Universitat Autònoma de Barcelona

Susceptibility, severity and immunological factors against SARSCoV-2 infection in patients with Multiple Sclerosis

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Susceptibility, severity and immunological factors against SARS-CoV-2 infection in patients with Multiple Sclerosis

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ABBREVIATIONS

Ab	Antibody
ACE2	Angiotensin-converting enzyme 2
ADRS	Acute Distress Respiratory Syndrome
AID	Autoimmune diseases
ALZ	Alemtuzumab
Anti-CD20s	Treatments against CD20 receptor include ocrelizumab, rituximab and ofatumumab
BAU/ml	Binding Antibody Units per milliliter
BMI	Body Mass Index
CD19	Cluster of differentiation 19
CD3	Cluster of differentiation 3
CD4	Cluster of differentiation 4
CD8	Cluster of differentiation 8
Cemcat	Multiple Sclerosis Centre of Catalonia
CI	Confidence interval
CIS	Clinically isolated syndrome
CLA	Cladribine
CLIA	Chemiluminescence Immunoassays
CNS	Central nervous system
CSF	Cerebrospinal fluid
COVID-19	Novel coronavirus disease 2019
DIS	Dissemination in space
DIT	Dissemination in time
DMT	Disease-modifying treatment

DMF	Dimethyl fumarate
DNA	Deoxyribonucleic acid
EDSS	Expanded disability status scale
ELISA	Enzyme-linked immunosorbent assay
EU	ELISA units
FTY	Fingolimod
GA	Glatiramer acetate
GFAP	Glial fibrillary acidic protein
HLA	Human leukocyte antigen
HIV	Human Immunodeficiency Virus
ICU	Intensive Care Unit
IFN-y	Interferon gamma
IFN	Interferons
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgG4-RD	Immunoglobulin G4 related disease
IgG-S	Immunoglobulin G antibody against SARS-CoV-2 spike protein
IgG-N	Immunoglobulin G antibody against SARS-CoV-2 nucleotide protein
IgM	Immunoglobulin M
IQR	Interquartile range
IL-6	Interleukin 6
IFN	Interferon
JCV	John Cunningham virus
LDH	Lactate dehydrogenase
MOGAD	Myelin Oligodendrocyte Glycoprotein Antibody Disorders

MRI Magnetic resonance imaging

mRNA Messenger ribonucleic acid

MS Multiple sclerosis

NFKβ Nuclear factor kappa beta

NK Natural Killer cells

NMO Neuromyelitis Optica

NMOSD Neuromyelitis Optica spectrum disorders

NRF2 Nuclear factor erythroid 2-related factor 2

NTZ Natalizumab

OCB Oligoclonal bands

OCR Ocrelizumab

OR Odds Ratio

PBMC Peripheral blood mononuclear cells

PCR Polymerase Chain Reaction

PML Progressive multifocal leukoencephalopathy

PwMS Patients with Multiple Sclerosis

RRMS Relapsing remitting multiple sclerosis

RT-PCR Reverse Transcription Polymerase Chain Reaction

RTX Rituximab

SAR-EM SARS-CoV-2 humoral and cellular response in patients with multiple sclerosis and immunosuppressant treatments study

SARS-CoV-2 Severe Acute Respiratory Syndrome Coronavirus 2

SD Standard deviation

SPMS Secondary progressive multiple sclerosis

SP1RM Sphingosine 1-phosphate receptor modulator

TF Teriflunomide

UNIEMTG Neuroimmunology and MS unit of the Doctor Trueta/Santa Caterina University Hospital of Girona

VELOCE Effect of ocrelizumab on vaccine responses in patients with multiple sclerosis

VHIR Vall d'Hebron Institut de Recerca

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RESUM | SUMMARY

Resum

Considerant que els pacients amb esclerosi múltiple (EM) presenten un major risc de morbimortalitat secundari a infeccions i que aquest risc es troba incrementat en aquells amb tractament immunosupressors, quan la pandèmia de COVID-19 va començar va quedar palès que era crucial estudiar quins pacients amb EM presentaven major risc de COVID-19 i com influïa el tractament en aquest risc. A més a més, donat que la majoria de tractaments alteren la resposta immunitària humoral i/o cel·lular, aquesta resposta immunitària a la infecció o a la vacuna per SARS-CoV-2 es podria veure influenciada pels diferents tractaments. Per tot això, els objectius d'aquest estudi són els d'analitzar la incidència, la susceptibilitat i els factors de risc per a presentar un COVID-19 greu en pacients amb EM; el d'estudiar la resposta humoral i cel·lular després de la infecció i la vacunació en aquests pacients i el d'analitzar com s'ha modificat la pràctica clínica arran de la pandèmia. Amb aquest objectius en ment, vam dur a terme varis projectes de recerca.

Durant la primera onada de la pandèmia, vam enviar una enquesta per correu electrònic a tots els nostres pacients per a detectar quins havien presentat la COVID-19, el que ens va permetre establir una incidència del COVID-19 en la nostra cohort del 6.3%. Addicionalment, vam trobar que els pacients més joves, amb una EM de més llarga evolució, que vivien a Barcelona ciutat i que havien estat amb contacte amb una persona infectada presentaven un risc més alt de COVID-19. En la nostre cohort, l'edat va ser l'únic factor independent que es va relacionar amb la severitat del COVID-19. Cap tractament va augmentar la susceptibilitat o la severitat del COVID-19.

Seguidament, vam avaluar la resposta immunològica humoral i cel·lular al SARS-CoV-2 dels pacients amb EM que havien presentat COVID-19. La resposta humoral es trobava disminuïda en els pacients tractats amb anti-CD20s i augmentada en aquells amb sexe masculí. Per contra, la resposta cel·lular es trobava disminuïda en pacients amb formes progressives de la malaltia.

Quan les vacunes contra el SARS-CoV-2 van estar disponibles, vam realitzar l'estudi de resposta humoral i cel·lular després de la vacunació amb col·laboració de la unitat de neuroimmunologia i EM de l'hospital universitari de Girona Doctor Trueta/ Santa Caterina. Vam detectar que la resposta humoral es troba reduïda en els pacients tractats amb moduladors del receptors de la esfingosina 1-fosfats (SP1RM) o amb anti-CD20s. En aquests darrers, la resposta humoral augmentava quan més temps hagués passat entre la vacuna i la darrera infusió. A més a més, també vam detectar que una major durada del

tractament disminuïa la resposta humoral post-vacunal. La resposta cel·lular després de la vacuna estava disminuïda en aquells pacients amb SP1RM i majors de 50 anys.

Finalment, vam avaluar com la pandèmia va impactar la practica clínica en el nostre centre mesurant el nombre de visites i ressonàncies magnètiques realitzades i el nombre i tipus de prescripció de tractaments fetes durant l'any 2020 i comparant-los amb els anys previs. Vam trobar que durant la pandèmia tant les visites com l'activitat radiològica es va mantenir però es va disminuir el nombre de prescripcions i es va modificar el patró de prescripció de fàrmacs d'alta eficàcia. Concretament, es va disminuir la prescripció d'anti-CD20s i es va augmentar la de natalizumab.

Summary

Given that MS patients are at higher risk of morbimortality due to infection and that this risk is increased in those under immunosuppressant treatments, when the COVID-19 pandemic started it was crucial to study which MS patients where at higher risk of COVID-19 and how DMT influenced this risk. Additionally, as most MS treatments alter humoral and/or cellular immunity, these immunological responses to SARS-CoV-2 infection and vaccination may be modified by the different treatments. Therefore, the aim of this study is to analyze the incidence, susceptibility and severity risk factors for COVID-19 in MS patients, their humoral and cellular response to SARS-CoV-2 infection and vaccination and the modification in the clinical practice caused by the pandemic. With these objectives in mind, several research proposals were considered.

First, we sent an online survey to all our patients to detect those who have had COVID-19, allowing us to estimate an incidence of COVID-19 of 6.3% in our cohort. Additionally, we found that younger patients, with a longer disease duration, living in Barcelona city and who had had contact with an infected person presented a higher risk of COVID-19. In our cohort, age was the only independent factor related to severity. No DMT was related to an increased risk of COVID-19 susceptibility or severity.

Next, we evaluated the humoral and cellular immunological response of MS patients who had presented COVID-19. After COVID-19, humoral response decreased in in anti-CD20s-treated patients and increased in male patients, whereas progressive forms decreased cellular response.

When SARS-CoV-2 vaccines were available, we performed a humoral and cellular immunological response study after vaccination in collaboration with the Neuroimmunology and MS unit of the Doctor Trueta/Santa Caterina University Hospital of Girona. We detected that humoral response was reduced in SP1RM-treated and anti-CD20s-treated patients. In the later, the humoral response rate increased the longer the interval between last infusion and vaccination. A longer treatment duration also decreased humoral response to vaccines. The cellular response after vaccination was also blunted by SP1RM treatment and in patients over 50 years of age.

Finally, we evaluated how the pandemic impacted the clinical practice in our center by analyzing the number of clinical visits, the number of magnetic resonance imaging studies and the number and type of treatment prescriptions during the year 2020 compared to 2019. We found that, during the pandemic, the clinical and radiological activity at our center were

maintained but the number of treatment prescriptions was reduced, and the pattern modified. Specifically, there was a change in the high efficacy prescription pattern where anti-CD20s therapy prescription was diminished while natalizumab prescription increased.

1. INTRODUCTION

1.1 SARS-CoV-2 and COVID-19

1.1.1 Epidemiology

The 2019 novel coronavirus disease (COVID-19) is a pandemic infection caused by severe acute respiratory syndrome (SARS) coronavirus two (SARS-CoV-2). The first infections were reported in China in late 2019. It rapidly spread around China and the rest of the world. The World Health Organization declared a global pandemic on 11 March 2020 (1). As of April 2022, the disease has spread all around the world with more than 510 million confirmed cases and 6.2 million deaths (2).

1.1.2 Coronavirus virology and pathogenesis

Coronavirus are enveloped positive-stranded RNA viruses. SARS-CoV-2 is a betacoronavirus as is the severe acute respiratory syndrome (SARS) virus. The viral envelope is coated by a spike (S) glycoprotein and by envelope (E) and membrane (M) proteins (3).

The host receptor for SARS-CoV-2 cell entry is the angiotensin-converting enzyme 2 (ACE2).(1) SARS-CoV-2 binds to ACE2 through the receptor-binding domain of its spike protein. Replication of the virus in the lung cells leads to non-specific symptoms such as fever, myalgia, headache, and respiratory symptoms. The distribution of ACE2 receptors in different tissues may explain the broad spectrum of COVID-19 symptoms from gastrointestinal symptoms (diarrhea) to neurological symptoms (anosmia) (4). However, the understanding of COVID-19 is constantly evolving.

Like any other virus, SARS-CoV-2 virus has evolved over time with new mutations in its genome. Certain variants have become variants of concern because of their evidence of rapid transmission or clinical implications (5). In late 2020, the first variant of concern was identified and named alpha variant (B.1.1.7 lineage) and subsequently became the globally dominant variant. This variant was 50 to 75% more transmissible than the previous strains. The next global strain was the delta variant (B.1.617.2 lineage) and was first described in December 2020. In comparison with the alpha variant, it was said to be more transmissible and associated to a higher risk of severity. The last and actual global strain is the omicron variant (B.1.1.529 lineage) that was first described in November 2021 and by the end of December 2021 was the predominant global variant. This variant has been associated with increased transmissibility, increased risk of reinfection after the infection with other variants,

decreased susceptibility to neutralizing antibodies and decreased severity compared to other variants. Other variants of concern are the beta (B.1.351 lineage) and the gamma (P.1 lineage) variant which did not become a global variant (6,7). It is expected that the ongoing rapid antigenic evolution of SARS-CoV-2 is likely to produce new variants that may escape immunity and be more severe (8).

1.1.3 COVID-19 disease spectrum

The COVID-19 disease course is highly heterogeneous, ranging from infectious but asymptomatic to severe disease and death. Initially, it was characterized by dry cough, fever, dyspnea, fatigue, anosmia and lymphopenia. 15% of infected patients present a severe disease with interstitial pneumonia because of alveolar damage that can lead to an acute distress respiratory syndrome (ADRS) or even death. ADRS is caused by the host reaction to the virus, which can also include antibody-mediated inflammation and a cytokine release syndrome that are thought to have a major impact on outcome (9).

1.1.4 Severity risk factors

Severe COVID-19 is defined as SARS-CoV-2 infection resulting in hospitalization, admission to the intensive care unit (ICU), intubation or mechanical ventilation, or death. It has been associated to advanced age and previous comorbidities (10).

In the general population, older age is associated with a higher risk of hospitalization and mortality with 80% of deaths occurring in those aged ≥65 years (1,10–12). Male sex has also been associated to an increased mortality in multiple cohorts (12,13). Additionally, Black, Hispanic and Sothern Asian individuals are overrepresented in the severe cases in countries such as United States or United Kingdom which could be relate to underlying socioeconomical disparities (12,14).

In relation to comorbidities, most of the severe cases present at least one comorbidity (1,10,12). Those that increase the risk of severe COVID-19 in a least one metanalysis are: cancer, cerebrovascular disease, chronic kidney disease, chronic lung disease, chronic liver disease, diabetes mellitus type 1 and type 2, Down syndrome, heart conditions (such as heart failure, coronary artery disease, or cardiomyopathies), Human Immunodeficiency Virus (HIV) infection, mental health disorders, dementia, obesity (Body Mass Index [BMI] ≥30 kg/m2) and overweight (BMI 25 to 29 kg/m2), pregnancy, smoking (current and former),

sickle cell disease or thalassemia, solid organ or blood stem cell transplantation, tuberculosis and use of corticosteroids or other immunosuppressive medications. Nevertheless, there are some comorbidities such as hypertension or asthma where evidence of the increased severe risk is mixed (15).

Numerous studies have demonstrated that COVID-19's severity is closely related to the patient's immune response. In this sense, some laboratory findings have been associated with an increased risk of severe COVID-19, these are: lymphopenia, thrombocytopenia, elevated liver enzymes, elevated lactate dehydrogenase, elevated inflammatory markers and inflammatory cytokines (interleukin 6 and tumor necrosis factor-alpha) and elevated D-dimer (16). In some observational studies, hypovitaminosis D has also been associated to a poorer outcome, but multiple confounders likely impact the observed associations (17).

The introduction of vaccines and the appearance of new milder variants such as the omicron variant has led to a decrease in the rates of intensive care unit admission (4 vs 21%) and death (1 vs 4.5%) (18,19). However, risk factors for a severe COVID-19 remain the same (age, previous comorbidities and male sex) specially in unvaccinated patients (20,21).

1.1.5 SARS-CoV-2 vaccination

SARS-CoV-2 vaccines are considered the most promising approach to control the pandemic. By the end of 2020, more than 180 vaccines against SARS-CoV-2 candidates, based on several different platforms, were in development. The platforms can be divided into 'traditional' approaches (inactivated or live-virus vaccines), platforms that have recently resulted in licensed vaccines (recombinant protein vaccines and vectored vaccines), and platforms that have yet to result in a licensed vaccine (RNA and DNA vaccines) such as the Pfizer/BioNTech and Moderna vaccines (22). Clinical trials of different vaccines started in late April 2020, demonstrating in some of them both safety and high efficacy, thus leading to the first emergency approval of a SARS-CoV-2 vaccine on December 2 (Pfizer/BioNTech vaccine) in the United Kingdom. This vaccine was shortly after granted an emergency authorization by the Food and Drug Administration and the European Medicines Agency (23). The Moderna vaccine was approved by the European Medicine Agency on January 6th 2021 (24). In Spain, general vaccination started on December 27th with the Pfizer/BioNTech vaccine and it currently includes the COVID-19 mRNA vaccine BNT162b2 (Pfizer-BioNTech COVID-19 vaccine), the COVID-19 mRNA vaccine mRNA-1273 (Moderna COVID-19 vaccine), the adenoviral vector vaccine Ad26.COV2.S (Janssen COVID-19

vaccine) (25) and the adenoviral vector vaccine ChAdOx1 nCoV-19/AZD1222 (University of Oxford/AstraZeneca COVID-19 vaccine) (26).

Initially, SARS-CoV-2 vaccines' phase 3 clinical trials excluded subjects with autoimmune diseases or immunosuppressive treatment, rending the efficacy and safety of the vaccine to be determined. Post-commercialization studies have corroborated the efficacy of many of these vaccines in these patients.

1.1.6 Immune response to SARS-CoV-2

1.1.6.1 Humoral and cellular immune response following infection

After SARS-CoV-2 infection, most patients develop detectable antibodies against the receptor-binding domain of the viral spike protein and the nucleocapsid protein (27). Antibodies with the capacity to restrict virus growth known as neutralizing antibodies correlate with IgG anti-spike protein (28). The magnitude of the antibody response is associated with the severity of the disease, as some patients with asymptomatic or mild disease fail to produce neutralizing antibodies (29,30). In general, antibody titers can increase during the first two months after infection and decline progressively afterwards. However, neutralizing activity has been reported up to 12 months after infection (31–34).

Neutralizing activity is the best current evidence associated with protection from subsequent infection and reinfection (28). Long-term memory humoral response has been demonstrated by some studies that have detected specific memory B cells and plasma cells against the spike protein (33,34).

Specific SARS-CoV-2 CD4 and CD8 T cell response has also been identified in convalescent COVID-19 patients (35,36). The T-cell response develops over a period of 10-20 days post-infection. Similarly to the humoral response, increasing disease severity is associated with a more robust T-cell response (35). In several studies, the magnitude of cellular responses in COVID-19 patients correlated with antibody titers.

Robust long-term specific cellular response has been described at least 12 months after infection (33,34,37). Memory T-cells can improve future immune responses by offering a more efficient support to activated B cells responding to the spike protein (memory CD4 T cells) or through direct lysis of SARS-CoV-2 infected cells (CD8 memory T cells) (38,39).

1.1.6.2 Immune response following vaccination

All approved SARS-CoV-2 vaccines have demonstrated to induce anti-Spike IgG antibodies and neutralizing antibodies with high proportions of seroconversion (40,41). Some vaccine regimens have shown higher anti-S titers and neutralization capacity after vaccination compared to natural infection (28).

Different studies have shown that SARS-CoV-2 vaccines induce rapid antigen-specific CD4+ T cell responses after the first dose, whereas CD8+ T cell responses develop more gradually (41). Specific cellular response correlated with neutralizing antibodies (42). However, the significance of cellular responses for susceptibility, independent of memory B cell responses, remains unclear (43).

1.2 Multiple sclerosis

1.2.1 Epidemiology and pathophysiology

Multiple Sclerosis (MS) is a chronic immune-mediated demyelinating disease of the central nervous system (CNS). The prevalence in Catalonia is of 73-80 cases per 100.000 people and is the leading cause of disability in young adults (44). Although the etiology of the disease is unknown, a complex interaction between multiple genetic and environmental factors is thought to be responsible for disease development. MS starts around 30 years of age and 80% of patients present with an acute, often monofocal, episode, which is known as clinically isolated syndrome (CIS). A CIS can have diverse clinical manifestations (visual disorder, walking impairment, sensory symptoms, urinary problems..) (45). The diagnosis of MS is confirmed by a combination of clinical, biological and radiological criteria (McDonald criteria 2017).(46) Natural history studies show that patients suffer successive clinical attacks and with time, recovery from each episode is incomplete and persistent symptoms accumulate. Eventually, around 65% of patients enter a progressive phase. Although different risk factors for disability progression have been suggested, currently it is not yet possible to successfully identify which patients will develop a progressive phase and which will show a faster progression of disability at the individual level (47). The impact of the disease is associated with lower employment rate (66% of patients stop working at 15 years of illness), limitations in social interaction, and greater dependence, greatly reducing the quality of life of people with MS (48).

1.2.2 Differential diagnosis

The differential diagnosis of MS includes a several inflammatory, vascular, infectious, genetic, granulomatous, and other demyelinating disorder, but depends on the clinical setting. The differential diagnosis differs depending on the syndrome, the topography and the characteristics of the MRI lesions and demographic-related characteristics of the patient (49). At the time of the CIS, red flags such as hyperacute presentation or encephalopathy, should arise the suspicion of alternative diagnoses (50,51).

Some inflammatory diseases such as systemic lupus erythematosus, Sjögren's disease, Behçet's disease, Susac's syndrome, and sarcoidosis; infections like Lyme neuroborreliosis, neurosyphilis and retroviral; or mitochondrial diseases such as cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) can produce multiple T2 hyperintense lesions on MRI that can be mistaken by MS (52).

A number of inflammatory-demyelinating disorders of the CNS must be considered in the differential diagnosis of MS, including acute disseminated encephalomyelitis (ADEM), Neuromyelitis Optica Spectrum Disorder (NMOSD) and myelin oligodendrocyte glycoprotein (MOG) antibody-associated disease (MOGAD) (49). The physiopathology of these disorders and MS is different in many pathways (53), however they all present aberrant B-cell responses like impairment of B regulatory activity, heightened production of proinflammatory cytokines, and complement activation (54). Therefore, they all may benefit from B-cell depleting therapies such as anti-CD20s (54).

ADEM is an autoimmune demyelinating disease of the CNS that presents with rapid development of focal or multifocal neurologic dysfunction and can begin following a viral infection or vaccination (55,56).

NMOSD is an inflammatory disorder of the CNS characterized by severe, immune-mediated demyelination and axonal damage predominantly targeting the optic nerves and spinal cord, but also the brain and brainstem, that is caused by pathogenic immunoglobulin G antibodies directed at the astrocytic endfoot aquaporin 4 water channel (57).

MOGAD is another disorder that starts with a variety of manifestations related to CNS demyelination (optic neuritis, ADEM, transverse myelitis, encephalitis, etc.) and can present a monophasic or relapsing course. Its diagnosis is supported by a serum positivity for MOG antibody (58,59).

1.2.3 Disease Modifying Treatments (DMT)

1.2.3.1 DMTs types and mode of action

There is currently no cure for MS, but existing immunomodulatory and immunosuppressive disease-modifying therapies (DMTs) reduce the number of clinical relapses, radiological activity and may be helpful to decrease the probability of disability progression (60). Approved immunosuppressive treatments include cladribine, fingolimod, siponimod, alemtuzumab, ocrelizumab and natalizumab and the approved immunomodulatory treatments are interferons, glatiramer acetate, teriflunomide and dimethyl fumarate. Rituximab, an anti-CD20 therapy, is being used off-label in relapsing and progressive MS form (60,61). **Table 1** summarizes the mode of action and effect on the immune system of each DMT and how said mechanism of action may influence response to vaccination.

Table 1. Mode of action, effect on the immune system, viral infection risk and vaccine response of the different treatments in MS

Class DMT	Mode of action (60)	Effect on immune system (62)	Viral infection risk (62)	Vaccine response (63,64)
Interferon-beta	Immunomodulatory, pleiotropic	Lymphopenia	Possible antiviral effect	Adequate immune responses to a variety of vaccine mechanisms
Glatiramer acetate	Immunomodulatory	Rare leukocytosis or mild leukopenia	Herpesvirus (single cases)	Reduced immune responses to influenza compared to controls (inactivated vaccine)
Teriflunomide	Dihydro-orotate dehydrogenase inhibitor (reduced de novo pyrimidine synthesis),anti-proliferative	Neutropenia	Case reports in MS patients with other immunosuppressive treatments	Modest negative effects on immune response to influenza and rabies vaccines. Incompatibility with attenuated vaccines
Dimethyl fumarate	Pleotropic, Nrf2 activation, downregulation of NFKβ	Lymphopenia	JCV in patients with MS and psoriasis and in patients treated with other immunosuppressive treatment	Adequate immune responses to influenza. Incompatibility attenuated vaccines

SP1RM (Fingolimod or siponimod)	Sphingosine 1-phosphate receptor functional antagonist	Peripheric lymphopenia	Herpesvirus	Blunted responses to influenza inactivated and toxoid vaccines. Incompatibility attenuated vaccines
Natalizumab	Anti-α4-integrin antibody	Diminished immune surveillance in the CNS	JCV (PML) and herpesvirus	Adequate immune responses to influenza.
Cladribine	Synthetic purine nucleoside analogue that inhibits DNA synthesis selectively, mainly in circulating T cells and B cells	Depletion: immature B cells (6-9 months), memory B cells (>1 year), CD4 (40-50%), CD8 (30-40%), NK (50%)	Limited increased risk (some herpesvirus)	No relevant studies
Anti-CD20s (ocrelizumab, rituximab or ofatumumab)	Anti-CD20 antibody	Depletion: Immature B cells, memory B cells, CD4 (40-50%), CD8 (30-40%), NK (50%)	Long-term use increases severe infection risk Hepatitis B and C viruses' reactivation JCV (PML)	Impaired vaccine responses, especially to neoantigens and T cell-independent antigens. Incompatibility attenuated vaccines
Alemtuzumab	Monoclonal anti-CD52 antibody	Depletion: immature B cells (3-6m), memory B cells (>1 year), CD4 (70-90%), CD8 (70-90%), NK (40%), Monocytes (1-2 months), Neutrophiles	Early infection risk (Herpesvirus)	Responses to multiple vaccine types but blunted for vaccinations within 6 months of dosing

1.2.3.2 DMTs and infection risk

Patients with MS have been shown to be at a higher risk of viral infections and related mortality than the general population (65,66). Some studies suggest that infections in MS patients may increase the risk of relapses, radiological activity o increase progression (67,68). Additionally, with the rise of immunosuppressive treatment use, the infection rate is MS patients has increased. These new treatments may alter humoral and/or cellular

immunity, leading to an increased risk of latent infections reactivation or acquiring new infections (69,70).

