhar A, *et al.* Immunization responses in rheumatoid arthritis patients treated with rituximab: results from a controlled clinical trial. *Arthritis Rheum* 2010;62:64–74.
- 28 Chavarot N, Leruez-Ville M, Scemla A, *et al.* Decline and loss of anti-SARS-CoV-2 antibodies in kidney transplant recipients in the 6 months following SARS-CoV-2 infection. *Kidney Int* 2021;99:486–8.
- 29 Frasca D, Reidy L, Cray C, *et al.* Influence of obesity on serum levels of SARS-CoV-2-specific antibodies in COVID-19 patients. *PLoS One* 2021;16:e0245424.
- 30 Gudbjartsson DF, Norddahl GL, Melsted P, *et al.* Humoral immune response to SARS-CoV-2 in Iceland. *N Engl J Med* 2020;383:1724–34.
- 31 Ibarondo FJ, Fulcher JA, Goodman-Meza D, *et al.* Rapid decay of anti-SARS-CoV-2 antibodies in persons with mild Covid-19. *N Engl J Med* 2020;383:1085–7.
- 32 Marot S, Malet I, Leducq V, *et al.* Rapid decline of neutralizing antibodies against SARS-CoV-2 among infected healthcare workers. *Nat Commun* 2021;12:844.
- 33 National SARS-CoV-2 Serology Assay Evaluation Group. Performance characteristics of five immunoassays for SARS-CoV-2: a head-to-head benchmark comparison. *Lancet Infect Dis* 2020;20:1390–400.
- 34 Vauloup-Fellous C, Maylin S, Périllaud-Dubois C. Performance of 30 commercial SARS-CoV-2 serology assays in testing symptomatic COVID-19 patients. *Eur J Clin Microbiol Infect Dis* 2021 doi:10.1007/s10096-021-04232-3
- 35 Sekine T, Perez-Potti A, Rivera-Ballesteros O, *et al.* Robust T cell immunity in convalescent individuals with asymptomatic or mild COVID-19. *Cell* 2020;183:158–68.
- 36 Kister I, Krogsgaard M, Mulligan MJ. Preliminary results of ongoing, prospective study of antibody and T-cell responses to SARS-CoV-2 in patients with MS on ocrelizumab or other disease-modifying therapies. 73rd Congress of the American Academy of Neurology (AAN) Virtual 2021. 17–22 April 2021; Presentation Number P15.014.