It is unclear whether immunosuppressive therapy may be beneficial or detrimental to the immune response against SARS-CoV-2 infection: while it might impair the immune system's ability to control viral replication, an exaggerated immune response has been associated with a severe disease in the general population (71). Preliminary data suggest that although MS patients do not have an increased risk of COVID-19 compared to the general population, anti-CD20s therapies may increase both the risk and the severity of COVID-19 in MS patients (72–74).

1.2.3.3 DMTs and vaccine response

Although some studies have shown that vaccines are safe in patients with MS (75,76), questions remain about the effectiveness of vaccines in patients already under immunosuppressive drugs because they can interfere with the immune response to the vaccine (77). The few available studies show different immune response depending on the DMT, with a good response to influenza vaccine in patients under treatment with interferon, teriflunomide, and dimethyl fumarate, but a certain reduction in the vaccine response in patients undergoing treatment with fingolimod, natalizumab or ocrelizumab compared with healthy controls (63).

The evidence regarding the immunogenicity of vaccines in patients with MS comes from a few clinical trials conducted in the context of the preclinical and clinical drug development (78–80). Specifically in B-cell-depleting treatments, the clinical trial VELOCE showed that in patients treated with ocrelizumab, vaccine responses to neoantigens and T cell-independent antigens are significantly impaired with a diminished humoral response (81).

2. THESIS JUSTIFICATION

Considering that MS patients present an increased risk of morbimortality due to infections, getting to know the relationship between SARS-CoV-2 infection and MS patients is critical, especially in those with immunosuppressive treatment.

Although there are some studies on the risk factors for COVID-19 susceptibility and severity, humoral and cellular response to COVID-19 and clinical trials demonstrating the efficacy and safeness of SARS-CoV-2 vaccines, most were conducted in healthy people without autoimmune diseases. Therefore, there are still important gaps in knowledge at three main levels: 1) the COVID-19 susceptibility and severity risk factors in patients with immunosuppressive treatments, 2) the humoral and cellular response to COVID-19 in patients with immunosuppressive treatments and 3) the unknown immune response and effectiveness of SARS-CoV-2 vaccination in the context of immunosuppressive therapies.

Therefore, understanding the risk factors for severe COVID-19 in MS patients, how the impact of different treatments on the immune response against SARS-CoV-2 infection varies in MS and other autoimmune diseases, and how this may affect the effectiveness of the vaccines is imperative for the safe management of MS patients during the COVID-19 pandemic. Consequently, the aims of this study are to investigate the susceptibility and severity risk factors of COVID-19, the serological and cellular response to COVID-19 infection or SARS-CoV-2 vaccination in patients with MS and other autoimmune disease under immunosuppressive treatments and to evaluate the changes implemented in clinical practice due to these risk factors and the COVID-19 pandemic.

3. HYPOTHESES

1. COVID-19 incidence, susceptibility and severity are higher in MS patients who receive immunosuppressive treatments than in those without immunosuppressive treatments or untreated, and susceptibility can be influenced by other demographical, clinical or laboratory variables.

2. MS patients treated with immunosuppressive treatments who had COVID-19 have a reduced and shorter-lived humoral and cellular immunity against SARS-CoV-2 infection than MS patients with other treatments or no treatment.

3. People with MS or other autoimmune disease treated with immunosuppressive treatments who have been vaccinated against SARS-CoV-2 have a reduced humoral and cellular immunity against SARS-CoV-2 vaccine compared to MS patients with other treatments or no treatment.

4. During the COVID-19 pandemic the number of clinical visits, magnetic resonance imaging studies and immunosuppressive treatment prescriptions will be reduced compared to the year prior to the pandemic.

4. OBJECTIVES

Main objective

To investigate the susceptibility and severity risk factors of COVID-19 and the immunological responses to SARS-CoV-2 infection and vaccination in patients with MS and other autoimmune disease under immunosuppressive treatments and to evaluate the changes implemented in clinical practice due to these factors.

Secondary objectives

1. To determine the incidence of SARS-CoV-2 infection and susceptibility and severity risk factors in MS patients.

2. To determine the humoral and cellular responses against SARS-CoV-2 infection and their persistence in patients with MS treated with immunosuppressive treatments compared to patients with MS under other treatments or without treatment.
    a. To determine the demographic, clinical and laboratory factors that influence the presence and persistence of humoral and cellular immune responses to SARS-CoV-2 infection.

3. To determine the humoral and cellular responses against SARS-CoV-2 vaccines in patients with MS or other autoimmune disease treated with immunosuppressive treatments compared to patients with MS under other treatments or without treatment.
    a. To determine the demographic, clinical and laboratory factors that influence the presence of humoral and cellular immune responses to SARS-CoV-2 vaccine.

4. To describe the impact of the COVID-19 pandemic on the number of clinical visits, magnetic resonance scans and treatment prescriptions at Cemcat compared to the year prior to the pandemic.

5. COMPENDIUM OF PUBLICATIONS

5.1 Article 1

COVID-19 in multiple sclerosis patients: susceptibility, severity risk factors and serological response

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ORIGINAL ARTICLE

COVID-19 in multiple sclerosis patients: susceptibility, severity risk factors and serological response

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Abstract

Background and purpose: Information regarding multiple sclerosis (MS) patients with the 2019 novel coronavirus disease (COVID-19) is scarce. The study objective was to describe the incidence and characteristics of MS patients with COVID-19, to identify susceptibility and severity risk factors and to assess the proportion of positive severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) serologies according to disease-modifying treatments.

Methods: This was a retrospective study of an MS cohort analysing data collected between February and May 2020. Cases were identified through an email survey and clinical visits. The relationship of demographic and MS characteristics with COVID-19 and of the disease-modifying treatments with SARS-CoV-2 serostatus were examined.

Results: Data from 48 suspected cases out of 758 valid respondents and from 45 COVID-19 cases identified through clinical visits were collected. Incidence was 6.3%. Nineteen (20.3%) patients were hospitalized and two (2.2%) died. Multivariable models determined that age (odds ratio [OR] per 10 years 0.53, 95% confidence interval [CI] 0.34–0.85), contact with a confirmed case (OR 197.02, 95% CI 56.36–688.79), residence in Barcelona (OR 2.23, 95% CI 1.03–4.80), MS duration (OR per 5 years 1.41, 95% CI 1.09–1.83) and time on anti-CD20 treatment (OR per 2 years 3.48, 95% CI 1.44–8.45) were independent factors for presenting COVID-19 and age (OR per 10 years 2.71, 95% CI 1.13–6.53) for a severe COVID-19. Out of the 79 (84.9%) with serological test, 45.6% generated antibodies, but only 17.6% of those on anti-CD20 therapies. Lymphopaenia or immunoglobulin levels did not relate to COVID-19.

Conclusions: Multiple sclerosis patients present similar incidence, risk factors and outcomes for COVID-19 as the general population. Patients treated with an anti-CD20 therapy for a longer period of time might be at a higher risk of COVID-19 and less than 20% generate an antibody response. Only age was related to severity.

KEYWORDS
COVID-19, disease-modifying therapy, multiple sclerosis, risk factors, SARS-CoV-2

INTRODUCTION

The 2019 novel coronavirus disease (COVID-19) is a pandemic infection caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [1] The region of Catalonia has been one of the hardest hit in Europe with around 241,570 cases and 14,140 deaths reported on 3 November 2020 [2,3]

People with multiple sclerosis (MS) on disease-modifying therapies (DMTs), particularly immunosuppressants, have been shown to be at a higher risk of infection and related mortality than the general population [4,5]

It is unclear whether immunosuppressive therapy is beneficial or detrimental to the immune response against SARS-CoV-2 infection [6] On this line, some of the recent reports suggest that, although MS patients do not have an augmented risk of COVID-19 compared to the general population, anti-CD20 treatment may increase the risk of COVID-19 and of a severe infection [7,8] However, whether these treatments may impact the antibody production against SARS-CoV-2 or the immune reaction to a future vaccine is still unknown.

Therefore, the aims of this study were to investigate the incidence of COVID-19 in patients with MS followed at our centre, to describe their characteristics, to identify candidate risk factors for COVID-19 susceptibility and for a severe disease in our cohort and to describe SARS-CoV-2 serological results according to the different DMTs.

PATIENTS AND METHODS

This is a retrospective study on a cohort of MS patients, conducted at the Multiple Sclerosis Centre of Catalonia (Cemcat) in Barcelona between 1 February and 7 May 2020.

Study population

All patients followed at our centre who consented to be contacted by email were invited to complete a self-administered survey sent via email that retrospectively collected COVID-19 symptoms, diagnosis of COVID-19 by a physician, hospitalization due to COVID-19, and number of cohabitants/cohabitants with symptoms/cohabitants with confirmed COVID-19 (positive SARS-CoV-2 polymerase chain reaction [PCR]). An invitation to participate with the link to access the survey online and the informed consent form was sent every 2 weeks starting on 15 April and finishing on 6 May.

From the patients who answered the survey, those older than 18 years of age with the following diagnoses were included: clinically isolated syndrome, relapsing–remitting MS, secondary progressive MS or primary progressive MS [9] Patients without MS, without a clear diagnosis or without follow-up after February 2019 were excluded. MS patients with COVID-19 were also detected through spontaneous phone consultations from patients or during follow-ups.

Following the European Centre for Disease Prevention and Control guidance [10] patients with fever, dyspnoea, persistent cough or sudden onset of anosmia, ageusia or dysgeusia after February 2020 or radiological images compatible with COVID-19 were considered suspected cases and those with a positive SARS-CoV-2 PCR were considered confirmed cases. Due to regional health policies, at that time PCR was generally restricted to patients admitted to hospitals.

Patients from the survey were classified according to the COVID-19 case definition in suspected COVID-19 and non-COVID-19 cases. Suspected COVID-19 cases were interviewed by phone by experienced professionals from our department to collect data in relation to COVID-19. Data were collected in a REDCap-based [11] electronic case report form.

Demographic and clinical data

Demographic, clinical, laboratory and MS information, including DMT use, were retrieved from the hospital electronic health records of all survey responders and patients detected spontaneously. As a proxy for socioeconomic status, the patient's postal code was used to extract the average annual wage and it was categorized by personal income tax brackets (<€22,200, €22,000–€35,200, >€35,200) [12] Laboratory findings measured after February 2019 included absolute lymphocyte count (cell/m^3) (ALC) and vitamin D (ng/ml). In patients treated with anti-CD20 therapies, CD19+ lymphocytes (cell/μl; per cent of ALC) and immunoglobulins (IgM, IgG and IgA; mg/dl) were also collected. Laboratory variables were categorized in relation to their low-range normal value (i.e., vitamin D, 30; IgM, 40; IgG, 700; IgA, 70) and lymphopaenia was categorized according to the common terminology criteria for adverse events [13]

In COVID-19 patients, data related to symptoms, SARS-CoV-2 PCR, laboratory and radiological variables (i.e., lactate dehydrogenase [UI/l], D-dimer [ng/ml] and interleukin-6 [pg/ml]), outcome at the moment of analysis (recovered, improving, worsening or death) and MS relapses within 1 month of COVID-19 were collected. COVID-19 severity was categorized as (1) mild–moderate disease if patients had no signs or symptoms of pneumonia or a mild pneumonia and (2) severe–critical disease if they presented dyspnoea, or a respiratory rate of ≥30 breaths per minute or a blood oxygen saturation of ≤93%, or a ratio of the partial pressure of arterial oxygen to the fraction of inspired oxygen of <300 mmHg, or infiltrates in >50% of the lung field within 24–48 h from the onset of symptoms and/or organ or multiple organ failure [14] Serological SARS-CoV-2 testing was performed in suspected COVID-19 cases with chemiluminescence immunoassays and, in cases of unclear result, confirmed with an enzyme-linked immunosorbent assay.

Statistical analysis

For the purpose of this study, the data capture was locked on 7 May 2020. Descriptive statistics were used to compare demographics

and disease characteristics according to the classification of suspected cases and severity.

Univariable logistic regression models were performed on identified variables to assess their association with COVID-19 susceptibility or severity. Expanded Disability Status Scale was segmented into three categories (<3.0, 3–5.5 and ≥6.0). DMTs were categorized according to theoretical COVID-19 risk [6] (no risk, interferon beta and glatiramer; low–intermediate risk, teriflunomide, dimethylfumarate, natalizumab and fingolimod; high risk, cladribine and alemtuzumab, anti-CD20 therapy). Pearson's chi-squared test, Fisher's exact test, Student's *t* test and the Mann–Whitney *U* test were used for comparisons as appropriate. Odds ratios (ORs) with 95% confidence intervals (CIs) were estimated with a logistic regression model. A multivariable logistic regression model was performed to determine which variables are independently associated with presenting COVID-19 or a severe COVID-19. Variable selection was done through backward elimination (*p* value out <0.2) and forward selection (*p* value in <0.2) by Akaike's information criterion minimization.

The incidence of COVID-19 was estimated by dividing the number of suspected or confirmed COVID-19 cases detected through the survey by the total number of survey responders.

For the purpose of this analysis, COVID-19 susceptibility was studied using the data from patients who answered the survey and COVID-19 severity was characterized using the total number of suspected COVID-19 cases (survey and spontaneous detection methods). In order to address the susceptibility risk of COVID-19 in patients treated with anti-CD20 therapies, the demographic and laboratory characteristics of these patients were also analysed separately, and a sensitivity analysis of the cases was performed. COVID-19 severity and serological status were studied in those patients with COVID-19 suspicion.

Statistical tests were performed at the 0.05 level of significance using the IBM SPSS Statistics version 22.0 (SPSS Inc., Chicago, IL, USA) and GraphPad Prism version 7.0 (GraphPad Software, La Jolla, CA, USA).

The study was approved by the ethics committee of the Vall d'Hebron University Hospital. Patient consent was obtained.

RESULTS

Patient identification and COVID-19 incidence

Out of the 2903 surveys sent, a total of 875 were answered with a response rate of 30.1%. Of these, 117 (13.4%) patients were excluded for not meeting the general inclusion criteria. Of the remaining cases, 48 met the definition criteria of COVID-19 and the remaining 710 were classified as non-COVID-19. The incidence of COVID-19 in our cohort was estimated at 6.3% (95% CI 4.6%–8.1%). Additionally, 45 suspected COVID-19 cases were detected through the spontaneous method. Overall, 93 suspected COVID-19 cases were identified (Figure 1).

COVID-19 susceptibility

Demographic, clinical and laboratory characteristics of the COVID-19 and non-COVID-19 patients who responded to the survey are summarized in Table 1. Demographic, MS characteristics and

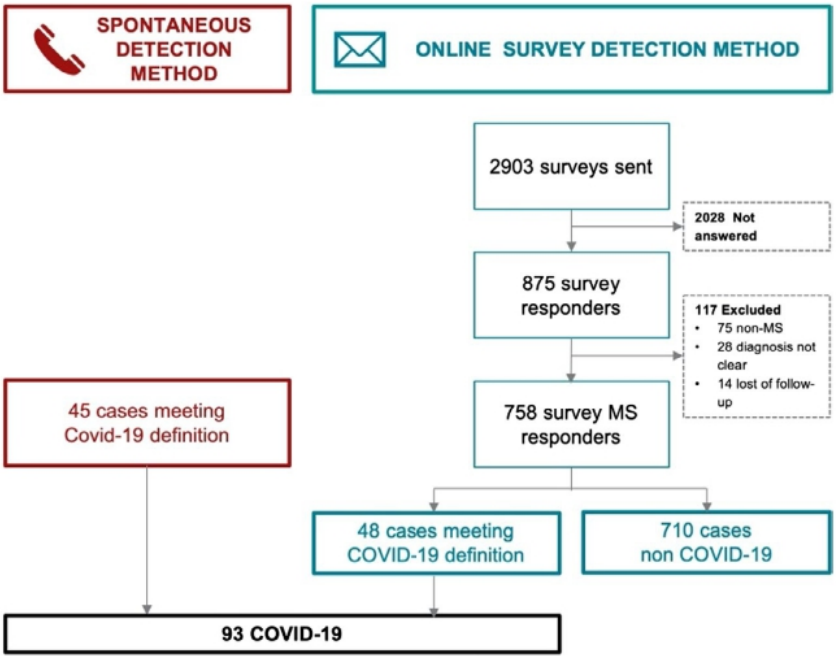

FIGURE 1 Study flow diagram. Patients selected through the online survey detection method (green box), spontaneous detection method (red box), excluded (grey dotted box) and total COVID-19 cases (black box)

TABLE 1 Demographic characteristics, COVID-19 previous contact, MS characteristics, DMTs and previous laboratory findings of the survey respondents in relation to COVID-19 disease

	COVID-19 *n* = 48	Non-COVID-19 *n* = 710
Demographics		
Age, years, mean (SD)	43.95 (9.22)	45.03 (14.86)
Female sex, *n* (%)	35 (72.9)	490 (69.0)
Average net wage <€20,200, *n* (%)[a]	4 (14.3)	39 (11.8)
Average net wage €20,200–€35,200, *n* (%)[a]	23 (82.1)	255 (77.0)
Average net wage >€35,200, *n* (%)[a]	1 (3.6)	35 (10.6)
Residence in Barcelona, *n* (%)	26 (54.2)	255 (35.9)
Caucasian ethnicity, *n* (%)	47 (97.9)	708 (99.7)
Pregnancy, *n* (%)	0 (0)	3 (0.6)
Previous COVID-19 contact		
Number of cohabitants, median (IQR)	2 (2)	2 (2)
Cohabitants with symptoms, *n* (%)	24 (50.0)	4 (0.6)
Contact with PCR+ (home, community, healthcare), *n* (%)	22 (45.8)	5 (0.7)
Comorbidities		
Smoker, *n* (%)	7 (14.6)	99 (13.9)
Any comorbidity, *n* (%)	17 (35.4)	150 (21.1)
Two or more comorbidities, *n* (%)	15 (31.3)	131 (18.5)
Lung disease, *n* (%)	3 (6.3)	20 (2.8)
Cardiac disease, *n* (%)	–	11 (1.5)
Stroke, *n* (%)	–	9 (1.3)
Haematological, *n* (%)	–	21 (3.0)
Diabetes, *n* (%)	2 (4.2)	13 (1.8)
Obesity, *n* (%)[b]	6 (13.6)	71 (15.9)
Hypertension, *n* (%)	7 (14.6)	60 (8.5)
Liver disease, *n* (%)	1 (2.1)	1 (0.1)
Chronic kidney disease, *n* (%)	–	1 (0.1)
Cancer, *n* (%)	2 (4.2)	16 (2.3)
HIV, *n* (%)	–	–
MS clinical characteristics		
Type MS, *n* (%)		
CIS	2 (4.2)	31 (4.4)
RRMS	35 (72.9)	501 (70.6)
SPMS	7 (14.6)	126 (17.7)
PPMS	4 (8.3)	52 (7.3)
Disease duration, years, median (IQR)	14.46 (13.1)	10.93 (15.0)
EDSS, median (IQR)	2.0 (3.5)	2 (3.0)
Corticosteroids past 3 months, *n* (%)	2 (4.2)	23 (3.3)
Disease-modifying treatment		
DMT, *n* (%)	43 (89.6)	558 (79)
Type DMT, *n* (%)		
First line	24 (51.1)	299 (42.4)
Second line	18 (38.3)	235 (33.3)
Other	1 (2.1)	24 (3.4)
Specific DMT, *n* (%)		
Interferon	12 (25)	102 (14.4)
Copaxone	2 (4.2)	57 (8.1)
Dimethylfumarate	5 (10.4)	96 (13.6)
Teriflunomide	5 (10.4)	45 (6.4)
Fingolimod	4 (8.3)	30 (4.2)
Natalizumab	2 (4.2)	30 (4.2)
Cladribine	–	10 (1.4)
Alemtuzumab	2 (4.2)	36 (5.1)
Anti-CD20	10 (13.15)	130 (18.31)
Ocrelizumab	3 (6.3)	55 (7.8)
Rituximab	7 (14.6)	74 (10.5)
Other	1 (2.1)	1 (0.1)
Treatment duration, years, median (IQR)	3.22 (4.10)	1.54 (3.41)
Previous laboratory findings		
Absolute lymphocyte count, median (IQR)[b]	1545 (1100)	1660 (1030)
Absolute lymphocyte count <1000/μl, *n* (%)[b]	13 (29.5)	131 (25.4)
Lymphopaenia grade 1, *n* (%)[b]	11 (25.0)	87 (16.9)
Lymphopaenia grade 2, *n* (%)[b]	1 (2.3)	36 (7.0)
Lymphopaenia grade 3, *n* (%)[b]	1 (2.3)	8 (1.6)
Lymphopaenia grade 4, *n* (%)[b]	–	–
Vitamin D, median (IQR)[c]	24.70 (9.8)	25.6 (19.78)
Vitamin D <30 ng/ml, *n* (%)	8 (66.7)	215 (62.1)

Note: Count of total cases of variables: [a]COVID 28, non-COVID 340; [b]COVID 44, non-COVID 533; [c]COVID 12; non-COVID 357.

Abbreviations: CIS, clinical isolated syndrome; COVID-19, 2019 novel coronavirus disease; DMT, disease-modifying therapy; EDSS, Expanded Disability Status Scale; HIV, human immunodeficiency virus; IQR, interquartile range; MS, multiple sclerosis; PCR, polymerase chain reaction; PPMS, primary progressive multiple sclerosis; RRMS, relapsing–remitting multiple sclerosis; SPMS, secondary progressive multiple sclerosis.

proportion of specific DMTs were similar in COVID-19 and non-COVID-19 cases. Although lymphopaenia was detected in 144 patients, neither the presence of lymphopaenia nor its grade increased the risk of COVID-19. The multivariable analysis revealed that younger age, contact in the community with a confirmed COVID-19

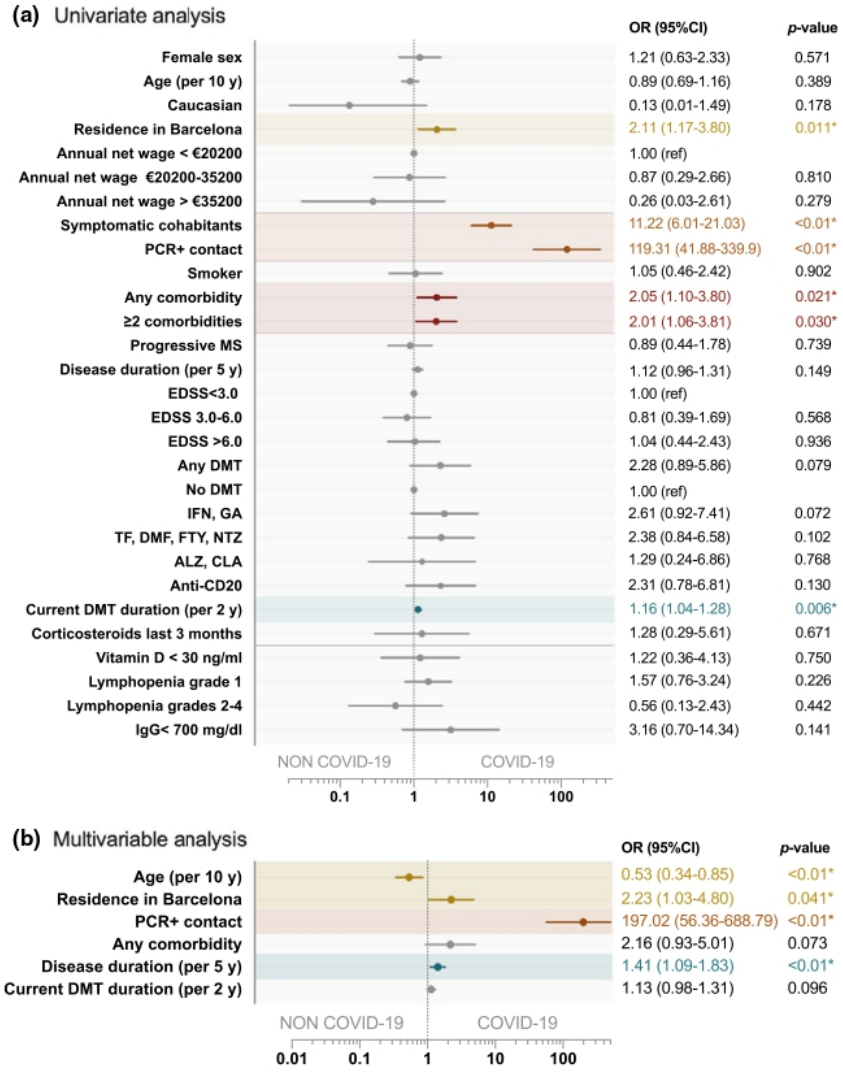

FIGURE 2 Risk factors for COVID-19 susceptibility. (a) Univariate analysis. (b) Multivariable analysis. Forest plot depicting unadjusted odds ratio (OR) for presenting COVID-19 in our cohort. Demographic and clinical characteristics, comorbidities and laboratory data are represented with OR, 95% confidence interval (95% CI) and *p* value. Variables with a *p* value ≤0.05 are highlighted with colours and marked with an asterisk. In dichotomous variables, the reference is not specified. PCR, polymerase chain reaction; any comorbidity and two or more comorbidities include obesity, lung disease, cardiovascular disease, diabetes, hypertension, haematological benign disease, chronic kidney disease, liver disease, HIV or malignancy; progressive MS includes secondary progressive MS and primary progressive MS; DMT, disease-modifying therapy; IFN, interferon; GA, glatiramer acetate; TFN, teriflunomide; DMF, dimethylfumarate; NTZ, natalizumab; FTY, fingolimod; CLA, cladribine; ALZ, alemtuzumab; anti-CD20 therapies include ocrelizumab and rituximab; lymphopaenia grade 1, ALC 1000–800 c/μl; lymphopaenia grade 2–4, ALC <800 c/μl; IgG, immunoglobulin G

case, living in Barcelona city and longer MS disease duration persisted to be risk factors for COVID-19 (Figure 2).

When exploring the 144 patients treated with anti-CD20 therapies who answered the survey, 10 (6.9%) had suspected COVID-19 infection. Amongst anti-CD20 treated patients, demographic and MS characteristics of patients with COVID-19 were similar to those without COVID-19 except for the median (interquartile range [IQR]) anti-CD20 treatment duration which was slightly longer in COVID-19 cases (2.77, IQR 3.14, vs. 1.19, IQR 1.32 years, $p = 0.069$). Regarding laboratory findings, three COVID-19 cases had IgG hypogammaglobulinaemia whereas no non-COVID-19 cases presented with it. No differences of IgM or IgA levels or CD19+ proportion were found. Lymphopaenia was present in a similar proportion in both groups (Data S1). The multivariable analysis showed that younger patients (OR per 10 years 0.032, 95% CI 0.12–0.85, $p = 0.023$) and a longer anti-CD20 treatment duration increased the risk of COVID-19 (OR per 2 years 3.48, 95% CI 1.44–8.45, $p < 0.01$).

COVID-19 severity

The demographic and clinical characteristics of the 93 suspected/confirmed COVID-19 cases in our centre are summarized in Table 2. Nineteen (20.4%) patients were hospitalized, nine (9.7%) had a severe or critical disease course and two (2.2%) patients died. In the

TABLE 2 Demographic characteristics, MS characteristics, DMTs, previous laboratory findings, COVID-19 symptoms, COVID-19 diagnostic tests, laboratory findings and outcome of total COVID-19 suspected cases and according to COVID-19 severity

	Total *n* = 93	Mild–moderate *n* = 84	Severe–critical *n* = 9
Demographics			
Age, years, mean (SD)	45.63 (10.79)	44.20 (9.57)	58.96 (12.93)
Female sex, *n* (%)	62 (66.7)	55 (65.5)	7 (77.8)
Average net wage <€20,200, *n* (%)[a]	5 (9.8)	4 (8.7)	1 (20.0)
Average net wage €20,200–€35,200, *n* (%)[a]	43 (84.3)	40 (87.0)	3 (60.0)
Average net wage >€35,200, *n* (%)[a]	3 (5.9)	2 (4.3)	1 (20)
Any comorbidity, *n* (%)	32 (34.4)	26 (31.0)	6 (66.7)
Two or more comorbidities, *n* (%)	27 (29.0)	21 (25.0)	6 (66.7)
MS characteristics			
EDSS, median (IQR)	2.0 (2.0)	2.0 (2.0)	3.0 (2.5)
Disease duration, years, median (IQR)	15.1 (10.54)	14.93 (12.0)	20.09 (10.9)
Type of MS, *n* (%)			
CIS	5 (5.4)	5 (6.0)	–
RRMS	66 (71.0)	62 (72.8)	4 (44.4)
SPMS	17 (18.3)	13 (15.5)	4 (44.4)
PPMS	5 (5.4)	4 (4.8)	1 (11.1)
DMT, *n* (%)	72 (77.4)	69 (82.1)	3 (33.3)
Type of DMT, *n* (%)			
None	21 (22.6)	15 (17.9)	6 (66.7)
First line	38 (40.9)	38 (45.2)	–
Second line	31 (33.3)	29 (34.5)	2 (22.2)
Other	3 (3.2)	2 (2.4)	1 (11.1)
Treatment duration, years, median (IQR)	3.18 (4.68)	3.18 (4.68)	3.56 (12.04)
Corticosteroids last 3 months, *n* (%)	5 (5.4)	5 (6.0)	–
COVID-19 symptoms			
Fever, *n* (%)	39 (41.9)	47 (56.0)	7 (77.8)
Cough, *n* (%)	59 (63.4)	55 (65.5)	4 (44.4)
Dyspnoea, *n* (%)	35 (37.6)	30 (35.7)	5 (55.6)
Hyposmia/anosmia, *n* (%)	40 (43.0)	38 (45.2)	2 (22.2)
Dysgeusia, *n* (%)	37 (39.8)	35 (41.7)	2 (22.2)
Isolated anosmia/ageusia, *n* (%)	4 (4.3)	4 (4.84)	–
Headache, *n* (%)	47 (50.5)	42 (50.0)	5 (55.6)
Fatigue, *n* (%)	47 (50.5)	44 (52.4)	3 (33.3)
Arthralgia, *n* (%)	29 (31.2)	27 (32.1)	2 (22.2)
Diarrhoea, *n* (%)	25 (26.9)	24 (28.6)	1 (11.1)
Chills, *n* (%)	23 (24.7)	20 (23.8)	3 (33.3)
Sore throat, *n* (%)	23 (24.7)	22 (26.2)	1 (11.1)
Muscle aches, *n* (%)	15 (16.1)	15 (17.9)	–
Nasal congestion, *n* (%)	16 (17.2)	15 (17.9)	1 (11.1)
Sputum, *n* (%)	16 (17.2)	14 (16.7)	2 (22.2)
Red/itchy eyes, *n* (%)	2 (2.2)	2 (2.4)	–
Laboratory and X-ray findings			
Previous lymphocyte count, median (IQR)[b]	1630 (980)	1660 (1030)	1500 (900)
Absolute lymphocyte count <1000/μl, *n* (%)[b]	21 (25.0)	19 (25.3)	2 (22.2)

(Continues)

TABLE 2 (Continued)

	Total *n* = 93	Mild–moderate *n* = 84	Severe–critical *n* = 9
Lymphopaenia grade 1, *n* (%)[b]	16 (19.0)	15 (20.0)	1 (11.1)
Lymphopaenia grade 2–4, *n* (%)[b]	5 (6.0)	4 (5.3)	1 (11.1)
Vitamin D, median (IQR)[c]	25.2 (13.4)	27.6 (12.8)	14.4 (0)
Vitamin D <30 ng/ml, *n* (%)[c]	14 (51.9)	13 (50.0)	1 (100.0)
Leukocyte count, median (IQR)[d]	4295 (4750)	4295 (4550)	4050 (3710)
Lymphocyte count, median (IQR)[e]	900 (1010)	1390 (1010)	550 (400)
Haemoglobin (mg/dl), median (IQR)[e]	12.0 (2.0)	13.0 (2.0)	11.0 (1.0)
Platelets, median (IQR)[f]	156000 (96000)	139000 (96000)	201000 (6000)
D-dimer, median (IQR)[g]	320 (274)	240 (115)	424 (234)
LDH, median (IQR)[h]	292 (183)	212 (203)	351 (202)
IL-6, median (IQR)[i]	17.39 (40.85)	12.48 (10.06)	85.06 (16.62)
IL-6 >40 ng/ml, *n* (%)[i]	2 (25.0)	–	2 (100.0)
Abnormal thorax X-ray[j]	21 (22.8)	12 (14.5)	9 (100.0)
MS relapse associated with COVID-19			
MS symptom worsening, *n* (%)	4 (4.3)	4 (4.76)	–
MS relapse during COVID-19	4 (4.3)	4 (4.76)	–
COVID-19 outcome			
Hospitalization, *n* (%)	19 (20.4)	10 (11.9)	9 (100.0)
Fully recovered, *n* (%)	72 (77.4)	69 (82.1)	3 (33.3)
Improving, *n* (%)	18 (19.4)	15 (17.9)	3 (33.3)
Worsening, *n* (%)	1 (1.1)	–	1 (11.1)
Death, *n* (%)	2 (2.2)	–	2 (22.2)
PCR SARS-CoV-2			
Positive PCR, *n* (%)[k]	22/32 (68.8)	13/23 (56.5)	9/9 (100.00)
Positive serologies, *n* (%)[l]	36/79 (45.6)	33/73 (45.2)	3/6 (50.0)
Months after debut of COVID-19 of serologies, mean (IQR)	3.02 (0.59)	3.04 (0.59)	2.89 (0.69)
Any test positive, *n* (%)[m]	47/86 (54.7)	38/77 (49.4)	9/9 (100.00)

Notes: Detection method of the 93 suspected COVID-19 cases: 48 online survey method and 45 spontaneous method through telephone calls.

Count of total cases of variables with missing information: [a]mild–moderate, 46; severe, 5; [b]mild–moderate, 75; severe, 9; [c]mild–moderate, 26; severe, 1; [d]mild–moderate, 6; severe, 4; [e]mild–moderate, 9; severe, 4; [f]mild–moderate, 7; severe, 2; [g]mild–moderate, 7; severe, 7; [h]mild–moderate, 6; severe, 4; [i]mild–moderate, 6; severe, 2; [j]mild-moderate, 83; severe, 9; [k]mild–moderate, 23; severe, 9; [l]mild–moderate, 73; severe, 6; [m]mild–moderate, 77; severe, 9.

Abbreviations: CIS, clinical isolated syndrome; COVID-19, 2019 novel coronavirus disease; DMT, disease-modifying therapy; EDSS, Expanded Disability Status Scale; IL-6, interleukin 6; IQR, interquartile range; LDH, lactate dehydrogenase; MS, multiple sclerosis; PCR, polymerase chain reaction; PPMS, primary progressive multiple sclerosis; RRMS, relapsing–remitting multiple sclerosis; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SPMS, secondary progressive multiple sclerosis.

univariate analysis, older patients with longer disease duration, a higher disability, a progressive form and previous comorbidities presented a higher risk of a severe COVID-19 disease. In the multivariable analysis, only age remained as an independent risk factor of a severe COVID-19 (Figure 3). Neither previous lymphopaenia nor lymphopaenia during the COVID-19 disease was associated with a more critical disease course. In the mild–moderate group, all patients had either completely recovered or were improving at the time of our analysis. By contrast, all patients in the severe group required hospitalization and two of them required mechanical ventilation and died. Both were aged 68 years, had previous comorbidities and a progressive form of MS. Neither of them was on a DMT and their Expanded Disability Status Scale scores were 4.5 and 8.5, respectively. During the infection, both had elevated inflammatory biomarkers (D-dimer, interleukin 6 or ferritin levels). Two patients with a mild infection presented an MS relapse within the first month of COVID infection, both requiring corticosteroid treatment.

SARS-CoV-2 PCR and serological tests

In relation to SARS-CoV-2 testing, 84 out of the 93 (90.3%) COVID-19 studied cases were tested through PCR, serological test or both. Nine (100%) severe cases and 38 (38/77; 49.4%) of the mild–moderate

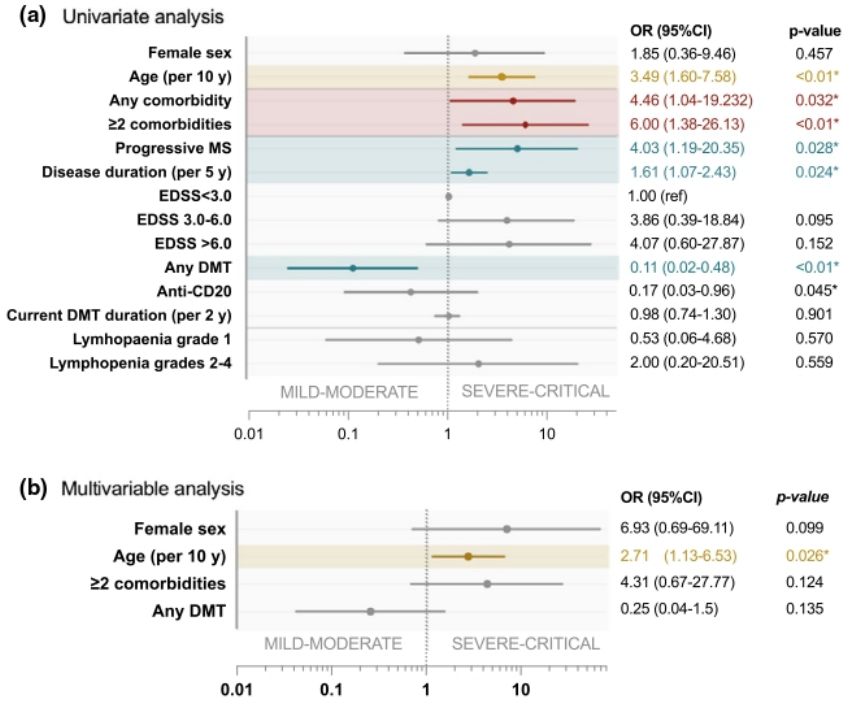

FIGURE 3 Risk factors for a severe–critical course of COVID-19. (a) Univariate analysis. (b) Multivariable analysis. Forest plot depicting unadjusted odds ratio (OR) for presenting severe COVID-19 in our cohort. Demographic and clinical characteristics, comorbidities and laboratory data are represented with OR, 95% confidence interval (95% CI) and *p* value. Variables with a *p* value ≤0.05 are highlighted with colours and marked with an asterisk. In dichotomous variables, the reference is not specified. Any comorbidity and two or more comorbidities include obesity, lung disease, cardiovascular disease, diabetes, hypertension, haematological benign disease, chronic kidney disease, liver disease, HIV or malignancy; progressive MS includes secondary progressive MS and primary progressive MS; DMT, disease-modifying therapy; anti-CD20 therapies include ocrelizumab and rituximab; lymphopaenia grade 1, ALC 1000–800 c/μl; lymphopaenia grade 2–4, ALC <800 c/μl

cases were confirmed COVID-19 cases through PCR, serological or both tests (Table 2). No differences in positive PCR results in relation to treatment were found (anti-CD20, 4/6 [66.7%]; other DMTs, 8/13 [61.5%]; and patients without DMTs, 10/13 [76.9%]). Serological tests were performed in 79 (84.9%) patients around 3 months after COVID-19 symptom onset (median 3.12 months, IQR 2.83–3.25). Patients on anti-CD20 therapies presented a lower proportion of positive serological tests (3/19; 15.8%) than those with other DMTs (20/41; 48.8%; $p = 0.045$) or without DMTs (13/19; 68.4%; $p = 0.003$). Although a serological response was found in patients with all types of DMTs, including anti-CD20s, the proportion of positive serological tests varied depending on the DMT (Figure 4). Four cases presented a positive PCR and were afterwards seronegative: two on rituximab, one on alemtuzumab (83.3 weeks after last alemtuzumab infusion) and one without treatment. Conversely, three cases had an initial negative PCR with positive serology (two on interferon and one on rituximab).

DISCUSSION

As of 7 May 2020, the incidence of COVID-19 amongst our cohort was estimated at 6.3% (95% CI 4.6%–8.1%), similar to the highly affected region of Catalonia (6.1%). As in the general population, the incidence is increased in Barcelona city residents [15]

Our data indicate that clinical characteristics do not differ greatly in COVID-19 patients with MS from non-infected MS patients. This is in line with other reports of COVID-19 in MS patients [8,16–18] and the demographics of COVID-19 outpatients in the Catalan region [19] except for COVID-19 cases being younger and with a longer disease duration in our cohort. It could be argued that the younger age of COVID-19 cases is a reflection of the online selection method, with young people being more prone to participate, or because this age group is less inclined to follow stringent physical distancing measures. However, this results could also be due to the reduced number of participants. In this sense, we participated in a study to assess COVID-19 susceptibility factors with two American MS centres with a larger number of responders (3028), where age did not remain an independent factor for COVID-19. [18]

As previously described, having had contact with a PCR-positive person [8,18] and living in a very populated place such as Barcelona was strongly associated with COVID-19 [20] as well as a longer MS disease duration [8] In fact, the province of Barcelona is one of the most affected areas in Spain, and the one with the highest seroprevalence of SARS-CoV-2 in Catalonia [15] Although in other regions the risk of SARS-CoV-2 infection and of COVID-19 severe course and hospital admission was higher in low income areas [21] no differences regarding socioeconomic status were found. However, it is possible that the annual mean wage lacks enough precision and

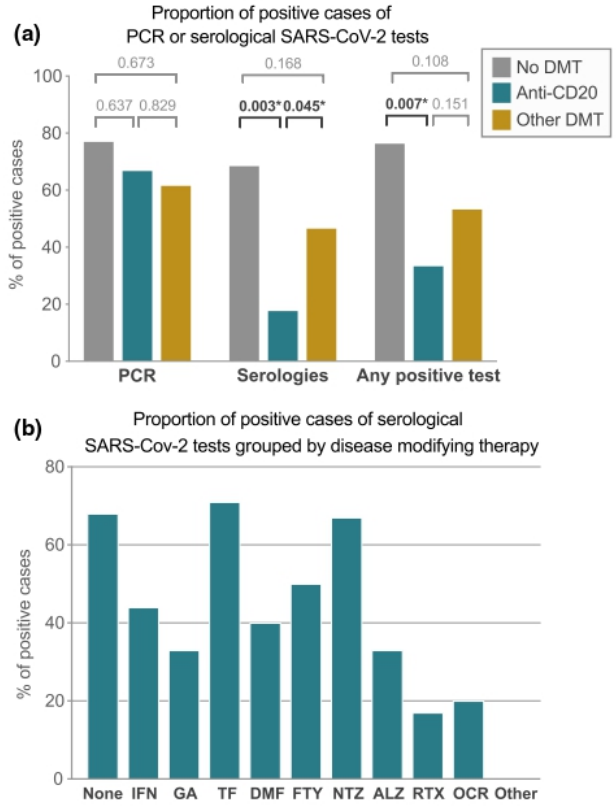

FIGURE 4 Proportion of positive cases of PCR or serological SARS-CoV-2 tests according to treatment. (a) Proportion of positive cases of SARS-CoV-2 PCR (polymerase chain reaction), SARS-CoV-2 serological test or any of the two in relation to patient's treatment. Patients' treatments were classified as no DMT (disease-modifying therapy) and anti-CD20 therapy which included ocrelizumab and rituximab and other DMTs (interferon, glatiramer acetate, teriflunomide, dimethylfumarate, cladribine, fingolimod or alemtuzumab). Count of total cases analysed for each test: PCR, no DMT 13, anti-CD20 6, other DMT 13; serologies, no DMT 19, anti-CD20 17, other DMT 43; any positive test, no DMT 21, anti-CD20 18, other DMT 47. Significant p values ($p \leq 0.05$) are marked with an asterisk. (b) Proportion of positive cases to serological test or any of the two in relation to patient's treatment. IFN, interferon; GA, glatiramer acetate; TFN, teriflunomide; DMF, dimethylfumarate; FTY, fingolimod; NTZ, natalizumab; ALZ, alemtuzumab; RTX, Rituximab; OCR, Ocrelizumab

accuracy as a socioeconomic proxy, leading to inconclusive results. A higher number of COVID-19 patients presented one or more comorbidities. None of those comorbidities was independently associated with the susceptibility to COVID-19 in contrast with previous experiences [22,23] possibly due to the low frequency of individual comorbidities in our sample.

In relation to previous laboratory data, despite reports suggesting that low vitamin D levels may play a role in COVID-19 susceptibility [24] no differences between COVID-19 and non-COVID-19 patients were found. Likewise, neither previous lymphocyte counts nor the degree of lymphopaenia increased COVID-19 risk.

Data regarding the relationship between MS therapy and COVID-19 are scarce. It has been suggested that, as most DMTs do not particularly target the innate immune system, which is responsible for the initial response against infections, the majority of them do not increase the susceptibility to SARS-CoV-2 infection [6,25] No clear association of COVID-19 with immunotherapy or with the use of low or high efficacy therapies was found, which is consistent with some of the reported experience in MS and other autoimmune diseases [26,27] However, our results suggest that, amongst anti-CD20-treated patients, COVID-19 susceptibility was higher in patients treated for a longer period of time (median duration 2.8 years in COVID-19 vs. 1.2 years in non-COVID-19), independently of age, previous comorbidities and MS characteristics. Moreover, some of these patients presented low IgG levels and it is known that IgG hypogammaglobulinaemia is more frequent with repeated infusions, which could potentially contribute to the infection susceptibility [28,29] These results are consistent with previously published literature but, given the small sample size of our cohort, further confirmation is needed.

The proportion of severe and hospitalized COVID-19 cases in our cohort was similar to that of other COVID-19 and MS cohorts (20.4% vs. 21.0%) [26] It is confirmed that advanced age is a risk factor for severe disease [22] Similar to the French cohort [17] pre-existing comorbidities, patients with progressive forms and a longer disease duration increased the risk of a critical disease but, in our cohort, none of them showed an independent association in the multivariable analysis. A lower proportion of patients with severe COVID-19 were on DMTs, compared to mild cases. This should be interpreted with caution given our small sample and that published data in MS and other immune-mediated diseases are inconsistent [7,17,30–32] Even though lymphopaenia during the infection is associated with a severe disease course [33] it was not possible to replicate those findings in our sample. The two deceased patients were untreated and had several of the known mortality predictors [19] Overall, these findings suggest that preventive infection measures should be focused on older MS patients with progressive phenotypes.

Many potential factors influence SARS-CoV-2 seroconversion such as time of test performance and severity of the disease [34] Although serological tests were performed 3 months after the beginning of COVID-19, less than half of COVID-19 patients (45.6%) in our cohort had generated antibodies, independently of the severity of the disease, and this frequency increased to 100% in severe cases. A noteworthy finding in our study was the much lower rate of positive serological tests (17.6%) in anti-CD20-treated patients compared to patients treated with other DMTs (48.8%) or without treatment (68.4%). This is in line with the VELOCE study, in which humoral responses were attenuated in patients treated with ocrelizumab [35] A similar situation has already been described. In the first report, two MS COVID-19 cases with anti-CD20 therapies present no seroconversion but a favourable outcome [30] In another study, the authors analyse the serological status of 13 MS or neuromyelitis optica spectrum disorder patients and serology was negative for the five patients treated with anti-CD20 treatments [36] On the other hand, data on robust memory T cell response in antibody-seronegative cases is encouraging and could explain how immunity is achieved in this type of patients [37]

The limitations of our study include the following. The retrospective nature of our study makes it prone to recall bias and missing data. Given the high non-respondent rate and limited access to PCR, it is likely that asymptomatic, mild or atypical COVID-19 cases were missed, limiting our conclusions. Nevertheless, the rate of PCR testing in our province was restricted as well, so the incidence estimates of the general population are subject to the same limitations. Moreover, this might be compensated by a potential response bias where COVID-19 cases might have been overrepresented amongst the responders of our survey. Another limitation is the small sample size of COVID-19 cases and especially of those with a severe course, probably leading to overestimation of associations and a high degree of uncertainty. Our strengths derive from having an important sample of controls, serological data and valuable information on previous laboratory data.

In conclusion, our study shows that the incidence of COVID-19 in our cohort is similar to the general population and that COVID-19 susceptibility and severity are largely determined by the predictors reported in non-MS patients. Overall prognosis is favourable in the majority of MS patients. However, patients treated with an anti-CD20 therapy for a longer period of time might be at a higher risk of COVID-19 and less than one in five generate an antibody response. Age is the only prognostic factor for severity.

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CONFLICT OF INTEREST

The author(s) declared the following potential conflicts of interest with respect to the research, authorship and/or publication of this article. A.Z. has received travel expenses for scientific meetings from Biogen-Idec and Novartis, speaking honoraria from Eisai and a study grant from Novartis. S.C.-R. is an ECTRIMS clinical fellowship awardee; has received travel expenses for scientific meetings from Biogen-Idec and Genzyme; compensation for consulting services or participation in advisory boards from Roche and Novartis; and speaking honoraria from Novartis. P.T. is an ECTRIMS clinical fellowship awardee; has received travel expenses for scientific meetings from Roche. G.A. has received compensation for consulting services or participation in advisory boards from Sanofi, Merck and Roche; research support from Novartis; travel expenses for scientific meetings from Novartis, Roche, Stendhal and ECTRIMS; speaking honoraria from Sanofi and Merck; and is a member of the International Women in Multiple Sclerosis (iWiMS) network executive committee. S.O.-R. has received compensation for consulting services from Biogen-Idec and Genzyme, and research support from Novartis. P.C. has received travel expenses from Biogen. P.C.'s yearly salary is supported by a grant from Biogen to Fundació privada Cemcat towards statistical analysis. A.V.-J. receives support for contracts Juan Rodes (JR16/00024) from Fondo de Investigaciones Sanitarias, Instituto de Salud Carlos III, Spain, and has received speaking honoraria and travel expenses from Novartis, Roche, Teva, Biogen and Genzyme-Sanofi. J.R. has received speaking honoraria and personal compensation for participating on advisory boards from Almirall, Bayer Schering Healthcare, Biogen-Idec, Genzyme. A.C.-C has received grants from Instituto de Salud Carlos III, Spain; JR19/00007. M.C. has received compensation for consulting services and speaking honoraria from Bayer Schering Pharma, Merck Serono, Biogen-Idec, Teva Pharmaceuticals, Sanofi-Aventis and Novartis. C.N. has received funding for travel from Biogen-Idec and F. Hoffmann-La Roche Ltd, and speaker honoraria from Novartis. J.S.-G. has received compensation for consulting services and speaking honoraria from Almirall, Bayer, Biogen, Celgene, Sanofi, Merck, Novartis, Roche, Bial, Biopass and Teva, is member of the editorial committee of *Multiple Sclerosis Journal*, and director of Revista de Neurología. M.T. has received compensation for consulting services and speaking honoraria from Almirall, Bayer Schering Pharma, Biogen-Idec, Genzyme, Merck-Serono, Novartis, Roche, Sanofi-Aventis Viela-Bio and Teva Pharmaceuticals. Dr Tintore is co-editor of *Multiple Sclerosis Journal—Experimental, Translational and Clinical*. X.M. received speaking honoraria and travel expenses for scientific meetings, has been a steering committee member of clinical trials or participated in advisory boards of clinical trials in the past 3 years with Actelion, Alexion, Bayer, Biogen, Celgene, EMD Serono, EXCEMED, Genzyme, MedDay, Merck, MSIF, Nervgen, NMSS, Novartis, Roche, Sanofi-Genzyme, Teva Pharmaceutical and TG Therapeutics. M.R., B.R.-A., J.L.R., L.M., I.G. and J.C. report no disclosures.

AUTHOR CONTRIBUTIONS

Ana Zabalza: conceptualization (equal); data curation (equal); formal analysis (lead); methodology (equal); writing original draft (lead); writing review editing (lead). Simón Cárdenas-Robledo: conceptualization (equal); data curation (equal); formal analysis (equal); methodology (equal); validation (equal); writing original draft (equal). Paula Tagliani: conceptualization (equal); data curation (equal); methodology (equal); validation (equal); writing original draft (equal). Georgina Arrambide: conceptualization (equal); data curation (equal); formal analysis (equal); methodology (equal); supervision (equal); validation (equal); writing original draft (equal); writing review editing (equal). Susana Otero-Romero: supervision (equal); validation (equal); writing original draft (equal); writing review editing (equal). Pere Carbonell-Mirabent: formal analysis (equal); methodology (equal); supervision (equal); writing review editing (equal). Marta Rodriguez-Barranco: data curation (equal). Breogán Rodríguez-Acevedo: data curation (equal). Juan Luis Restrepo Vera: data curation (equal). Mireia Resina-Salles: data curation (equal). Luciana Midaglia: data curation (equal). Angela Vidal-Jordana: data curation (equal). Jordi Río: data curation (equal). Ingrid Galan: data curation (equal). Joaquin Castillo: data curation (equal). Álvaro Cobo-Calvo: data curation (equal). Manuel Comabella: validation (equal); writing review editing (equal). Carlos Nos: validation (equal); writing review editing (equal). Jaume Sastre-Garriga: conceptualization (equal); methodology (equal); supervision (equal); validation (equal); visualization (equal); writing original draft (equal); writing review editing (equal).

Mar Tintore: conceptualization (equal); methodology (equal); supervision (equal); validation (equal); visualization (equal); writing original draft (equal); writing review editing (equal). Xavier Montalban: conceptualization (equal); methodology (equal); supervision (equal); validation (equal); visualization (equal); writing original draft (equal); writing review editing (equal).

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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5.1.1 Supplementary material

Table 1. Demographic characteristics, COVID-19 previous contact, MS characteristics, DMTs and previous laboratory findings of the survey respondents treated with anti-CD20 monoclonal antibodies according to COVID-19 disease

	COVID-19 n=10	NON COVID-19 n=129
DEMOGRAPHICS AND MS		
Age years, mean (SD)	40.89 (9.42)	45.58 (10.24)
Female, n (%)	6 (60.0)	79 (61.2)
Any comorbidity, n(%)	2 (20.0)	29 (22.5)
2 or more comorbidities, n(%)	1 (10.0)	24 (18.6)
RRMS, n(%)	5 (50.0)	53 (41.1)
Progressive MS, n(%)‡	5 (50.0)	76 (58.9)
Disease duration in years, median (IQR)	15.79 (11.17)	10.93 (15)
EDSS, median (IQR)	4.0 (4.5)	2 (3)
Corticoids past 3 months, n (%)	1 (10.0)	2 (1.6)
Treatment duration in years, median (IQR)	2.77 (3.14)	1.19 (1.32)
PREVIOUS LABORATORY FINDINGS		
Lymphocyte count, median (IQR)[1]	1400 (1000)	1720 (980)
Absolute lymphocyte count <1000/ul, n (%)[1]	2 (22.2)	17 (16.2)
Lymphopenia grade 1, n(%)[1]	2 (22.2)	16 (15.2)
Lymphopenia grade 2-4, n(%)[1]	-	1 (1.0)
CD19+ count, median (IQR)[2]	0 (0)	0 (0)
CD19+ %, median (IQR)[2]	0.01 (0.01)	0.03 (0.16)
IgM, median (IQR)[3]	52 (12)	83 (71)
IgM < 40, n(%)[3]	-	13 (16.0)
IgA, median (IQR)[3]	194 (57)	190 (126)
IgA <70, n(%)[3]	-	2 (2.5)
IgG, median (IQR)[4]	794 (534)	943 (351)
IgG <700, n(%)[4]	3 (42.9)	13 (15.5)

Count of total cases of variables with missing information: [1]Covid=9. Non covid=110; [2]Covid=5. Non covid=75; [3]Covid=5. Non covid=81; [4]Covid=7. Non covid=84.

‡Progressive MS includes PPMS and SPMS

Significant p-values (p≤0.05) are marked with *

5.2 Article 2

Humoral and Cellular Responses to SARS-CoV-2 in Convalescent COVID-19 Patients with Multiple Sclerosis

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Abstract

Background and Objectives

Information about humoral and cellular responses to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and antibody persistence in convalescent (COVID-19) patients with multiple sclerosis (PwMS) is scarce. The objectives of this study were to investigate factors influencing humoral and cellular responses to SARS-CoV-2 and its persistence in convalescent COVID-19 PwMS.

Methods

This is a retrospective study of confirmed COVID-19 convalescent PwMS identified between February 2020 and May 2021 by SARS-CoV-2 antibody testing. We examined relationships between demographics, MS characteristics, disease-modifying therapy (DMT), and humoral (immunoglobulin G against spike and nucleocapsid proteins) and cellular (interferon-gamma [IFN-γ]) responses to SARS-CoV-2.

Results

A total of 121 (83.45%) of 145 PwMS were seropositive, and 25/42 (59.5%) presented a cellular response up to 13.1 months after COVID-19. Anti–CD20-treated patients had lower antibody titers than those under other DMTs ($p < 0.001$), but severe COVID-19 and a longer time from last infusion increased the likelihood of producing a humoral response. IFN-γ levels did not differ among DMT. Five of 7 (71.4%) anti-–CD20-treated seronegative patients had a cellular response. The humoral response persisted for more than 6 months in 41/56(81.13%) PwMS. In multivariate analysis, seropositivity decreased due to anti-CD20 therapy (OR 0.08 [95% CI 0.01–0.55]) and increased in males (OR 3.59 [1.02–12.68]), whereas the cellular response decreased in those with progressive disease (OR 0.04 [0.001–0.88]). No factors were associated with antibody persistence.

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Go to Neurology.org/NN for full disclosures. Funding information is provided at the end of the article.

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Glossary

CIS = clinically isolated syndrome; **CLIA** = chemiluminescence immunoassay; **COVID-19** = coronavirus disease 2019; **DMT** = disease-modifying therapy; **EDSS** = Expanded Disability Status Scale; **IFN** = interferon; **Ig** = immunoglobulin; **IGRA** = interferon-gamma release immunoassay; **IQR** = interquartile range; **MS** = multiple sclerosis; **PwMS** = patients with MS; **RRMS** = relapsing-remitting MS; **RT-PCR** = reverse transcription–PCR; **SARS-CoV-2** = severe acute respiratory syndrome coronavirus 2.

Discussion

Humoral and cellular responses to SARS-CoV-2 are present in COVID-19 convalescent PwMS up to 13.10 months after COVID-19. The humoral response decreases under anti-CD20 treatment, although the cellular response can be detected in anti–CD20-treated patients, even in the absence of antibodies.

Coronavirus disease 2019 (COVID-19) is a pandemic infection caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and has caused almost 5 million deaths worldwide.[1] Although patients with multiple sclerosis (MS) do not have an increased risk of COVID-19 compared with the general population, risk factors for severe COVID-19 in patients with MS include older age, male sex, comorbidities, progressive forms, and higher disability.[2-5] In relation to disease-modifying therapy (DMT), only anti-CD20 therapies appear to increase the risk of COVID-19 severity, and interferon (IFN) may play a protective role.[2-4]

Emerging evidence shows that DMTs alter immunologic responses to both SARS-CoV-2 infection and vaccination against the disease. Some DMTs may induce immunomodulation, whereas others deplete T cells, B cells, or both. In this regard, some studies have shown a decreased humoral response to SARS-CoV-2 infection in patients treated with anti-CD20 therapies.[6-9] For SARS-CoV-2 vaccines, recent studies demonstrate that the humoral response is blunted not only in patients on anti-CD20 therapies but also in those on anti-SP1 receptor treatment.[10,11] Encouragingly, vaccinated patients with MS on anti-CD20 therapies seem to present a specific cellular response, even in the absence of a humoral response.[12,13] However, whether these treatments may affect cellular or long-term humoral responses against SARS-CoV-2 natural infection is still unknown. Therefore, the aims of this study were to investigate humoral and cellular responses to SARS-CoV-2 in COVID-19 convalescent patients with MS (PwMS), to identify factors for developing humoral and cellular responses, and to evaluate factors for humoral response persistence.

Methods

This is a retrospective study involving a cohort of PwMS conducted at the Multiple Sclerosis Centre of Catalonia (Cemcat) in Barcelona between February 1, 2020, and May 22, 2021.

Study Population

We included PwMS with all of the following criteria: older than 18 years, not vaccinated against SARS-CoV-2, COVID-19 convalescence, and a serologic study performed at any time point during the observation period. Following the European Centre for Disease Prevention and Control guidelines,[14] COVID-19-confirmed cases were defined as a positive SARS-CoV-2 reverse transcription–PCR (RT-PCR) or a positive antibody test. Data were collected using a REDCap-based electronic case report form.

Demographic and Clinical Data

Demographic, clinical, laboratory, and COVID-19 data were retrieved from hospital electronic health records. Demographic data included age, sex, and ethnicity. Clinical data included comorbidities (obesity, lung disease, cardiovascular disease, diabetes, hypertension, hematologic disease, chronic kidney disease, liver disease, other autoimmune disease, HIV, or malignancy), MS phenotype (clinically isolated syndrome [CIS], relapsing-remitting MS [RRMS], secondary progressive MS, and primary progressive MS), MS disease duration, Expanded Disability Status Scale (EDSS), DMT at the time of COVID-19, treatment duration, and, for patients on anti-CD20 therapy, cladribine, or alemtuzumab, time since last administration. The absolute lymphocyte count (cell/m^3) was retrieved for all patients. In anti–CD20-treated patients, immunoglobulins (IgM, IgG, and IgA; mg/dL) and flow cytometry lymphocyte phenotypes (total lymphocytes: $CD3^+$, CD4 T cells: $CD4^+$, CD8 T cells: $CD8^+$, B cells: $CD19^+$) were also collected. COVID-19 data included the presence or absence of symptoms and SARS-CoV-2 RT-PCR. COVID-19 severity was categorized as mild-moderate disease or severe-critical disease, as previously described.[6]

Humoral and Cellular Response Studies

Humoral and cellular responses were analyzed in the clinical microbiology and immunology laboratories of Vall d'Hebron's hospital. The qualitative humoral response was analyzed using different commercial chemiluminescence immunoassays (CLIAs) targeting specific SARS-CoV-2 antibodies against spike and nucleocapsid, as per clinical practice. Qualitative and quantitative CLIA studies were performed in a group of selected patients: SARS-CoV-2 antibodies (IgG, IgM, and IgA) against the nucleocapsid protein (Ig-N) were

detected by the Elecsys Anti-SARS-CoV-2 test (Roche Diagnostics, Mannheim, Germany) with a cutoff of 1.0 index performed using a Cobas 8800 system autoanalyzer (Roche Diagnostics, Basel, Switzerland); IgG antibodies against the spike protein (IgG-S) were measured by the LIAISON TrimericS IgG SARS-CoV-2 IgG test (DiaSorin, Stillwater, MN) with a cutoff point of 13.0 AU/mL performed using an XL Analyzer (DiaSorin, Saluggia, Italy).

The cellular response was assessed in a group of selected patients according to DMT with consecutive sampling, prioritizing those on anti-CD20 therapy. The cellular response was assessed using IFN-γ release immunoassay (IGRA) methodology with 2 QuantiFERON SARS-CoV-2 RUO tubes from Qiagen (Hilden, Germany), proprietary mixes of SARS-CoV-2 S protein (Ag1 and Ag2) selected to activate both CD4 and CD8 T cells, as per the manufacturer's instructions. IFN-γ (IU/mL) was measured in these plasma samples using ELISA (QuantiFERON-TB Gold Plus; Qiagen) tests. According to the manufacturers, a test was considered positive if IFN-γ was higher than 0.15 IU/mL.

Statistical Analysis

For the purpose of this study, data capture was locked on May 22, 2021. Descriptive statistics were used to compare demographics and disease characteristics according to classification of the presence or absence of humoral and cellular responses.

Univariable logistic regression models were performed on identified variables to assess their association with the presence of humoral and cellular responses and antibody persistence. Age was treated as a continuous variable, and the EDSS was categorized into 2 bins (EDSS <3.0 and ≥3.0). DMTs were categorized into 3 categories (untreated, anti-CD20 therapies and other DMTs). The Pearson χ^2, Fisher exact test, Student *t* test, and Mann-Whitney *U* tests were used for comparisons, as appropriate. ORs with 95% CIs were estimated using a logistic regression model.

Multivariate logistic regression was applied to determine variables independently associated with presenting a humoral response, a cellular response, and humoral response persistence over 6 months. The model included age, sex, presenting any comorbidity, MS phenotype (CIS/RRMS vs progressive forms), EDSS, DMTs, and COVID-19 severity. Other variables, such as months after COVID-19 of serologic determination or SARS-CoV-2 RT-PCR, were included in the models if considered clinically relevant or if a *p* value <0.1 was obtained by univariate analysis. Disease duration was excluded from the model because collinearity between age and disease duration was detected.

For the purpose of this analysis, seropositive patients were those with positive serologic testing for IgG-S and/or Ig-N at any time point during follow-up. Patients with a cellular response were those with positive results for antigen mix 1 and/or 2.

Humoral response persistence was analyzed only in patients with at least 2 serologic determinations: 1 performed within the first 6 months of COVID-19 diagnosis and another at least 6 months thereafter. The humoral response was considered persistent when serologic determinations were positive both before and after 6 months or when presenting a positive determination after 6 months. Unless otherwise specified, statistical tests were performed at the 0.05 level of significance using Stata version 14.0 (Stata Statistical Software, College Station, TX) and GraphPad Prism version 9.0 (GraphPad Software, San Diego, CA).

Standard Protocol Approvals, Registrations, and Patient Consents

The study was approved by the ethics committee of Vall d'Hebron University Hospital [EOM(AG)003/2021(5768)]. Patient consent was obtained.

Data Availability

Anonymized data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Results

Patient Identification

Until May 22, 2021, 256 patients with suspected or confirmed COVID-19 were identified in our center. Of those, 243 (94.92%) had MS; the other 13 had inflammatory-demyelinating diseases of the central and peripheral nervous systems. Of the 243 with MS, 187 had confirmed COVID-19; 56 had clinically suspected COVID-19. Only those with confirmed COVID-19 and at least 1 serologic determination (n = 145; 77.54%) were included in this study (eFigure 1, links.lww.com/NXI/A693). The distribution of patient DMTs is specified in Table 1. The median follow-up time after COVID-19 of these patients was 10.5 months (interquartile range [IQR] 8.2 months).

SARS-CoV-2 Humoral and Cellular Responses

One hundred twenty-one (83.44%) of the 145 PwMS included in the study had a positive serologic determination at some time point. Positive humoral responses were detected at 0–13.10 months after COVID-19 diagnosis. Demographic, clinical, and laboratory characteristics of patients according to positive or negative antibody results are summarized in Table 1. Demographic, MS, and COVID-19 characteristics and previous laboratory findings were similar in those with positive and negative antibodies. SARS-CoV-2 RT-PCR was performed for 106 (73.1%) of the patients, which was positive in 72 (59.50%) seropositive patients and in all seronegative patients. Nineteen (13.1%) PwMS presented severe or critical COVID-19; 11 (7.6%) had asymptomatic disease. Patients with severe disease were mostly on anti-CD20 therapies

Table 1 Clinical and Demographic Characteristics of the Cohort in Relation to SARS-CoV-2 Serostatus

	Total (N = 145)	Negative serology (n = 24)	Positive serology (n = 121)	OR (95% CI)[a]	*p* Value[a]
Age, y, mean (SD)	46.87 (11.25)	46.39 (12.90)	46.97 (10.97)	1.00 (0.97–1.04)	0.817
Male sex, n (%)	52 (35.86)	4 (16.67)	48 (39.67)	3.29 (1.03–10.45)	**0.032**
Any comorbidity, n (%)[b]	69 (75.59)	15 (62.50)	54 (44.63)	0.48 (0.19–1.20)	0.111
Obesity, n (%)	27 (18.62)	3 (12.50)	24 (19.83)	1.73 (0.47–6.34)	0.401
Progressive MS, n (%)[c]	27 (18.62)	9 (37.50)	18 (14.88)	0.29 (0.11–0.78)	**0.010**
EDSS ≥3.0, n (%)	54 (37.24)	13 (54.17)	41 (33.88)	0.43 (0.17–1.07)	0.061
Disease duration, y, median (IQR)	14 (11.0)	12.55 (11.1)	14.7 (11.1)	1.01 (0.95–1.06)	0.793
Corticosteroids last 3 mo, n (%)	2 (1.38)	1 (4.17)	1 (0.83)	0.19 (0.01–3.25)	0.203
DMTs, n (% of the row)					
No treatment	30 (20.69)	2 (6.67)	28 (93.33)	Ref	Ref
Interferon-β	19 (13.10)	3 (15.79)	16 (84.21)	0.38 (0.06–2.63)	0.309
Glatiramer acetate	13 (8.97)	1 (7.69)	12 (92.31)	0.86 (0.07–10.69)	0.905
Dimethyl fumarate	18 (12.41)	2 (11.11)	16 (88.89)	0.57 (0.07–4.58)	0.594
Teriflunomide	12 (8.28)	—	12 (100.0)	—	0.365
Fingolimod	6 (4.14)	—	6 (100.0)	—	0.521
Natalizumab	4 (2.76)	—	4 (100.0)	—	0.600
Alemtuzumab	7 (4.83)	2 (28.57)	5 (71.43)	0.18 (0.02–1.79)	0.097
Cladribine	2 (1.38)	—	2 (100.0)	—	0.711
Ocrelizumab	7 (4.83)	2 (28.57)	5 (71.43)	0.18 (0.18–1.78)	0.097
Rituximab	22 (15.17)	10 (45.45)	12 (54.55)	0.086 (0.01–0.56)	**0.001**
Other anti-CD20	4 (2.76)	2 (50.0)	2 (50.0)	0.07 (0.00–1.10)	**0.013**
Other DMTs	1 (0.69)	—	1 (100.0)	—	0.793
Anti-CD20	33 (22.76)	14 (42.42)	19 (57.58)	0.13 (0.05–0.37)	**0.000**
Treatment duration, y, median (IQR)	2.7 (4.9)	1.85 (3.0)	3.0 (5.0)	1.04 (0.94–1.16)	0.405
Time of COVID-19 since last infusion, mo, median (IQR)[1]	4.0 (5.2)	3.15 (5.10)	5.0 (8.7)	1.08 (0.96–1.22)	0.203
Time of serology since last infusion, mo, median (IQR)[1]	4.47 (6.83)	5.42 (6.64)	4.47 (9.36)	1.06 (0.96–1.16)	0.269
Previous lymphocyte count, median (IQR)[2]	1,700 (1,000)	1,400 (900)	1,730 (1,000)	1.00 (0.99–1.00)	0.381
Previous IgG count, median (IQR)[3]	914 (287)	942.5 (338)	869 (317.5)	1.00 (0.99–1.00)	0.440
Previous IgM count, median (IQR)[3]	88 (50)	78.5 (52.5)	90.5 (50.5)	1.02 (0.98–1.04)	0.264
Previous IgA count, median (IQR)[3]	192 (109)	182 (41)	221 (76)	1.01 (0.99–1.02)	0.094
Negative RT-PCR, n (%)	10 (6.90)	—	10 (8.26)	Ref	Ref
Positive RT-PCR, n (%)	96 (66.21)	24 (100.0)	72 (59.50)	2.10 (0.71–6.21)	0.170
RT-PCR not performed, n (%)	39 (26.90)	—	39 (32.23)	0.83 (0.28–2.43)	0.730
COVID-19 symptoms, n (%)	134 (90.34)	20 (83.33)	111 (91.74)	2.22 (0.63–7.86)	0.205

Continued

Table 1 Clinical and Demographic Characteristics of the Cohort in Relation to SARS-CoV-2 Serostatus *(continued)*

	Total (N = 145)	Negative serology (n = 24)	Positive serology (n = 121)	OR (95% CI)[a]	*p* Value[a]
COVID-19 severe-critical course, n (%)[d]	19 (13.10)	3 (12.50)	16 (13.22)	1.07 (0.28–4.01)	0.924
Time of serologies after COVID-19 diagnosis, months, mean (IQR)	3.19 (2.40)	3.38 (4.42)	3.15 (2.3)	0.98 (0.85–1.12)	0.747

Abbreviations: COVID-19 = coronavirus disease 2019; DMT = disease-modifying therapy; EDSS = Expanded Disability Status Scale; IgG, IgM, IgA = immunoglobulin G, M, or A; IQR = interquartile range; MS = multiple sclerosis; RT-PCR = reverse transcription-PCR; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2. Percentage is the proportion of patients of the column with that variable if not otherwise specified. Bold indicates statistically significant *p* value <0.05. Count of total cases of variables with missing information: $n^1 = 73$, $n^2 = 129$, and $n^3 = 28$.
[a] Statistical analysis was performed using a not adjusted logistic regression model.
[b] Any comorbidity includes obesity, lung disease, cardiovascular disease, diabetes, hypertension, hematologic benign disease, chronic kidney disease, liver disease, HIV, or malignancy.
[c] Progressive MS includes secondary progressive multiple sclerosis and primary progressive multiple sclerosis.
[d] COVID-19 severity is categorized as (1) mild-moderate disease if patients had no signs or symptoms of pneumonia or a mild pneumonia and (2) severe-critical disease if they presented dyspnea, or a respiratory rate of ≥30 breaths per minute or a blood oxygen saturation of ≤93%, or a ratio of the partial pressure of arterial oxygen to the fraction of inspired oxygen of <300 mm Hg, or infiltrates in >50% of the lung field within 24–48 hours from the onset of symptoms and/or organ or multiple organ failure.

(42.1%) or untreated (47.4%) (data not shown, eTable 5, links.lww.com/NXI/A693). Multivariable analysis revealed that males were more likely to become seropositive (OR 3.59, 95% CI 1.02–12.68, $p < 0.05$), whereas PwMS under anti-CD20 therapy had a higher risk of remaining seronegative than untreated patients (OR 0.08, 95% CI 0.01–0.55, $p = 0.01$) (Figure 1A).

When exploring the 33 patients on anti-CD20 therapy, 19 (57.6%) had a positive humoral response. In multivariable analysis, only severe COVID-19 infection (OR 14.06, 95% CI 1.02–192.68, $p = 0.048$) and a longer time between the last treatment infusion and COVID-19 disease (OR per month 1.51, 95% CI 1.01–2.24, $p = 0.042$) were significantly associated with a higher probability of developing a humoral response after COVID-19 (eTable 1, links.lww.com/NXI/A693).

Antibody titers were measured in 124 patients with demographic and MS characteristics similar to those of the total cohort (data not shown). Anti–CD20-treated patients presented lower IgG-S (15.4 [IQR 60.0]) and Ig-N median (0.08 [IQR 0.13]) titers than those on other DMTs (37.8 [IQR 68.3], $p > 0.05$, and 19.55 [IQR 42.92], $p < 0.001$) or untreated patients (74.3 [IQR 182.4], $p < 0.05$, and 34.3 [IQR 128.8], $p < 0.001$). Patients on fingolimod presented lower median titers of IgG-S (17.0 [IQR 13.5]) and Ig-N (2.16 [IQR 2.21]) than those on other DMTs, although no significant differences were found due to the small number of cases. A higher proportion of seropositivity according to each DMT was observed for antibodies against nucleocapsid compared with those against spike, especially in patients treated with IFN (61.5% vs 38.5%) (Figure 1, B and C).

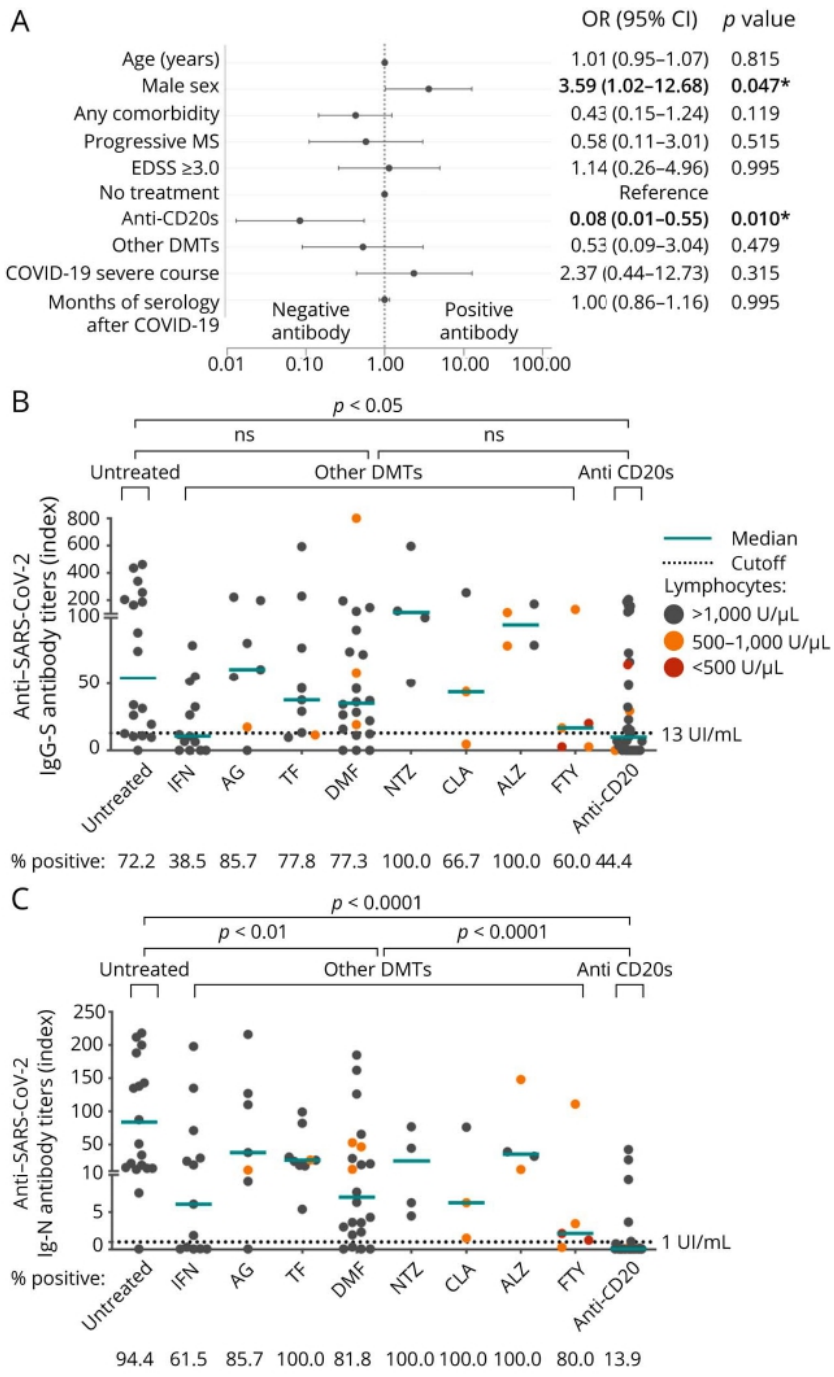
In patients treated with anti-CD20 therapy, the number of months since the last infusion of COVID-19 correlated with IgG-S titer ($r = 0.50$ [95% CI 0.13–0.75]; $p < 0.01$). However, no correlation was found with Ig-N antibodies or with antibody titers and treatment duration. In patients treated with cladribine and alemtuzumab, the association of antibody titer with time since last treatment administration or treatment duration could not be analyzed due to the low number of cases (eFigures 2 and 3, links.lww.com/NXI/A693).

The cellular response was analyzed in 42 patients selected according to DMT: 22 on anti-CD20 therapy, 5 without treatment, and 15 on other DMTs. Twenty-five (59.5%) of these 42 patients presented a cellular response, which was detected 0.6–13.0 months after COVID-19, with a median time of 7.0 months (IQR 7.2 months). No differences were found in demographic and MS variables between positive and negative responders (eTable 2, links.lww.com/NXI/A693). Nonetheless, all patients with severe COVID-19 presented a cellular response ($p = 0.018$). Five of these patients were on anti-CD20 therapy, 1 was on dimethyl fumarate, and 1 was untreated. In multivariable analysis, the occurrence of a cellular response was decreased with progressive MS forms (OR 0.04, 95% CI 0.001–0.88, $p < 0.05$) (Figure 2A).

A cellular response was detected in patients with all types of DMT, except for glatiramer acetate (n = 0/2). IFN-γ titers against antigen 1 or 2 did not differ between anti–CD20-treated patients, untreated patients, or those with other DMTs, although the individual proportions of positive determinations varied among DMTs. However, no significant differences were observed due to the low number of cases per DMT (Figure 2, B and C).

Twenty (47.6%) of the 42 patients analyzed presented both humoral and cellular responses. In severe cases, the proportion of those with both responses increased up to 85.7% (6 of 7) (eTable 3, links.lww.com/NXI/A693). In seronegative patients, a cellular response was observed in 5 of 7 (71.4%) anti–CD20-treated patients and in none of the patients treated with other DMTs or untreated patients. Mean titers of IFN-γ in anti–CD20-treated patients were similar between seropositive and seronegative PwMS (IFN-γ against Ag.1: 1.4

Figure 1 Humoral Response to SARS-CoV-2



(A) Forest plot depicting adjusted ORs for presenting positive antibodies against SARS-CoV-2 (n = 145). Demographic and clinical characteristics, comorbidities, and laboratory data are represented with OR, 95% CI, and *p* values. In dichotomous variables, the reference is not specified. Statistical analysis was performed using a logistic regression model adjusted for age, sex, presenting any comorbidity, MS phenotype EDSS, DMTs, COVID-19 severity, and months of the serology after COVID-19. (B and C) Median titers of IgG against SARS-CoV-2 spike (IgG-S; A) antibody and total Igs against SARS-CoV-2 nucleocapsid (Ig-N; B), each dot represents a different subject. Statistical analysis was performed using a Mann-Whitney test analysis; only statistically significant differences are indicated. Cutoff values for antibody positivity are indicated by a dotted line. Spot's color indicates previous lymphocyte count >1,000 U/μL (gray), between 500 and 1,000 U/μL (orange), and <500 cells/mm^3 U/μL. Any comorbidity includes obesity, lung disease, cardiovascular disease, diabetes, hypertension, hematologic benign disease, chronic kidney disease, liver disease, HIV, or malignancy. *Statistically significant, *p* value <0.05. Ab = SARS-CoV-2 antibody; ALZ = alemtuzumab; CLA = cladribine; COVID-19 = coronavirus disease 2019; DMF = dimethyl fumarate; EDSS = Expanded Disability Status Scale; FTY = fingolimod; GA = glatiramer acetate; Ig = immunoglobulin; IFN = interferon; NTZ = natalizumab; OCR = ocrelizumab; other anti-CD20 = other anti-CD20 therapies; other DMTs = patients with disease-modifying treatment different from anti-CD20 therapies; progressive MS = secondary progressive MS and primary progressive MS; RTX = rituximab; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2; TFN = teriflunomide.

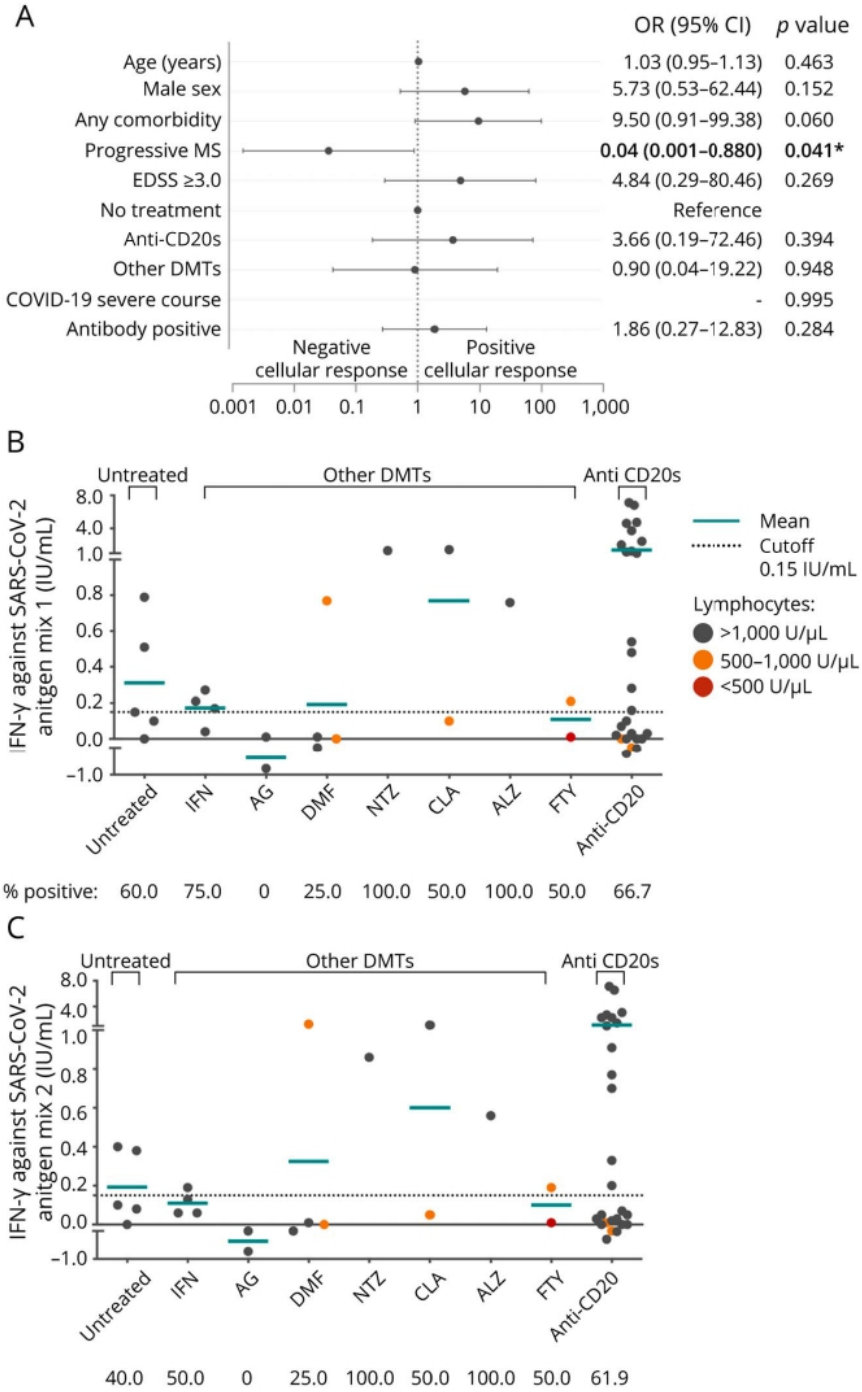
[SD: 2.0] vs 2.4 [SD: 2.8]; $p > 0.05$; Ag.2: 1.1 [SD: 1.8] vs 1.9 [SD: 2.6]; $p > 0.05$) (Figure 3). In anti–CD20-treated patients, mean titers of IFN-γ did not correlate with months since the last infusion or test or treatment duration (eFigures 2 and 3, links.lww.com/NXI/A693).

No correlation was found between antibody titers (Ig-N and IgG-S) and IFN-γ titers. Moreover, previous Ig levels, total lymphocyte counts, CD19$^+$ cells, CD4$^+$ cells, or CD8$^+$ cells did not correlate with antibody or IFN-γ titers (data not shown).

SARS-CoV-2 Humoral Response Persistence

To analyze humoral response persistence, a sensitivity analysis was performed in patients with 1 serology result within the first 6 months after COVID-19 and another after 6 months. Fifty-three PwMS were included, with a median follow-up after COVID-19 of 14.2 months (IQR 0.36). Of those PwMS, 41 (81.13%) were persistently positive or became positive during follow-up. Furthermore, a persistent humoral response to SARS-CoV-2 after more than 12 months after COVID-19 was found in patients treated with anti-CD20 therapy, other DMTs,

Figure 2 Cellular Response to SARS-CoV-2



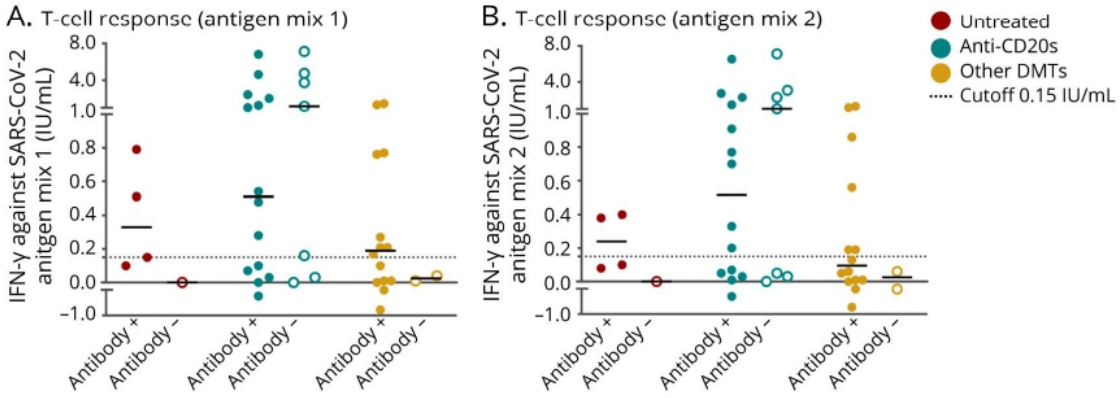
(A) Forest plot depicting adjusted ORs for presenting cellular response against SARS-CoV-2 (n = 42). Demographic and clinical characteristics, comorbidities, and laboratory data are represented with OR, 95% CI, and *p* values. In dichotomous variables, the reference is not specified. Statistical analysis was performed using a logistic regression model adjusted for age, sex, presenting any comorbidity, MS phenotype EDSS, DMTs, COVID-19 severity, and positive SARS-CoV-2 antibody. (B and C) Mean titers of interferon-gamma produced by T-cell against SARS-CoV-2 antigen mix 1 (A) and SARS-CoV-2 antigen mix 2 (B); each dot represents a different subject. Statistical analysis was performed using a Mann-Whitney test analysis, no statistical differences were found. Cutoff values for positive cellular response are indicated by a dotted line. Values over the lower line of the gray area are considered positive. Spot's color indicates previous lymphocyte count >1,000 U/μL (gray), between 500 and 1,000 U/μL (orange), and <500 cells/mm^3 U/μL. Any comorbidity includes obesity, lung disease, cardiovascular disease, diabetes, hypertension, hematologic benign disease, chronic kidney disease, liver disease, HIV, or malignancy. *Statistically significant, *p* value <0.05. Ab = SARS-CoV-2 antibody; ALZ = alemtuzumab; CLA = cladribine; COVID-19 = coronavirus disease 2019; DMF = dimethyl fumarate; EDSS = Expanded Disability Status Scale; FTY = fingolimod; GA = glatiramer acetate; IFN = interferon; ns = not significant; NTZ = natalizumab; OCR = ocrelizumab; other anti-CD20 = other anti-CD20 therapies; other DMTs = patients with disease-modifying treatment different from anti-CD20 therapies; progressive MS = secondary progressive MS and primary progressive MS; RTX = rituximab; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2; TFN = teriflunomide.

and untreated patients. Nevertheless, the percentage of positive serology in anti–CD20-treated patients was lower than that in patients on other DMTs and untreated patients (Figure 4A). In univariable analysis, patients with humoral persistence over 6 months presented a higher median lymphocyte count before COVID-19 than those without persistence (1,715 [IQR 685] vs 1,200 [IQR 100], $p < 0.05$) (eTable 4, links.lww.com/NXI/A693). Regardless, no factors were independently associated with humoral response persistence over 6 months in multivariable analysis (Figure 4B).

Discussion

In this study, we found that humoral and cellular responses to SARS-CoV-2 in convalescent COVID-19 PwMS are present for up to a year after COVID-19 diagnosis and that a cellular response can be present in anti–CD20-treated patients, even in the absence of a humoral response. There are 2 main pillars of an effective antiviral response. One is cellular immunity, specifically T-cytotoxic cells ($CD8^+$), which eliminate infected cells. The other is humoral immunity with plasma cells

Figure 3 Cellular Response to SARS-CoV-2 according to Serostatus and Treatment



(A and B) Mean IFN-γ titers produced by T cells against SARS-CoV-2 antigen mix 1 (A) and antigen mix 2 (B) according to SARS-CoV-2 antibody positivity (Ab+; Ab−) and treatment. Each dot represents a different subject. Cutoff values for positive cellular response are indicated by a dotted line. Statistical analysis was performed using a Mann-Whitney test analysis comparing differences between groups and between antibody positive and negative cases, no statistical differences were found. Ab = antibody; other DMTs = patients with disease-modifying treatment different from anti-CD20s therapies; IFN-γ = interferon-gamma; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2.

that secrete neutralizing antibodies and prevent viruses from infecting cells. After the initial response, T helper cells coordinate the long-term immune reaction, collaborating in the creation of long-lived plasma cells.[15]

The presence of neutralizing antibodies against SARS-CoV-2 provides the best current indication for protection against reinfection.[16] We assessed the humoral response to SARS-CoV-2 using a commercially available assay with a demonstrated correlation between IgG and antibody neutralization titers of 94.4%.[17] In our cohort, 83.4% of patients presented antibodies against SARS-CoV-2. In line with previous reports in PwMS, anti-CD20 therapy decreases both the probability of presenting a serologic response and the median titers of antibodies against SARS-CoV-2.[6-9] On the other hand, patients treated with other DMTs and untreated patients present an antibody positivity rate of more than 70%. Of interest, we found lower titers of anti-S IgG than Ig-N, especially in IFN-β–treated patients. These differences may be due to the glycosylation state of the spike protein, which makes it less immunogenic.[18] However, because a recent publication found higher IgG-S antibody titers in convalescent COVID-19 PwMS treated with IFN and glatiramer acetate compared with other DMTs,[9] our results should be interpreted with caution.

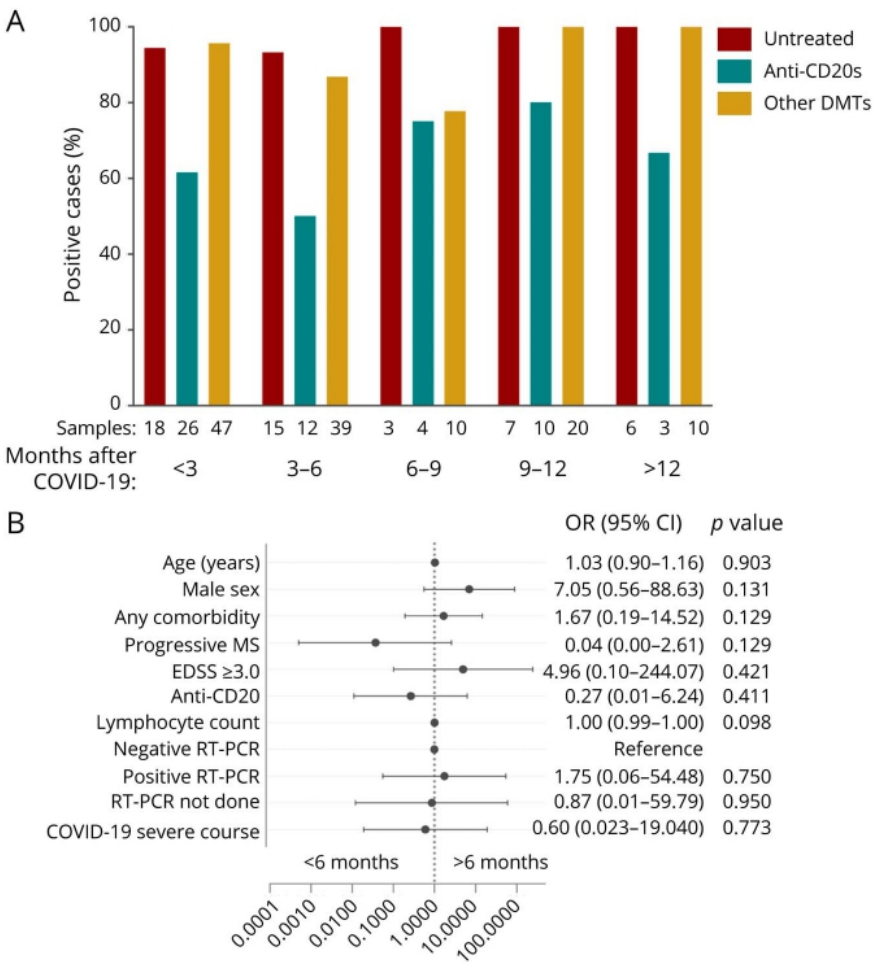
Anti-CD20 therapy affects the B-cell lineage, impairing differentiation into memory B cells or plasma cells. It is not surprising then that PwMS on such treatment fails to develop a humoral response. Among these PwMS, we found a higher humoral response in those with severe COVID-19.[19] Similarly, the longer it was since SARS-CoV-2 infection after the last anti-CD20 infusion, the higher was the serum anti-S IgG titer and proportion of humoral response positivity. This is probably due to an increasing repopulation of memory B cells over the months. These results are consistent with previous findings in patients treated with anti-CD20 therapy after COVID-19[9] and after vaccination,[20] but given our cohort's small sample size, further confirmation is needed.

Lymphodepleting therapies such as cladribine, alemtuzumab, or fingolimod might also modify immunologic responses to SARS-CoV-2 by reducing peripheral B-cell counts. We found an acceptable positive rate among all 3 treatments, as seen in previous studies.[8,9] Although there is growing evidence showing that the response to SARS-CoV-2 vaccination is blunted in patients on fingolimod,[10,11] controversy remains regarding the response after COVID-19.[8,9,21] Similar to other studies,[9,21] our results suggest that antibody titers are lower in patients on fingolimod than in those on other DMTs. Altogether, confirmation of these data in larger cohorts is needed. Our results also suggest that male sex increases the probability of seroconversion, which is consistent with some of the data published for the general population, in which higher antibody titers were found in male patients.[22,23]

The magnitude and profile of the T-cell response against SARS-CoV-2 is heterogeneous and may be a reflection of individual immunologic responses during acute infection.[24] We assessed the specific T-cell response using a commercially available IGRA kit[25] and found cellular responses in 59.5% of patients. As previously described in the general population,[26,27] the cellular response was associated with severe COVID-19 in univariable analysis. In fact, all patients with severe COVID-19 had detectable cellular responses. This suggests an increased immune response with higher viral loads and inflammatory mediators during acute infection.[28]

Nevertheless, we detected a specific cellular response despite the absence of a humoral response in 5 patients given anti-CD20 therapy but not in patients on other DMTs. Some studies of SARS-CoV-2 infection and vaccination have already described specific cellular responses in the absence of humoral responses.[12,13,29] In fact, a study of COVID-19 patients with hematologic cancer treated with rituximab showed that those with a higher proportion of T cells had a better outcome.[30]

Figure 4 SARS-CoV-2 Humoral Response Persistence



(A) Proportion of positive SARS-CoV-2 serologies according to the months since COVID-19 diagnosis and treatment. (B) Forest plot depicting adjusted ORs for presenting antibody response persistence over 6 months (n = 53). Only patients with 2 or more serologic determinations are included. Demographic and clinical characteristics are represented with OR, 95% CI and *p* values. In dichotomous variables, the reference is not specified. Statistical analysis was performed using a logistic regression model adjusted for age, sex, presenting any comorbidity, MS phenotype EDSS, DMTs, COVID-19 severity, lymphocyte count, and RT-PCR results (not done, negative, or positive). Patient's treatments are classified as untreated, anti-CD20 therapies (rituximab, ocrelizumab, and other anti-CD20s), and other DMTs (interferon, glatiramer acetate, teriflunomide, dimethyl fumarate, cladribine, fingolimod, or alemtuzumab). Any comorbidity includes obesity, lung disease, cardiovascular disease, diabetes, hypertension, hematologic benign disease, chronic kidney disease, liver disease, HIV, or malignancy. COVID-19 = coronavirus disease 2019; DMT = disease-modifying therapy; EDSS = Expanded Disability Status Scale; MS = multiple sclerosis; progressive MS = secondary progressive MS and primary progressive MS; RT-PCR = reverse transcription-PCR; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2.

Therefore, the cellular response might play an important role in COVID-19 recovery when humoral immunity is impaired.

In our cohort, progressive phenotypes were less likely to present humoral and cellular responses. In both cases, the decreased response might be justified by the older age of these patients or premature immunosenescence associated with progressive forms,[31] leading to a weakened immune response. However, potential confounders such as anti-CD20 therapy should be ruled out in future analyzes with larger cohorts.

Humoral and cellular responses against SARS-CoV-2 are detected within a few days of COVID-19 onset to up to 12 months.[32,33] We were able to detect both of them up to 13.10 months after COVID-19 disease. The humoral response persisted for more than 6 months in 81.1% of patients with 2 determinations. In the general population, increased severity of COVID-19 and younger age have been associated with longer SARS-CoV-2 humoral persistence,[34,35] although we did not find any association in this regard, probably because of the small cohort. Nonetheless, further information about long-term immunity in PwMS after SARS-CoV-2 vaccination is needed.

Limitations of our study are as follows. During the first wave of the pandemic, RT-PCR testing in our province was restricted to hospitalized patients. Therefore, our cohort's first cases were either severe cases or patients with positive serology performed in the convalescence phase. This might have led to an increased estimation of the positive serologic rate, as there might be an overrepresentation of patients with severe COVID-19 infection. In addition, all tests were performed according to clinical practice and not to established time points after COVID-19 diagnosis, which has led to variability in the time and frequency of testing after COVID-19, increasing the heterogeneity of the sample. Another limitation is the relatively small sample size of the study, probably leading to an overestimation of associations and a high degree of uncertainty. In the cellular response substudy in particular, there were few cases for each DMT, which prevented us from performing group comparisons. Our strengths include the deeply phenotyped cohort with valuable information on previous laboratory data and a long-term follow-up of PwMS and COVID-19. Moreover, to detect the cellular response, we used a commercially available test suitable for clinical laboratories and amenable to automation, making it potentially useful to

evaluate cellular response after COVID-19 or SARS-CoV-2 vaccination. However, it should be considered that previous studies on the SARS-CoV-2 cellular response in MS have used other methods, such as intracellular cytokine staining or other IGRAs, which may limit the reproducibility of our results.

In conclusion, convalescent COVID-19 patients with MS have preserved specific humoral and cellular responses to SARS-CoV-2 up to 13 months after COVID-19, although the humoral response is reduced in patients on anti-CD20 therapy. Overall, these data provide valuable information about the immune response in convalescent COVID-19 MS patients and can be used for clinical guidance.

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Disclosure

A. Zabalza has received travel expenses for scientific meetings from Biogen Idec, Merck Serono, and Novartis, speaking honoraria from Eisai, and a study grant from Novartis. G. Arrambide has received compensation for consulting services or participation in advisory boards from Sanofi, Merck, and Roche, research support from Novartis, travel expenses for scientific meetings from Novartis, Roche, Stendhal, and ECTRIMS, and speaking honoraria from Sanofi and Merck and is a member of the International Women in Multiple Sclerosis (iWiMS) network executive committee. P. Tagliani was an ECTRIMS clinical fellowship awardee in 2019–2020 and has received travel expenses for scientific meetings from Roche. S. Cárdena-Robledo was an ECTRIMS clinical fellowship awardee 2019-2020 and has received travel expenses for scientific meetings from Biogen Idec and Genzyme, compensation for consulting services or participation in advisory boards from Roche and Novartis, and speaking honoraria from Novartis. S. Otero-Robledo has received compensation for consulting services from Biogen Idec and Genzyme and research support from Novartis. J. Esperalba; C. Fernandez-Naval, J. Trocoli Campuzano, M. Martínez Gallo, M. Castillo, M. Bonastre, M. Resina Sallés, and J. Beltran report no disclosures relevant to the manuscript. P. Carbonell-Mirabent has received travel expenses from Biogen. P.C.-M's yearly salary is supported by a grant from Biogen to Fundació Privada Cemcat toward statistical analysis. M. Rodríguez-Barranco, S. López-Maza, P.J. Melgarejo Otálora, and M. Ruiz-Ortiz report no disclosures relevant to the manuscript. A. Papolla is an ECTRIMS clinical fellowship awardee. B. Rodríguez Acevedo has received honoraria for consulting services from Wellspect. L. Midaglia has received honoraria for consulting services and speaking honoraria from Roche and Novartis. A. Vidal-Jordana has received compensation for consulting services and speaking honoraria from Novartis, Roche, Teva, Mylan, Biogen, and Genzyme-Sanofi. A. Cobo-Calvo has received grant from Instituto de Salud Carlos III, Spain; JR19/00007. C. Tur is currently being funded by a Junior Leader La Caixa Fellowship; the fellowship code is LCF/BQ/PI20/11760008; she has also received the 2021 Fundación Merck Salud Award for the Investigation in Multiple Sclerosis (Spain); in 2015, she received an ECTRIMS Post-doctoral Research Fellowship and has received funding from the UK MS Society. I. Galán and J. Castilló report no disclosures relevant to the manuscript. J. Río has received speaking honoraria and personal compensation for participating on Advisory Boards from Almirall, Bayer Schering Healthcare, Biogen Idec, and Genzyme. C. Espejo reports no disclosures relevant to the manuscript. M. Comabella has received compensation for consulting services and speaking honoraria from Bayer Schering Pharma, Merck Serono, Biogen Idec, Teva Pharmaceuticals, Sanofi-Aventis, and Novartis. C. Nos has received funding for travel from Biogen Idec and F. Hoffmann-La Roche, Ltd., and speaker honoraria from Novartis. J. Sastre-Garriga has received compensation for consulting services and speaking honoraria from Almirall, Bayer, Biogen, Celgene, Sanofi, Merck, Novartis, Roche, Bial, Biopass, and Teva and is member of the editorial committee of *Multiple Sclerosis Journal* and director of Revista de Neurología. M. Tintore has received compensation for consulting services and speaking honoraria from Almirall, Bayer Schering Pharma, Biogen Idec, Genzyme, Merck Serono, Novartis, Roche, Sanofi-Aventis Viela Bio, and Teva Pharmaceuticals and is coeditor of *Multiple Sclerosis Journal–Experimental, Translational and Clinical*. X. Montalban has received speaking honoraria and travel expenses for scientific meetings, has been a steering committee member of clinical trials, or participated in advisory boards of clinical trials in the past 3 years with Actelion, Alexion, Bayer, Biogen, Celgene, EMD Serono, Genzyme, Immunic, MedDay, Merck, Mylan, NervGen, Novartis, Roche, Sanofi-Genzyme, Teva Pharmaceutical, TG Therapeutics, Excemed, MSIF, and NMSS. Go to Neurology.org/NN for full disclosures.

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Appendix *(continued)*

Name	Location	Contribution
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Humoral and Cellular Responses to SARS-CoV-2 in Convalescent COVID-19 Patients With Multiple Sclerosis

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5.2.1 Supplementary material

Table 1. Univariable and multivariable analysis of SARS-CoV-2 positive humoral response in anti-CD20 treated patients

	OR (95% CI)	p-value
UNIVARIABLE ANALYSIS[c]		
Age – mean (SD)	0.98 (0.92-1.05)	0.652
Male sex – n (%)	2.13 (0.42-10.89)	0.348
Any comorbidity[a]– n (%)	0.40 (0.09-1.77)	0.215
Obesity – n (%)	14.64 (0.43-50.38)	0.165
Progressive MS[b] – n(%)	0.72 (0.17-2.99)	0.658
EDSS 1. – n(%)	0.87 (0.17-1.07)	0.061
Disease duration, years – median (IQR)	1.05 (0.19-4.02)	0.854
Corticosteroids last 3 months – n (%)	-	-
DMTs – n (% of the row)		
Ocrelizumab	REF	
Rituximab	0.48 (0.07-3.19)	0.438
Other anti-CD20	0.40 (0.03-6.22)	0.498
Treatment duration, years – median (IQR)	0.82 (0.42-1.59)	0.551
Time of COVID-19 since last infusion, months – median (IQR)	1.28 (0.96-1.69)	0.088
Time of serology since last infusion, months –median (IQR)	1.08 (0.86-1.34)	0.518
Previous lymphocyte count –median (IQR)	1.00 (0.99-1.00)	0.862
Previous IgG count – median (IQR)[1]	1.00 (0.99-1.00)	0.284
Previous IgM count – median (IQR)[1]	1.02 (0.98-1.04)	0.283
Previous IgA count –median (IQR)[1]	1.02 (0.99-1.04)	0.051
Negative RT-PCR – n (%)	REF	REF
Positive RT-PCR – n (%)	-	0.072
RT-PCR not performed – n (%)	-	-
COVID-19 symptoms – n (%)	4.90 (0.40-59.64)	0.166
COVID-19 severe-critical course – n (%)	2.78 (0.44-17.56)	0.259
Time of serologies after COVID-19 diagnosis, months– mean (IQR)	1.08 (0.92-1.27)	0.343
MULTIVARIABLE ANALYSIS[d]		
Age	0.98 (0.87-1.11)	0.843
Male sex	1.38 (0.19-10.21)	0.755
Any comorbidity	0.29 (0.04-2.28)	0.242
Progressive MS	2.41 (0.15-38.25)	0.433
EDSS ≥3.0	0.25 (0.01-4-55)	0.348
Treatment duration, years	0.52 (0.20-1.31)	0.162
Time of COVID-19 since last infusion, months	1.51 (1.01-2.24)	**0.042***
COVID-19 severe-critical course	14.06 (1.02--192.68)	**0.048***

Total cases: 33; 14 (42.4%) with negative serology and 19 (57.6%) with positive serology. Count of total cases of variables with missing information: n[1]= 24. [a]Any comorbidity includes obesity, lung disease, cardiovascular disease, diabetes, hypertension, haematological benign disease, chronic kidney disease, liver disease, HIV or malignancy. [b]Progressive MS includes secondary progressive multiple sclerosis (SPMS) and primary progressive multiple sclerosis (PPMS). [c]Statistical analysis was performed using a not adjusted logistic regression model. [d]Statistical analysis was performed using a logistic regression model adjusted for age, sex, presenting any comorbidity, MS phenotype EDSS, DMTs, COVID-19 severity and months of the serology after COVID-19. Abbreviations: MS: multiple sclerosis; SD: standard deviation; IQR: interquartile range; EDSS: Expanded Disability Status Scale: DMT: disease modifying therapy; IgG, IgM, IgA: immunoglobulin G, M or A; RT-PCR: reverse transcription-polymerase chain reaction. % is the proportion of patients of the column with that variable if not otherwise specified.

Table 2. Clinical and demographic characteristics of the cohort in relation to SARS-CoV-2 cellular response

	TOTAL n=42	NEGATIVE CELL RESPONSE n=17	POSITIVE CELL RESPONSE n=25	OR (95% CI)[c]	p-value[c]
Age – mean (SD)	47.86 (11.91)	46.64 (12.59)	48.69 (12.59)	1.02 (0.96-1.07)	0.580
Male sex – n (%)	17 (40.48)	7 (41.18)	10 (40.0)	0.95 (0.27-3.39)	0.940
Any comorbidity[a]– n (%)	21 (50.0)	6 (35.29)	15 (60.0)	2.75 (0.73-10.42)	0.120
Obesity – n (%)	8 (19.05)	3 (17.65)	5 (20.0)	1.17 (0.23-5.81)	0.851
Progressive MS[b] – n(%)	11 (35.48)	8 (47.06)	8 (32.00)	0.53 (0.14-1.94)	0.330
EDSS ≥3.0 – n(%)	25 (59.52)	10 (58.82)	15 (60.0)	1.05 (0.30-3.74)	0.940
Disease duration, years – median (IQR)	14.1 (11.9)	14.0 (14.3)	14.7 (9.7)	1.02 (0.95-1.10)	0.604
Corticosteroids last 3 months – n (%)	0	0	0	-	-
DMTs – n (% of the row)					
No treatment	5 (100)	2 (40.0)	3 (60.0)	REF	
Interferon β	4 (100)	1 (25.0)	3 (75.0)	2.00 (0.09-44.35)	0.655
Glatiramer acetate	2 (100)	2 (100)	0	-	0.074
Dimethyl fumarate	4 (100)	3 (75.0)	1 (25.0)	0.22 (0.01-5.83)	0.322
Teriflunomide	0	0	0	-	-
Fingolimod	2 (100)	1 (50.0)	1 (50.0)	0.67 (0.02-23.88)	0.823
Natalizumab	1 (100)	0	1 (100)	-	0.653
Alemtuzumab	1 (100)	0	1 (100)	-	0.655
Cladribine	2 (100)	1 (50.0)	1 (50.0)	0.67 (0.02-23.88)	0.527
Ocrelizumab	2 (100)	0	2 (100)	-	0.527
Rituximab	15 (100)	6 (40.0)	9 (60.0)	1.00 (0.12-8.33)	1.000
Other anti-CD20	4 (100)	0	4 (100)	2.00 (0.09-44.35)	0.655
Other DMTs	0	0	0	-	-
Anti-CD20	22 (100)	7 (33.33)	14 (66.67)	1.81 (0.51-6.51)	0.351
Treatment duration, years – median (IQR)	1.9 (2.9)	1.2 (2.1)	2.1 (2.3)	1.04 (0.92-1.17)	0.489
Time of COVID-19 since last infusion, months – median (IQR)[1]	4.3 (5.2)	5.2 (5.5)	2.2 (4.1)	0.88 (0.70-1.10)	0.259
Time of cellular determination since last infusion, months – median (IQR)[1]	3.01 (4.5)	2.74 (4.66)	3.09 (4.6)	1.08 (0.86-1.36)	0.505
Previous lymphocyte count –median (IQR)	1400 (1000)	1600 (900)	1400 (940)	1.00 (0.99-1.00)	0.637
Previous IgG count – median (IQR)[2]	869 (282.5)	869 (349)	863 (289)	0.99 (0.99-1.00)	0.683
Previous IgM count – median (IQR)[2]	86 (48.5)	90.5 (48)	79.5 (56)	0.99 (0.96-1.03)	0.901
Previous IgA count – median (IQR)[2]	193 (112)	173.5 (70)	194 (92)	1.01 (0.99-1.03)	0.350
Negative PCR – n (%)	1 (2.38)	1 (5.88)	0	REF	
Positive PCR – n (%)	30 (71.43)	12 (70.59)	18 (72.0)	-	0.239
RT-PCR not performed – n (%)	11 (26.19)	4 (23.53)	7 (28.0)	-	0.237
COVID-19 symptoms – n (%)	40 (95.24)	15 (88.24)	25 (100.0)	3.00 (0.16-55.56)	0.082
COVID-19 severe-critical course – n (%)	7 (16.67)	0	7 (28.0)	-	**0.018***
Positive SARS-CoV-2 antibodies – n (%)	32 (76.19)	12 (70.59)	20 (80.0)	1.67 (0.39-7.18)	0.487
Time of cellular study after COVID-19 diagnosis, months – mean (IQR)	6.95 (7.20)	6.21 (5.56)	10.78 (7.69)	1.05 (0.91-1.23)	0.475

Count of total cases of variables with missing information: n^1= 25, , n^2= 16

[a]Any comorbidity includes obesity, lung disease, cardiovascular disease, diabetes, hypertension, hematological benign disease, chronic kidney disease, liver disease, HIV or malignancy. [b]Progressive MS includes secondary progressive multiple sclerosis (SPMS) and primary progressive multiple sclerosis (PPMS). [c]Statistical analysis was performed using a not adjusted logistic regression model. Abbreviations: MS: multiple sclerosis; SD: standard deviation; IQR: interquartile range; EDSS: Expanded Disability Status Scale: DMT: disease modifying therapy; IgG, IgM, IgA: Immunoglobulin G, M or A; RT-PCR: reverse transcription-polymerase chain reaction % is the proportion of patients of the column with that variable if not otherwise specified.

Table 3. Humoral and cellular response of the cohort in relation to treatment and COVID-19 severity

		NEGATIVE SEROLOGY		POSITIVE SEROLOGY		
		Negative cell response	Positive cell response	Negative cell response	Positive cell response	TOTAL
Untreated	Mild-moderate	1	0	1	2	4
	Severe-critical	0	0	0	1	1
	Total- n (% row)	**1 (20)**	**0**	**1 (20)**	**3 (60)**	**5**
Anti-CD20s	Mild-moderate	2	4	5	5	16
	Severe-critical	0	1	0	4	5
	Total- n (% row)	**2 (9.5)**	**5 (23.8)**	**5 (23.8)**	**9 (42.9)**	**21**
Other DMTs	Mild-moderate	2	0	6	7	15
	Severe-critical	0	0	0	1	1
	Total- n (% row)	**2 (12.5)**	**0**	**6 (37.5)**	**8 (50)**	**16**
TOTAL	Mild-moderate - n (% row)	5 (14.3)	4 (11.4)	12 (34.3)	14 (40)	35
	Severe-critical - n (% row)	0	1 (14.3)	0	6 (85.7)	7
	Total- n (% row)	**5 (11.9)**	**5 (11.9)**	**12 (28.6)**	**20 (47.6)**	**42**

COVID-19 severity is categorized as (1) mild–moderate disease if patients had no signs or symptoms of pneumonia or a mild pneumonia and (2) severe–critical disease if they presented dyspnea, or a respiratory rate of ≥30 breaths per minute or a blood oxygen saturation of ≤93%, or a ratio of the partial pressure of arterial oxygen to the fraction of inspired oxygen of <300 mmHg, or infiltrates in >50% of the lung field within 24–48 h from the onset of symptoms and/or organ or multiple organ failure

Table 4. Clinical and demographic characteristics of the cohort in relation to SARS-CoV-2 humoral persistence 6 months after COVID-19

	TOTAL n=53	NO PERSISTENCE n=10	PERSISTENCE MORE THAN 6 MONTHS n=43	OR (95% CI)[c]	p-value[c]
Age – mean (SD)	46.75 (12.15)	44.09 (10.60)	47.37 (12.51)	1.02 (0.96-1.09)	0.440
Male sex – n (%)	24 (45.28)	3 (30.00)	21 (48.84)	2.22 (0.49-10.08)	0.286
Any comorbidity[a] – n (%)	10 (33.96)	4 (40.00)	14 (32.56	0.72 (0.17-3.04)	0.658
Obesity – n (%)	7 (13.21)	0	7 (16.28)	-	0.175
Progressive MS[b] – n (%)	12 (22.64)	4 (40.0)	8 (18.60)	0.34 (0.07-1.56)	0.149
EDSS≥3.0 – n (%)	21 (100)	5 (50.0)	16 (37.21)	0.59 (0.15-2.41)	0.461
Disease duration, years – median (IQR)	14.7 (10.0)	16.2 (12.2)	14 (9.0)	0.98 (0.90-1.07)	0.693
Corticosteroids last 3 months – n (%)	0	0	0	-	-
DMTs – n (% of the row)					
No treatment	12 (100)	0	12 (100)	REF	
Interferon β	9 (100)	3 (33.3)	6 (66.7)	-	**0.035**
Glatiramer acetate	3 (100)	0	3 (100)	-	-
Dimethyl fumarate	4 (100)	1 (25.0)	3 (75.0)	-	0.083
Teriflunomide	3 (100)	0	3 (100)	-	-
Fingolimod	4 (100)	1 (25.0)	3 (75.0)	-	0.083
Natalizumab	2 (100)	0	2 (100)	-	-
Alemtuzumab	2 (100)	0	2 (100)	-	-
Cladribine	0	0	0	-	.
Ocrelizumab	3 (100)	0	3 (100)	-	.
Rituximab	9 (100)	4 (44.4)	5 (55.6)	-	**0.012**
Other anti-CD20	2 (100)	1 (50.0)	1 (50.0)	-	**0.013**
Other DMTs	0	0	0	.	**.**
Anti-CD20	11 (100)	5 (35.7)	9 (64.3)	0.26 (0.06-1.19)	0.063
Treatment duration, years – median (IQR)	2.8 (4.3)	2.1 (4.1)	3.0 (3.8)	1.09 (0.90-1.31)	0.383
Time of COVID-19 since last infusion, months – median (IQR)[1]	4.4 (5.2)	0.9 (4.0)	4.5 (8.3)	1.27 (0.84-1.92)	0.248
Time of cellular determination since last infusion, months – median (IQR) [1]	4.30 (6.9)	4.21 (1.74)	4.40 (7.92)	1.08 (0.88-1.33)	0.460
Previous lymphocyte count –median (IQR)[2]	1480 (1200)	1200 (100)	1715 (685)	1.00 (1.00-1.00)	**0.048**
Previous IgG count – median (IQR)[3]	842 (378)	772 (96)	1065 (320)	1.01 (0.99-1.03)	0.090
Previous IgM count – median (IQR)[3]	61 (46)	74 (78)	60 (30)	0.98 (0.95-1.02)	0.406
Previous IgA count –median (IQR)[3]	216.5 (103)	188 (118)	239 (92)	1.01 (0.99-1.02)	0.428
Negative RT-PCR – n (%)	5 (9.43)	1 (10.0)	4 (9.30)	REF	
Positive RT-PCR – n (%)	20 (37.74)	4 (40.0)	16 (37.21)	1.00 (0.08-12.19)	1.000
Not performed RT-PCR – n (%)	28 (52.83)	5 (50.0)	23 (53.49)	1.15 (0.10-13.10)	0.910
COVID-19 symptoms – n (%)	51 (96.23)	9 (90.0)	42 (97.67)	4.67 (0.25-87.20)	0.226
COVID-19 severe-critical course – n (%)	8 (15.09)	2 (20.00)	6 (13.95)	0.65 (0.11-3.90)	0.634
Time of first antibody determination after COVID-19 diagnosis, months– mean (IQR)	3.02 (0.92)	3.09 (1.75)	2.99 (0.89)	1.39 (0.72-2.65)	0.324
Time of second antibody determination after COVID-19 diagnosis, months– mean (IQR)	11.76 (1.38)	11.66 (1.25)	11.86 (1.41)	0.96 (0.60-1.52)	0.850

Count of total cases of variables with missing information: n^1= 18, n^2=47, n^3= 11.
[a]Any comorbidity includes obesity, lung disease, cardiovascular disease, diabetes, hypertension, hematological benign disease, chronic kidney disease, liver disease, HIV or malignancy. [b]Progressive MS includes secondary progressive multiple sclerosis (SPMS) and primary progressive multiple sclerosis (PPMS). [c]Statistical analysis was performed using a not adjusted logistic regression model. Abbreviations: MS: multiple sclerosis; SD: standard deviation; IQR: interquartile range; EDSS: Expanded Disability Status Scale: DMT: disease modifying therapy; IgG, IgM, IgA: immunoglobulin G, M or A; RT-PCR: reverse transcription-polymerase chain reaction
% is the proportion of patients of the column with that variable if not otherwise specified.

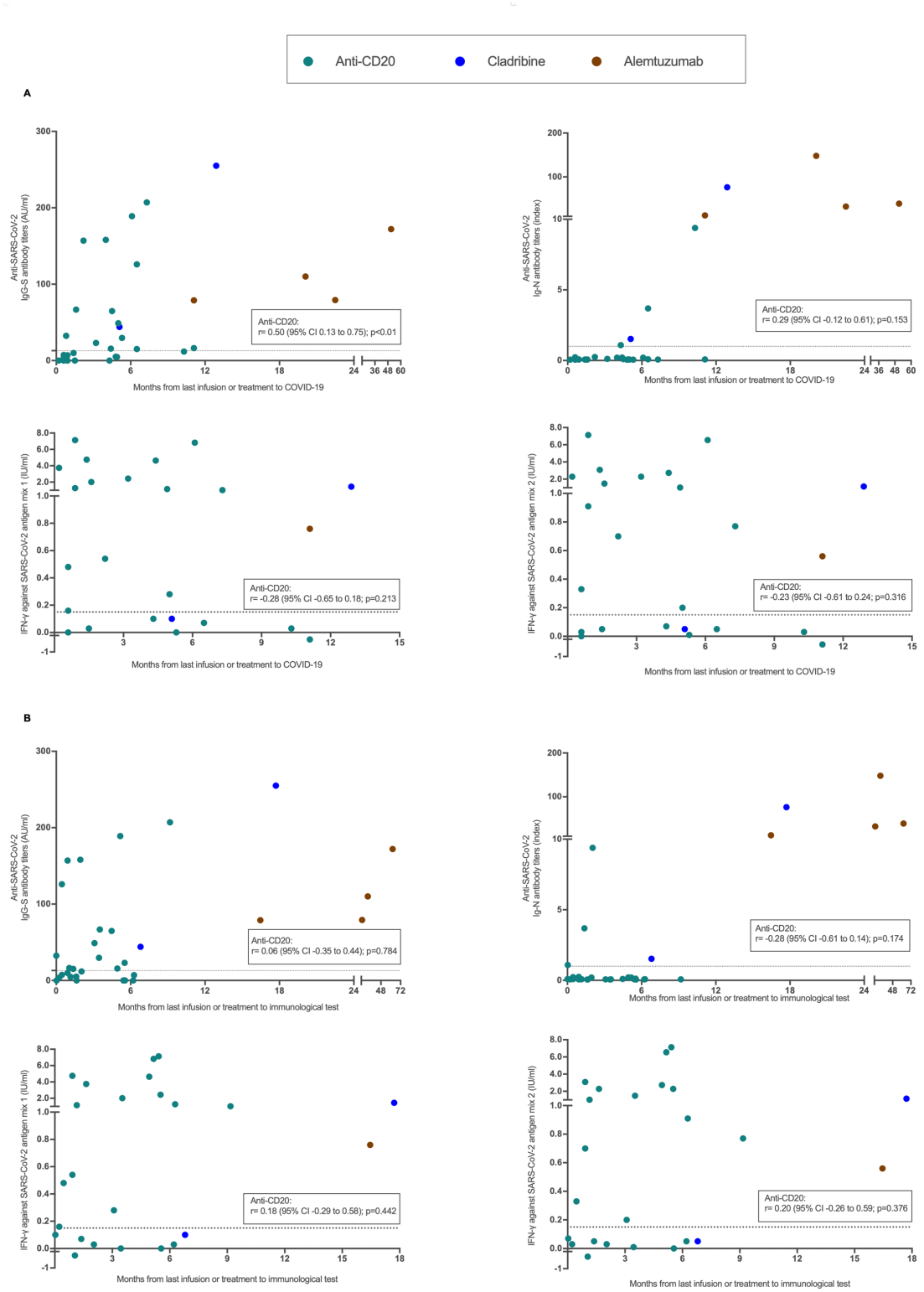
Table 5. COVID-19 severity according to treatment

	ASYMPTOMATIC COVID-19 (n=14; 9.7%)	MILD-MODERATE COVID-19 (n=112; 77.2%)	SEVERE-CRITICAL COVID-19 (n=19; 13.1%)	MILD-MODERATE vs SEVERE-CRITICAL* p-value
No treatment	0	21 (70.0)	9 (30.0)	
Interferon	3 (15.79)	16 (84.21)	0	**0.009**
Glatiramer acetate	2 (15.38)	10 (76.92)	1 (7.69)	0.116
Dimethyl fumarate	1 (5.56)	19 (88.89)	1 (4.76)	**0.046**
Teriflunomide	0	12 (100)	0	**0.034**
Fingolimod	1 (16.67)	5 (83.33)	0	0.127
Natalizumab	1 (25.0)	3 (75.0)	0	0.208
Alemtuzumab	1 (14.29)	6 (85.71)	0	0.100
Cladribine	1 (50.0)	1 (50.0)	0	0.369
Ocrelizumab	1 (14.29)	5 (71.43)	1 (14.29)	0.406
Rituximab	3 (13.64)	13 (59.09)	6 (27.27)	0.832
Other anti-CD20	0	3 (75.0)	1 (25.0)	0.839
Other DMTs	0	1 (100)	0	0.522

COVID-19 severity is categorized as (1) asymptomatic those without symptoms, (2) mild–moderate disease if patients had no signs or symptoms of pneumonia or a mild pneumonia and (3) severe–critical disease if they presented dyspnea, or a respiratory rate of ≥30 breaths per minute or a blood oxygen saturation of ≤93%, or a ratio of the partial pressure of arterial oxygen to the fraction of inspired oxygen of <300 mmHg, or infiltrates in >50% of the lung field within 24–48 h from the onset of symptoms and/or organ or multiple organ failure

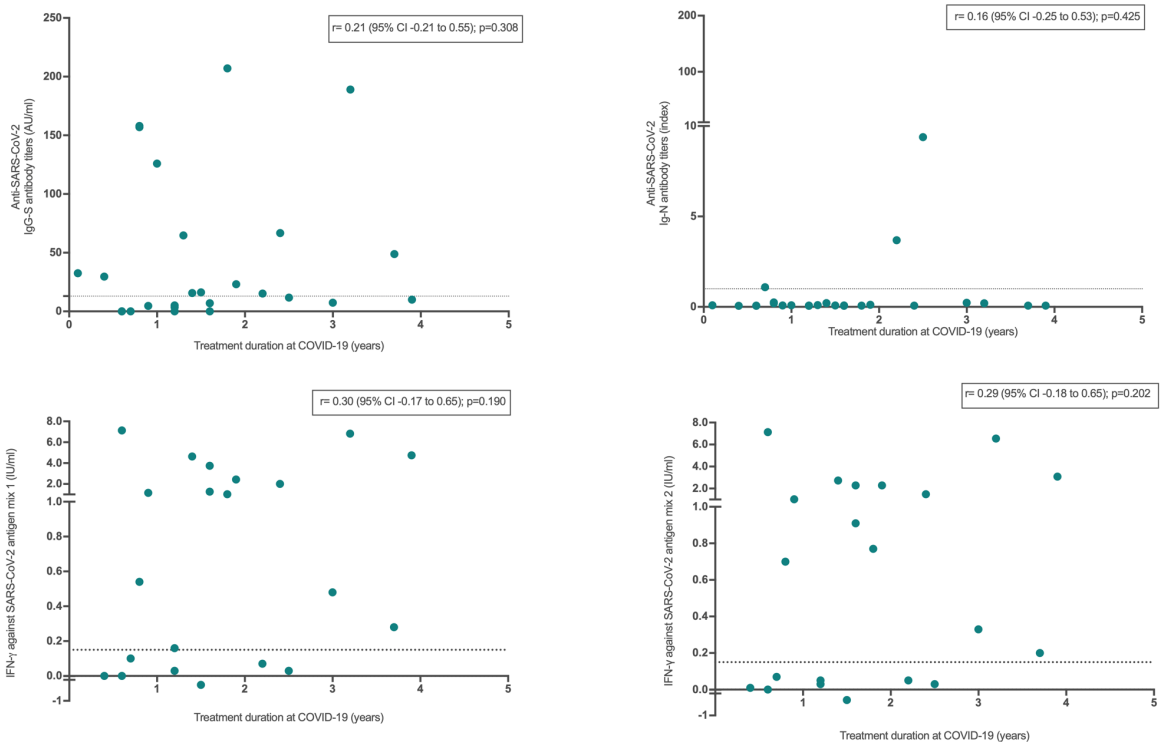
* Univariable analysis of the risk of presenting a severe-critical COVID-19. For this analysis, asymptomatic cases were included in the mild-moderate group.

Figure 1. Humoral and cellular response in relation to last treatment or infusion in patients treated with anti-CD20 therapies, cladribine or alemtuzumab



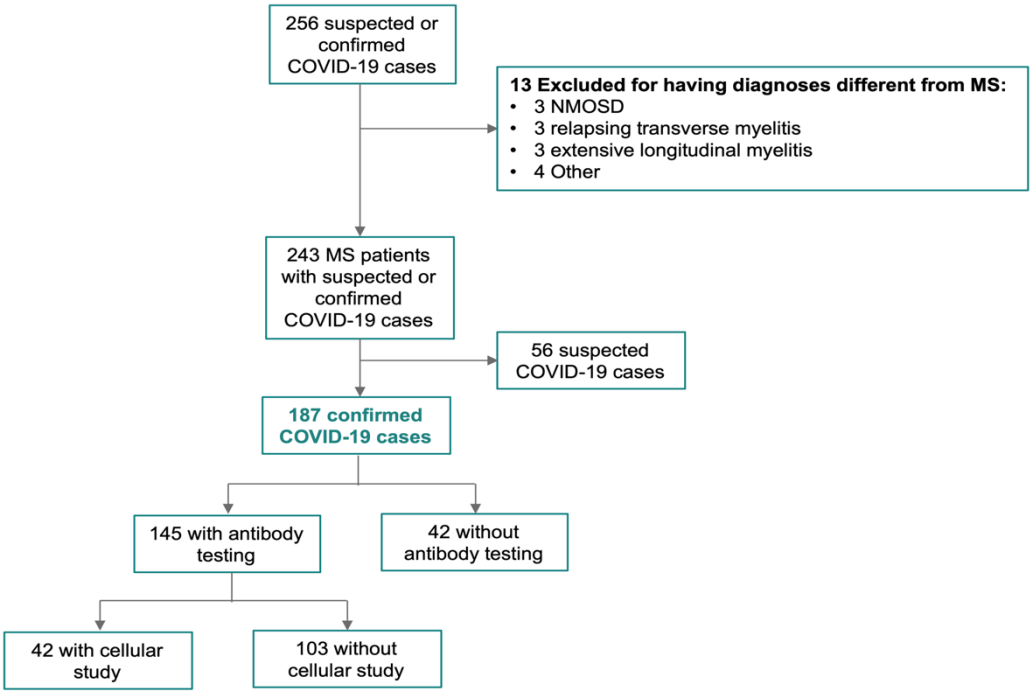
Spearman rank correlation (r) between different immunological responses to SARS-CoV-2 infection and months from last infusion or treatment to time of COVID-19 infection (A) and immunological testing (B). The studied immunological responses include: titers of titers of immunoglobulin G against SARS-CoV-2 IgG-S (upper left) and SARS-CoV-2 Ig-N (upper right); titers of interferon-gamma produced by T-cell against SARS-CoV-2 antigen mix 1 (lower left) and antigen mix 2 (lower right). Each dot represents a different subject. Cut-off values for antibody and cellular positivity are indicated by a dotted line. Abbreviations: SARS-CoV-2 IgG-S: SARS-CoV-2 IgG anti-spike antibody, SARS-CoV-2 Ig-N: total immunoglobulins against SARS-CoV-2 nucleocapsid

Figure 2. Correlation between humoral and cellular response and treatment duration at COVID-19 in patients treated with anti-CD20 therapy



Spearman rank correlation (r) between anti-CD20 therapy duration in years and: titers of immunoglobulin G against SARS-CoV-2 IgG-S (upper left) and SARS-CoV-2 Ig-N (upper right), titers of interferon-gamma produced by T-cell against SARS-CoV-2 antigen mix 1 (lower left) and antigen mix 2 (lower right). Each dot represents a different subject. Cut-off values for antibody and cellular positivity are indicated by a dotted line. Abbreviations: SARS-CoV-2 IgG-S: SARS-CoV-2 IgG anti-spike antibody, SARS-CoV-2 Ig-N: total immunoglobulins against SARS-CoV-2 nucleocapsid

Figure 3. Study flow diagram



5.4 Article 3

Is humoral and cellular response to SARS-CoV-2 vaccine modified by DMT in patients with multiple sclerosis and other autoimmune diseases?

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Short Report

Is humoral and cellular response to SARS-CoV-2 vaccine modified by DMT in patients with multiple sclerosis and other autoimmune diseases?

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Abstract:

Background: The effect of disease-modifying therapies on severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) vaccine response is unclear.

Objectives: We aim to determine the immunological responses to SARS-CoV-2 in multiple sclerosis (MS) and anti-CD20-treated patients with other autoimmune diseases (AID).

Methods: Humoral and cellular responses we determined before and 30-90 days after vaccination in patients with MS and anti-CD20-treated patients with other AID in two Catalan centers.

Results: 457 patients were enrolled. Findings showed that humoral response decreased under anti-CD20s or sphingosine 1-phosphate receptor modulators (S1PRM) and with longer treatment duration and increased after 4.5 months from the last anti-CD20 infusion. Cellular response decreased in S1PRM-treated. Patients on anti-CD20 can present cellular responses even in the absence of antibodies.

Conclusion: Anti-CD20s and S1PRM modify the immunological responses to SARS-CoV-2 vaccines.

Keywords: COVID-19, SARS-CoV-2, multiple sclerosis, disease-modifying therapy, humoral response, cellular response

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Introduction

Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome CoV 2 (SARS-CoV-2), resulted in a global pandemic. In response, many organizations worked together to make vaccines available to the public. Most approved SARS-CoV-2 vaccines induce humoral and cellular immune responses that reduce COVID-19 severity in the general population.[1]

Disease-modifying therapies (DMTs) can impact humoral and cellular immunity, which are both essential for protection against COVID-19 and vaccine response.[2] Studies regarding SARS-CoV-2 vaccines in patients with multiple sclerosis (pwMS) and other autoimmune diseases (AIDs) showed that humoral response was preserved under most DMTs, except for anti-CD20 and sphingosine-1-phosphate receptor modulators (SP1RM) therapies.[3–5] Cellular response studies, focused on anti-CD20s, demonstrated that it is generally present even in the absence of humoral response.[4–7] However, information regarding cellular response to SARS-CoV-2 vaccines in pwMS and other DMTs or in anti-CD20-treated AID patients is limited.[8]

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In this study, we determined the humoral and cellular responses after SARS-CoV-2 vaccination in pwMS with different DMTs or untreated and anti-CD20-treated patients with other AIDs.

Patients and methods

This prospective study was conducted between February and October 2021 in two Catalan hospitals. Inclusion criteria were (1) pwMS on any DMT or untreated and patients with other AIDs currently on anti-CD20s, (2) ⩾18 years old, (3) unvaccinated and willing to be vaccinated and (4) without previous known SARS-CoV-2 infection. Patients with other AID were patients with a neuroimmunological diseases not meeting multiple sclerosis criteria, systemic or nephrological AID who had received an anti-CD20-therapy in the previous year to the study enrolment.

One pre-vaccination sample per patient was collected before vaccination and another post-vaccination blood sample was taken after 30–90 days of the last vaccine dose. Fully vaccinated patients were considered those with two injections for the Pfizer, Moderna and AstraZeneca, and one injection for the Johnson & Johnson vaccine.

The humoral response was measured using commercial chemiluminescence immunoassays targeting specific antibodies against SARS-CoV-2 spike (IgG-S) and nucleocapsid proteins (Ig-N) before and after vaccination. Ig-N was detected using the Elecsys® test with a cut off of 1.0 index. IgG-S was measured using the LIAISON® TrimericS and Abbot® Quant test depending on the centre. Results are expressed in BAU/mL with a cutoff point of 33.8 BAU/mL. Seroconversion rate was calculated as the percentage of patients with a determination over the cutoff value.

The SARS-CoV-2 specific T-cell response was studied post-vaccination concurrently with the humoral response in 150 selected pwMS with consecutive sampling according to DMT and analysed using the whole blood Interferon-Gamma (IFN-γ) Release Immunoassay with Qiagen QuantiFERON® SARS-CoV-2 RUO according to the manufacturer. The test was considered positive if IFN-γ titers against antigen mix 1 and/or 2 were higher than 0.15 UI/mL.

Continuous variables were compared using the Mann–Whitney test. Correlations were addressed using Spearman's rank correlation coefficient. A multivariable logistic regression model was performed to determine factors that influence the absence of humoral or cellular responses to vaccines only in fully vaccinated patients. To avoid potential immunological modifications in the post-vaccination results, patients with positive serological tests (asymptomatic COVID-19) in the pre-vaccination sample or with post-vaccination COVID-19 were excluded. Statistical tests were performed on the 0.05 level of significance using the Stata version 14.0.

This study was approved by the local ethics committee (EOM (AG) 003/2021 (5768) and 2021.05). Informed consent was obtained from the patients.

Results

Overall, 457 participants were included: 421 pwMS and 36 with other AIDs. Table 1 shows the clinical and demographic variables. Of the 457 patients, 431 (94.3%) were fully vaccinated with an mRNA vaccine; 12 patients were excluded from the analysis due to positive SARS-CoV-2 antibodies in the pre-vaccination sample and 5 with COVID-19 post-vaccination.

Post-vaccination samples were collected in 430/440 (97.7%) patients within 2.0 (standard deviation 0.8) months after the last vaccine dose. Humoral responses were detected in patients with all types of DMTs. The seroconversion rate was >92.0%, except for patients on anti-CD20s and S1PRMs (45.6% and 51.4%, respectively) (Table 1). Patients on anti-CD20s and S1PRMs presented lower IgG titers compared to those untreated or on other DMTs ($p < 0.001$ for all comparisons; Figure 1(a1)). In the multivariable analysis, the absence of antibodies was associated with anti-CD20s, S1PRMs and longer treatment duration with any DMT (Figure 1(a2).

Regarding anti-CD20 therapy, IgG titres were associated with days elapsed between the last infusion and the first vaccine dose, treatment duration and previous IgG immunoglobulin levels (Figure 1(b1)–(b3)). Seroconversion was >80.0% from 4.5 months after the last infusion. In these patients, only a longer treatment duration was associated with the absence of antibodies (Figure 1(b4)).

Cellular responses were detected in 84.4% of patients. All DMTs presented a cellular response rate of >75.0%, except for patients on S1PRMs (11%). Furthermore, 91.4% of anti-CD20-treated patients without humoral response had a cellular response, and no significant differences were found in IFN-γ levels between those with presence or absence of humoral responses. Patients on S1PRMs presented lower IFN-γ levels compared to those on other DMTs or untreated ($p < 0.010$ for all comparisons). No relation with

Table 1. Clinical and demographic characteristics of the cohort in relation to DMT.

	All participants (*n*=457)	No DMT (*n*=28)	IFN Beta (*n*=31)	GA (*n*=26)	TF (*n*=31)	DMF (*n*=38)	CLA (*n*=30)	ALZ (*n*=30)	NTZ (*n*=32)	S1PRM (*n*=36)[a]	Anti-CD20s (*n*=139)	Anti-CD20s, other AIDs (*n*=36)[b]
Age – mean (*SD*)	48.2 (11.8)	61.0 (11.9)	55.3 (12.6)	46.2 (12.4)	47.4 (9.6)	45.6 (9.1)	41.2 (8.6)	42.4 (8.6)	43.8 (11.2)	48.1 (9.2)	48.9 (11.7)	50.0 (15.4)
Male sex – *n* (%)	143 (31.3)	6 (21.4)	13 (41.9)	11 (42.3)	11 (35.6)	13 (34.2)	4 (13.3)	7 (23.3)	7 (21.8)	9 (25.0)	62 (35.4)	16 (44.4)
Any comorbidity[c] – *n* (%)	205 (44.9)	22 (78.6)	14 (45.2)	15 (57.7)	13 (41.9)	16 (42.1)	11 (36.7)	14 (46.7)	12 (37.5)	13 (36.1)	75 (42.9)	10 (27.8)
Disease duration, years – median (range)	13.6 (6.8–20.8)	20.8 (16.8–26.8)	20.7 (12.7–25.7)	10 (3.2–18.2)	13.7 (6.8–20.0)	8.7 (4.8–13.5)	10.6 (3.3–15.8)	15.0 (7.7–21.2)	16.1 (6.8–20.8)	18.8 (13.9–23.7)	13.8 (7.7–21.7)	6.2 (2.3–10.7)
Progressive MS[d] – *n* (%)	124 (29.4)	10 (35.7)	6 (19.3)	6 (23.1)	2 (6.4)	1 (2.6)	–	3 (10.0)	7 (21.9)	10 (27.8)	79 (56.8)	–
EDSS – median (range)	3.0 (2.0–5.0)	4.0 (2.0–5.0)	2.5 (1.0–3.5)	2.0 (1.5–5.0)	2.0 (1.5–3.5)	2.0 (1.5–3.0)	2.0 (1.5–3.0)	3.5 (2.0–5.5)	3.5 (2.0–5.5)	3.5 (5.5–2.0)	4.0 (1.5–3.0)	2.0 (2.0–5.5)
Treatment duration, years – median (range)	2.5 (1.0–4.8)	–	9 (4.6–16.4)	4.9 (1.5–10.1)	3.3 (0.9–5.2)	2.4 (1.5–3.4)	1.1 (0.7–1.8)	3.3 (2.5–4.6)	8.5 (2.6–12.3)	5.5 (3.7–8.3)	1.6 (0.8–2.9)	1.9 (0.8–4.3)
Months between last infusion/treatment and first vaccine dose – median (range)	3.7 (1.8–6.4)	–	–	–	–	–	7.7 (4.8–9.0)	27.3 (16.7–37.7)	–	–	2.9 (1.6–4.6)	3.6 (2.3–4.8)
Previous lymphocyte count[e] – median (range)	1510 (1075–2100)	2200 (1590–2620)	1470 (1220–2040)	1870 (1360–2200)	1500 (1200–1800)	1185 (995–1535)	1040 (720–1600)	1480 (1050–1700)	3265 (2420–3850)	595 (430–660)	1600 (1200–2200)	1600 (1370–2200)
Vaccine type – *n* (%) Pfizer	29 (6.3)	1 (3.6)	6 (19.4)	4 (15.4)	2 (6.5)	2 (6.3)	1 (3.3)	–	1 (2.6)	4 (11.1)	8 (5.8)	–
Moderna	402 (88.0)	24 (85.7)	21 (67.7)	21 (80.8)	24 (77.4)	29 (90.6)	27 (90.0)	29 (96.7)	33 (86.8)	30 (83.3)	128 (92.1)	36 (100)
AstraZeneca	24 (5.6)	3 (10.7)	3 (9.7)	1 (3.9)	5 (16.1)	1 (3.1)	1 (3.3)	1 (3.3)	4 (10.5)	2 (5.6)	3 (2.2)	–
AZ + Pfizer	2 (0.4)	–	1 (3.2)	–	–	–	1 (3.3)	–	–	–	–	–
Positive IgG/N pre-vaccine, *n* (%)	12 (2.66)	3 (10.7)	2 (6.5)	1 (3.9)	1 (3.3)	1 (2.7)	1 (3.3)	2 (6.9)	–	1 (3.0)	–	–
Months of post-vaccine extraction after last vaccine dose, mean (*SD*)	2.0 (0.6)	1.8 (0.6)	2.1 (0.6)	2.1 (0.5)	2.0 (0.7)	2.1 (0.5)	2.1 (0.6)	2.1 (0.7)	1.9 (0.5)	2.2 (0.7)	2.0 (0.6)	2.0 (0.7)
Positive IgG-S, *n* (%) (≥33.8 BAU/mL)	320/430 (74.4)	24/24 (100.0)	25/27 (92.59)	23/23 (100)	29/29 (100)	36/36 (100)	29/29 (100)	27/27 (100)	30/31 (96.7)	18/35 (51.4)	67/134 (50.0)	12/35 (34.3)
IgG index in BAU/mL – mean (*SD*)	946.5 (897.6)	1628.6 (695.9)	1470.8 (795.5)	1305.9 (763.2)	1581.9 (716.6)	1665.3 (554.3)	1610.2 (623.3)	1718.6 (627.7)	1629.6 (675.1)	211.2 (493.2)	240.7 (487.2)	219.0 (417.4)
Positive anti-SARS-CoV-2 IFN-γ, *n* (%)	119/141 (84.4)	1/6 (85.7)	7/9 (77.8)	2/2 (100)	9/9 (100)	10/10 (100)	9/10 (90.0)	8/8 (100)	8/9 (88.9)	1/9 (11.11)	57/66 (86.4)	2/2 (100)

[a]Fingolimod *n*=35; Siponimod *n*=1.
[b]Other AIDs include neuromyelitis optica spectrum disorder (NMOSD) *n*=14, myelin oligodendrocyte glycoprotein antibody disorders (MOGAD) *n*=5, IgG-4-related diseases *n*=3, ANCA-associated vasculitis *n*=3, idiopathic membranous nephropathies *n*=2, minimal-change disease nephropathy *n*=2, primary angiitis of the central nervous system *n*=1, systemic lupus erythematosus vasculitis *n*=1, myelorradiculitis *n*=1, Tolosa-Hunt syndrome *n*=1, GFAP-encephalitis *n*=1 and Susac syndrome *n*=1.
[c]Any comorbidity includes obesity, lung disease, cardiovascular disease, diabetes, arterial hypertension, haematological benign disease, chronic kidney disease, liver disease, HIV or malignancy.
[d]Progressive MS includes secondary progressive multiple sclerosis (SPMS) and primary progressive multiple sclerosis (PPMS).
[e]Previous lymphocyte count was obtained in 412 participants.
EDSS: Expanded Disability Status Scale; IgG-S: IgG antibody against SARS-CoV-2 spike protein; IgG-N: antibody against SARS-CoV-2 nucleoprotein; IFN-γ: Interferon gamma. DMT: disease-modifying therapy; IFN: interferon; GA: glatiramer acetate; TF: teriflunomide; DMF: dimethyl fumarate; S1PRM, sphingosine 1-phosphate receptor modulator; NTZ: natalizumab; CLA: cladribine; ALZ: alemtuzumab; Anti-CD20s: includes ocrelizumab, rituximab and ofatumumab; AID: autoimmune disease.

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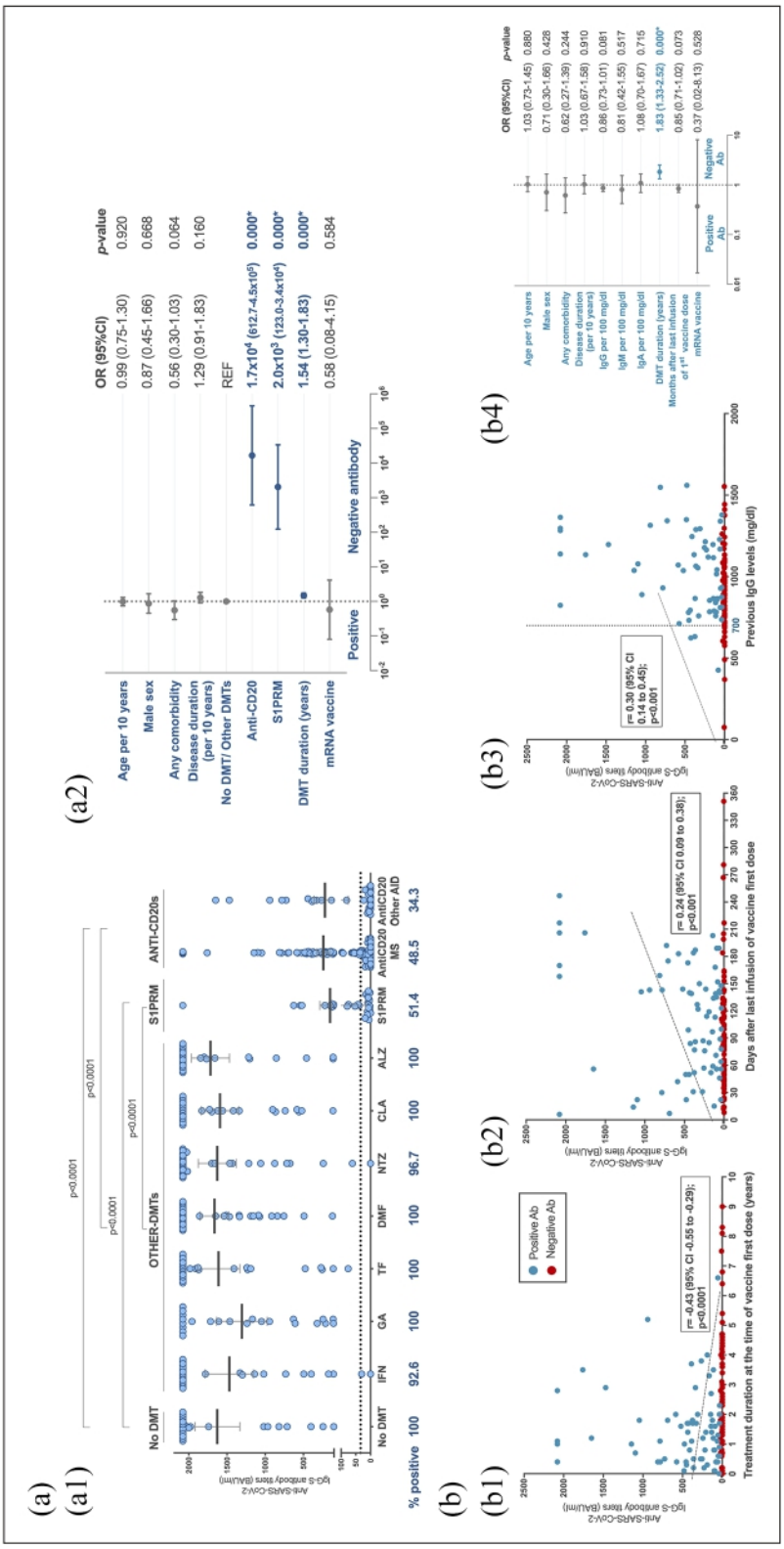

Figure 1. *(Continued)*

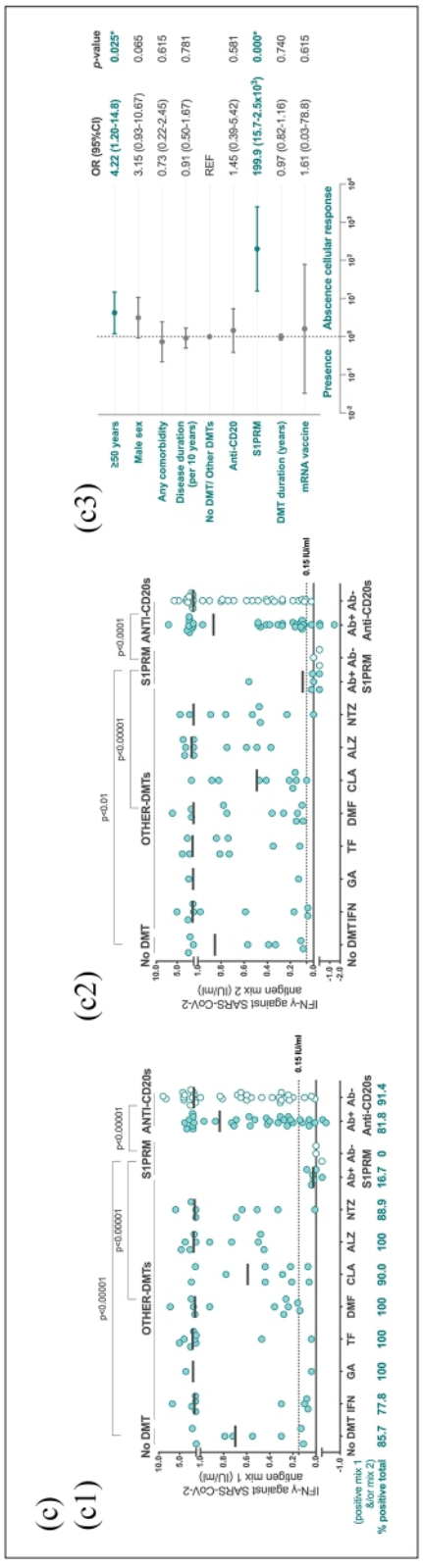

Figure 1. Humoral and cellular response to SARS-CoV-2 vaccines. (a) Humoral response to SARS-CoV-2 vaccine: (a1) Mean titres of immunoglobulin G against SARS-CoV-2 spike protein (IgG-S), each dot represents a different subject. The percentage of positive cases for each DMT is represented. The statistical analysis was performed using a Mann–Whitney test analysis, only statistically significant differences are indicated. Cut-off values for antibody positivity are indicated by a dotted line (33.8 BAU/mL). (a2) Forest plot depicting adjusted odds ratios (OR) for not presenting a humoral response against SARS-CoV-2 ($n = 430$). Demographic and clinical characteristics are represented with OR, 95% confidence interval (95% CI) and p-values. Statistical analysis was performed using a logistic regression model adjusted for age, sex, presenting any comorbidity, disease duration per 10 years, DMT (untreated or other DMT as reference, anti-CD20 therapies and S1PRM), DMT duration in year and type of vaccine (mRNA vaccine vs others). (b) Humoral response to SARS-CoV-2 vaccine in anti-CD20-treated patients. (b) IgG anti-spike titres according to time since last infusion (b1), treatment duration (b2) and previous immunoglobulin G levels (b3) at the time of the first vaccine dose. Spearman rank correlation, r is indicated. IgG seropositive (green) and seronegative (red) subjects are represented. Hypogammaglobulinemia is considered when IgG levels are below 700 mg/dL (vertical dotted line). (b4) Forest plot depicting adjusted ORs for not presenting a humoral response against SARS-CoV-2 in patients with anti-CD20 therapies ($n = 140$). Demographic and clinical characteristics are represented with OR, 95% CI and p values. Statistical analysis was performed using a logistic regression model adjusted for age; sex; presenting any comorbidity; disease duration per 10 years; previous IgG, IgM and IgA immunoglobulins levels per 100 mg/dL; tDMT duration in years; and type of vaccine (mRNA vaccine vs others). (c) Cellular response to SARS-CoV-2 vaccine. Mean titres of interferon-gamma produced by T-cell against SARS-CoV-2 antigen mix 1 (c1) and SARS-CoV-2 antigen mix 2 (c2); each dot represents a different subject. Statistical analysis was performed using a Mann–Whitney test analysis, only statistically significant differences are indicated. Cut-off values for positive cellular response are indicated by a dotted line. (c3) Forest plot depicting adjusted ORs for not presenting a cellular response against SARS-CoV-2 ($n = 141$). Demographic and clinical characteristics are represented with OR, 95% CI and p values. Statistical analysis was performed using a logistic regression model adjusted for age, sex, presenting any comorbidity, disease duration per 10 years, DMT (untreated or other DMT as reference, anti-CD20 therapies and S1PRM), DMT duration in years and type of vaccine (mRNA vaccine vs others).

MS: multiple sclerosis; Other AIDs: other autoimmune diseases include neuromyelitis optica spectrum disorder (NMOSD) $n = 14$, myelin oligodendrocyte glycoprotein antibody disorders (MOGAD) $n = 5$, IgG-4 related diseases $n = 3$, ANCA-associated vasculitis $n = 3$, idiopathic membranous nephropathies $n = 2$, minimal-change disease nephropathy $n = 2$, primary angiitis of the central nervous system $n = 1$, systemic lupus erythematosus vasculitis $n = 1$, myelorradiculitis $n = 1$, Tolosa-Hunt syndrome $n = 1$, GFAP-encephalitis $n = 1$ and Susac syndrome $n = 1$. IgG-S: IgG antibody against SARS-CoV-2 spike protein; IFN-γ: Interferon gamma; DMT: disease-modifying therapy; IFN: interferon gamma, GA: glatiramer acetate; TF: teriflunomide; DMF: dimethyl fumarate; NTZ: Natalizumab; CLA: Cladribine; ALZ: Alemtuzumab; S1PRM, sphingosine 1-phosphate receptor modulator (fingolimod and siponimod); Anti-CD20 MS: patients with MS treated with ocrelizumab, rituximab or ofatumumab; Anti-CD20 other AID: patients with other autoimmune diseases treated with rituximab. Any comorbidity includes obesity, lung disease, cardiovascular disease, diabetes, hypertension, haematological benign disease, chronic kidney disease, liver disease, HIV or malignancy; Ab: antibody.

previous lymphocyte count was found (data not shown). Cellular response decreased in S1PRM-treated and >50-year-old patients (Figure 1(c)). Cellular response was present in 78.2% in patients aged >50 years as compared to 88.4% in those <50 years ($p = 0.104$).

Discussion

We found that after SARS-CoV-2 vaccination, pwMS and other AIDs on anti-CD20s or S1PRMs and with longer treatment duration were more likely to remain seronegative. Patients on most DMTs presented a cellular response; however, it decreased in S1PRM-treated and >50-year-old patients.

In line with previous studies, our study showed a 90.0% seroconversion rate after SARS-CoV-2 vaccination in all patients, except those receiving anti-CD20 therapies or SP1RM. Patients on these two DMTs present lower IgG titers compared to those on other DMTs.[3,4]

The cellular response after SARS-CoV-2 vaccination appeared to be preserved under most DMTs.[8,9] Conversely, <12% of SP1RM-treated patients mounted a cellular response regardless of their serostatus or lymphocyte count. As highlighted in some studies, anti-CD20s present a high percentage of cellular response rate even in the absence of a humoral response.[4–7] However, the protective effect of cellular response against COVID-19 remains unknown.

It is not surprising that anti-CD20 therapies reduce the response to vaccines as they impair memory B-cell production. IgG titers correlate with months since last anti-CD20 infusion, treatment duration and IgG immunoglobulin levels. We observed that the seroconversion rate increased up to 80% when the vaccine was administered 4.5 months after the last anti-CD20 infusion, similar to other groups.[4–6] Therefore, optimizing the moment of vaccine administration could potentially lead to an increased antibody response. On the contrary, SP1RMs suppress lymphocyte egress from lymph nodes, affecting both humoral and cellular responses as observed in this study. In addition, cellular response seems weakened in patients aged >50 years, maybe due to immunosenesce.[10]

Our study has some limitations. First is the relatively small number of cases in some DMT groups. Second, previous studies on cellular response to SARS-CoV-2 vaccination used other methods, thus limiting the comparisons of our results. Third, information about the SARS-CoV-2 infection post-vaccination and its course is missing and therefore the protective immune effect is not clearly stablished.

Overall, these data provide valuable information about those pwMS or other AIDs on anti-CD20s that present a blunted immune response to SARS-CoV-2 vaccine and could possibly benefit from individualized vaccination strategies or prophylactic anti-SARS-CoV-2 monoclonal antibodies.

Declaration of Conflicting Interests

The author(s) declared the following potential conflicts of interest with respect to the research, authorship and/or publication of this article: A.Z. has received travel expenses for scientific meetings from Biogen-Idec, Merck Serono and Novartis; speaking honoraria from Eisai; and a study grant from Novartis. G.A. has received compensation for consulting services or participation in advisory boards from Sanofi, Merck and Roche; research support from Novartis; travel expenses for scientific meetings from Novartis, Roche, Stendhal and ECTRIMS; speaking honoraria from Sanofi and Merck; and is a member of the International Women in Multiple Sclerosis (iWiMS) network executive committee. S.O.-R. has received speaking and consulting honoraria from Genzyme, Biogen-Idec, Novartis, Roche, Excemed and MSD; as well as research support from Novartis. A.P. is an ECTRIMS clinical fellowship awardee. P.T. was an ECTRIMS clinical fellowship awardee in 2019–2020; has received travel expenses for scientific meetings from Roche. S.C.-R. was an ECTRIMS clinical fellowship awardee 2019–2020; has received travel expenses for scientific meetings from Biogen-Idec and Genzyme; compensation for consulting services or participation in advisory boards from Roche and Novartis; speaking honoraria from Novartis; and research grants from Biogen-Idec. M.M.-G. has received speaking honoraria by Beckman Coulter and Sobi. P.C.-M. has received travel expenses from Biogen. P.C.-M.'s yearly salary is supported by a grant from Biogen to Fundació privada Cemcat towards statistical analysis. F.M.-V. Has received compensation for consulting services from Alnylam and Pfizer. B.R.-A. has received honoraria for consulting services from Wellspect. L.M. has received honoraria for consulting services and speaking honoraria from Roche and Novartis. A.V.-J. has received compensation for consulting services and speaking honoraria from Novartis, Roche, Teva, Mylan, Biogen and Genzyme-Sanofi. A.C.-C. has received grant from Instituto de Salud Carlos III, Spain; JR19/00007. C.T. is currently being funded by a Junior Leader La Caixa Fellowship. The fellowship code is LCF/BQ/PI20/11760008. She has also received the 2021

Fundación Merck Salud Award for the Investigation in Multiple Sclerosis (Spain). In 2015, she received an ECTRIMS Post-doctoral Research Fellowship and has received funding from the UK MS Society. J.R. has received speaking honoraria and personal compensation for participating on Advisory Boards from Almirall, Bayer Schering Healthcare, Biogen-Idec, Genzyme. M.C. has received compensation for consulting services and speaking honoraria from Bayer Schering Pharma, Merck Serono, Biogen-Idec, Teva Pharmaceuticals, Sanofi-Aventis and Novartis. C.N. has received funding for travel from Biogen-Idec and F. Hoffmann-La Roche, Ltd.; and speaker honoraria from Novartis. J.S.-G. has received compensation for consulting services and speaking honoraria from Almirall, Bayer, Biogen, Celgene, Sanofi, Merck, Novartis, Roche, Bial, Biopass and Teva; is member of the editorial committee of *Multiple Sclerosis Journal*; and director of Revista de Neurología. L.R.T. has received compensation for consulting services and speaking honoraria from Almirall, Bayer Schering Pharma, Biogen-Idec, Genzyme, Merck Serono, Novartis, Roche, Sanofi-Aventis, Bristol-Myers-Squibb and Teva Pharmaceuticals. M.T. has received compensation for consulting services and speaking honoraria from Almirall, Bayer Schering Pharma, Biogen-Idec, Genzyme, Merck Serono, Novartis, Roche, Sanofi-Aventis Viela-Bio and Teva Pharmaceuticals. Dr. Tintore is co-editor of *Multiple Sclerosis Journal* – Experimental, Translational and Clinical. X.M. has received speaking honoraria and travel expenses for scientific meetings; has been a steering committee member of clinical trials or participated in advisory boards of clinical trials in the past 3 years with Actelion, Alexion, Bayer, Biogen, Celgene, EMD Serono, Genzyme, Immunic, Medday, Merck, Mylan, Nervgen, Novartis, Roche, Sanofi-Genzyme, Teva Pharmaceutical, TG Therapeutics, Excemed, MSIF and NMSS. S.L.-M., J.E., C.F.-N., M.C. M.B. M.R.-S., J.B. M.R.B., M.G. M.M. A.Q.-V., A.M., I.G., G.A., R.R., D.P.d.C., X.Q., M.J.S., I.A, I.G., J.C. and C.E. report no disclosures.

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5.5 Article 4

Impact of COVID-19 pandemic on frequency of clinical visits, performance of MRI studies, and therapeutic choices in a multiple sclerosis referral centre

Cobo-Calvo A, **Zabalza A**, Río J, Arrambide G, Otero-Romero S, Tagliani P, Cárdenas-Robledo S, Castillo M, Espejo C, Rodriguez M, Carbonell P, Rodríguez B, Midaglia L, Vidal-Jordana Á, Tur C, Galan I, Castillo J, Comabella M, Nos C, Auger C, Tintoré M, Rovira À, Montalban X, Sastre-Garriga J.

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ORIGINAL COMMUNICATION

Impact of COVID-19 pandemic on frequency of clinical visits, performance of MRI studies, and therapeutic choices in a multiple sclerosis referral centre

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Abstract

Introduction To evaluate the impact of the COVID-19 pandemic on (1) number of clinical visits, (2) magnetic resonance (MR) scans, and (3) treatment prescriptions in a multiple sclerosis (MS) referral centre.

Methods Retrospective study covering January 2018 to May 2021.

Results The monthly mean (standard deviation [SD]) of visits performed in 2020 (814[137.6]) was similar to 2018 (741[99.7]; $p=0.153$), and 2019 (797[116.3]; $p=0.747$). During the COVID-19 period (2020 year), 36.3% of the activity was performed through telemedicine. The number of MR scans performed dropped by 76.6% during the "first wave" (March 14 to June 21, 2020) compared to the mean monthly activity in 2020 (183.5[68.9]), with a recovery during the subsequent two months. The monthly mean of treatment prescriptions approved in 2020 (24.1[7.0]) was lower than in 2019 (30[7.0]; $p=0.049$), but similar to 2018 (23.8[8.0]; $p=0.727$). Natalizumab prescriptions increased in the "first wave" and onwards, whereas anti-CD20 prescriptions decreased during the COVID-19 period.

Conclusion Maintenance of the number of clinical visits was likely due to telemedicine adoption. Although the number of MR dramatically dropped during the "first wave", an early recovery was observed. Treatment prescriptions suffered a slight quantitative decrease during 2020, whereas substantial qualitative changes were found in specific treatments.

Keywords Multiple sclerosis · COVID-19 · SARS-CoV-2 · Standards of care

Introduction

The novel coronavirus, SARS-CoV-2, causing coronavirus disease 2019 (COVID-19) has produced a rapidly expanding worldwide pandemic with a deep impact on all aspects of healthcare [1]. According to updated information, COVID-19 susceptibility and severity risk factors in patients with immune-mediated inflammatory diseases appear to be similar to the general population [2–6]. However, contradictory data exist on whether people with multiple sclerosis (MS) are at higher risk of being infected by SARS-CoV-2 and develop a poorer outcome than non-MS patients [7–10]. In addition, MS disease-modifying drugs (DMD) have been proved to be safe during the pandemic, except for anti-CD20 therapies or intravenous

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methylprednisolone that may increase the risk of infection and worsen COVID-19 evolution [7, 9, 11, 12].

COVID-19 has excessively strained healthcare systems all around the world [13], compelling neurology departments and MS centres to adopt deep structural changes to maintain standards of care [13–18]. To buffer the impact of COVID-19 on both clinical activity and treatment administration procedures, the MS Centre of Catalonia (Cemcat) implemented a large internal reorganization: organization of healthcare professionals into non-contact stand-alone teams, adoption of telemedicine, prioritization of diagnostic tests, changes in medicine dispensation, etc.[18]. As others did [9, 19, 20], internal recommendations for the management of MS therapies were established, despite the unknown impact of DMD on the risk of COVID-19 susceptibility or outcomes at the beginning of the pandemic [18]. One year later, the evaluation of whether such urgent adaptations have enabled to keep standards of care in MS is warranted to face future extreme epidemiological situations.

The current study aims to assess the impact of the COVID-19 pandemic on (1) number of clinical visits, (2) magnetic resonance (MR) scans, and (3) treatment prescriptions in a MS referral centre.

Methods

The study was approved in the session Number 499 by the local Ethics Committee at Vall d'Hebron University Hospital (VHUH) in Barcelona, Spain.

Research site

Cemcat (Barcelona, Spain) is a specialized centre focusing on MS and other autoimmune disorders of the central nervous system (CNS). The centre provides healthcare coverage to patients with MS with a multidisciplinary approach based on neurological clinical visits, neuro-rehabilitation, neuropsychology, and a dedicated infusion centre. As a part of the Cemcat clinical activity, patients are routinely visited at an outpatient care centre every 3–12 months or at the time of new MS-related events. Intravenous acute or chronic treatments are administered at the infusion centre when medically needed. As part of the Cemcat supporting diagnostic tests activity, MR scans are performed at the Institut de Diagnostic per la Imatge (Section of Neuroradiology, VHUH), when needed. All potential DMD prescriptions are discussed in a weekly meeting attended by all involved treating neurologists, nurses, pharmacists and pharmacologists to reach consensus on any prescription issued.

Patients and data collection

This is a single-centre observational and retrospective study between January 2018 and May 2021. The number of first and follow-up visits at the outpatient care centre (clinical activity) was retrieved. Visits were classified into two types: face-to-face visits when patients moved to the outpatient care centre to be visited on-site, and telemedicine visits when the visit was performed by telephone or video call. The number of brain and spinal cord MR scans (radiological activity) was also retrieved. Both the number of visits and MR scans were anonymously extracted from the VHUH electronic database. The number and type of treatment prescription initiation (regardless of whether these were initiated in naïve or already treated patients–switches) were anonymously extracted from our Cemcat in-house clinical management software (eCemcat). Treatment prescriptions were classified into three groups: (1) first line (interferon, acetate glatiramer, teriflunomide, dimethyl-fumarate); (2) anti-CD 20 (rituximab, ocrelizumab); and (3) other second line (natalizumab, fingolimod, cladribine and alemtuzumab).

Study periods

Three different time periods were defined. The "COVID-19" period corresponds to 2020 (January 1 to December 31, 2020). Two control periods were established: (1) A first "control period" (named 2018 year) comprising activity registered between January 1 to December 31, 2018, and (2) a second "control period" (named 2019 year) comprising activity registered between January 1 to December 31, 2019. The term "first wave period" was used to define the period of time between March 14, and June 21, 2020, in which a complete population lockdown was enforced by the Government of Spain [18]. Since in holiday periods, all activities tend to decrease, the term "holiday period" defines the period of time between July 15th to August 31st and December 20th to January 10th in every year. Finally, an "extension period" comprising January 1 to May 31, 2021, was included as sensitivity analysis to confirm trends of treatment prescriptions during 2020 in comparison to control periods, if any.

Statistical analysis.

Distribution of continuous variables (number of visits, MR scans and treatment prescriptions) was described as mean and standard deviation (SD). Categorical variables were described as number and percentage (%).

Two different analyses were performed. First, the monthly number of clinical visits, MR scans and treatment prescriptions per period were compared between the control periods

and the COVID-19 period using the unpaired Student's t test. Second, a dynamic description of the monthly number of clinical visits, MR scans and treatment prescriptions during the COVID-19 and control periods was detailed. To provide an objective tool to detect significant activity changes across periods, values beyond ± 2SD from the mean monthly activity observed during the whole study period (i.e., monthly number of visits, MR scans and treatment prescriptions) were defined as *outliers*; otherwise variables were considered to remain stable during the time on study. In all analyses, type I error was set at $p < 0.05$. Statistical analyses and graphs were performed with STATA-12 software (64-bit, StataCorp, TX) and Prism 9 (9.01).

Results

Clinical activity

During the study period, a total of 28,230 visits were carried out at the outpatient care centre (8898 in 2018, 9564 in 2019, and 9768 in 2020) (Fig. 1a). No differences were found in the mean (SD) monthly number of visits performed in 2020 (814 [137.6]) compared to 2018 (741 [99.7]; $p = 0.153$), and 2019 (797 [116.3]; $p = 0.747$). In 2020, out of 9768 of total visits, 6218 (63.7%) visits were face-to-face and 3550 (36.3%) were performed either by phone or videoconference; routine clinical care was only performed face to face in the control periods. When evaluating clinical activity dynamics, a slight decrease in the monthly number of visits occurred during the first two months of the "first wave period", although *outlier* values were only observed in months including "holiday periods" (Fig. 1b).

Radiological activity

A total of 6765 MR scans were performed during the study period (4991 brain and 1774 spinal cord). MR scans were distributed as follows: 2207 during 2018, 2356 during 2019, and 2202 during 2020 (Fig. 2a). There were no differences in the mean (SD) monthly number of MR scans performed in 2020 (183.5 [SD 68.9]) compared to 2018 (183.9 [29.1]; $p = 0.984$) and 2019 (196 [17.5]; $p = 0.538$). When evaluating radiological activity dynamics, the monthly number of MR scans performed during the "first wave" decreased to reach an *outlier* value in April 2020 ($n = 43$), with a 76.6% drop compared to the mean monthly activity in 2020. A sharp increase was observed during the subsequent two months to reach an *outlier* high value in July 2020 ($n = 289$), a 58% increase (Fig. 2b).

Treatment prescription activity

The total number of treatment prescriptions was 925 throughout the whole study period (276 in 2018, 360 in 2019, and 289 in 2020) (Fig. 3a). The mean (SD) monthly number of treatment prescriptions in 2020 (24.1 [7.0]) was lower than in 2019 (30 [7.0]; $p = 0.049$), but similar to 2018 (23 [8.0]; $p = 0.727$). A trend towards a lower mean (SD) number of treatment prescriptions was observed during the extension period (first 5 months of 2021) (23.2 [5.5]) when compared to 2019 ($p = 0.072$),

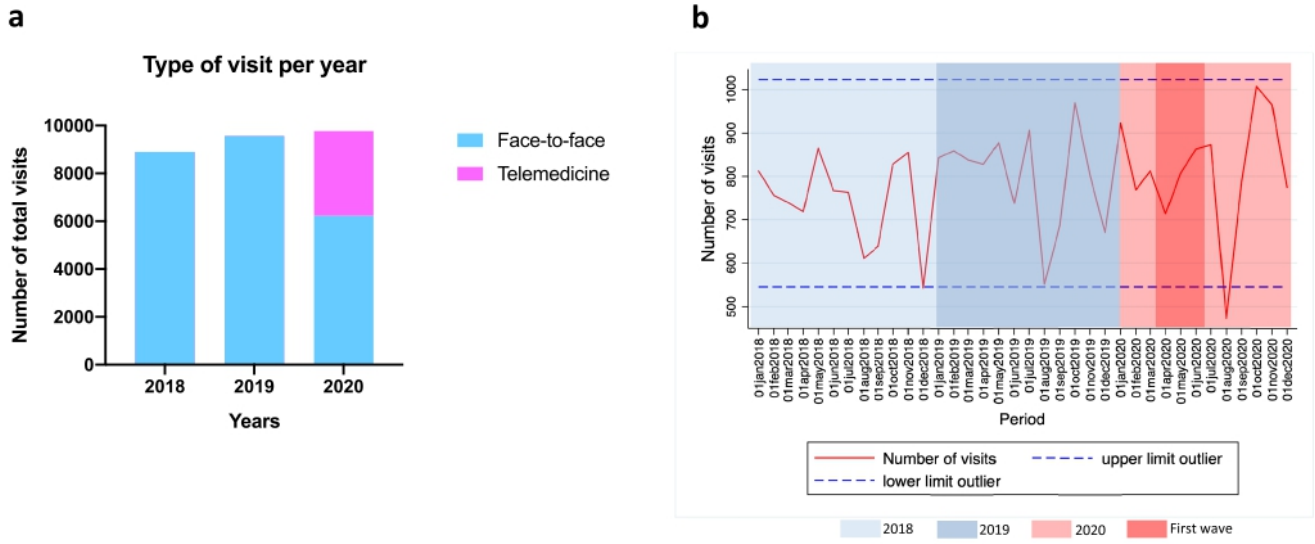

Fig. 1 Distribution of visits, according to 2020 year and control periods. **a** Total number of clinical visits in 2020, and control periods 2018 and 2019. **b** Longitudinal dynamics of the monthly number of visits during the study period. *Dash lines represent ± 2 standard deviations the mean number of clinical visits during the whole study period

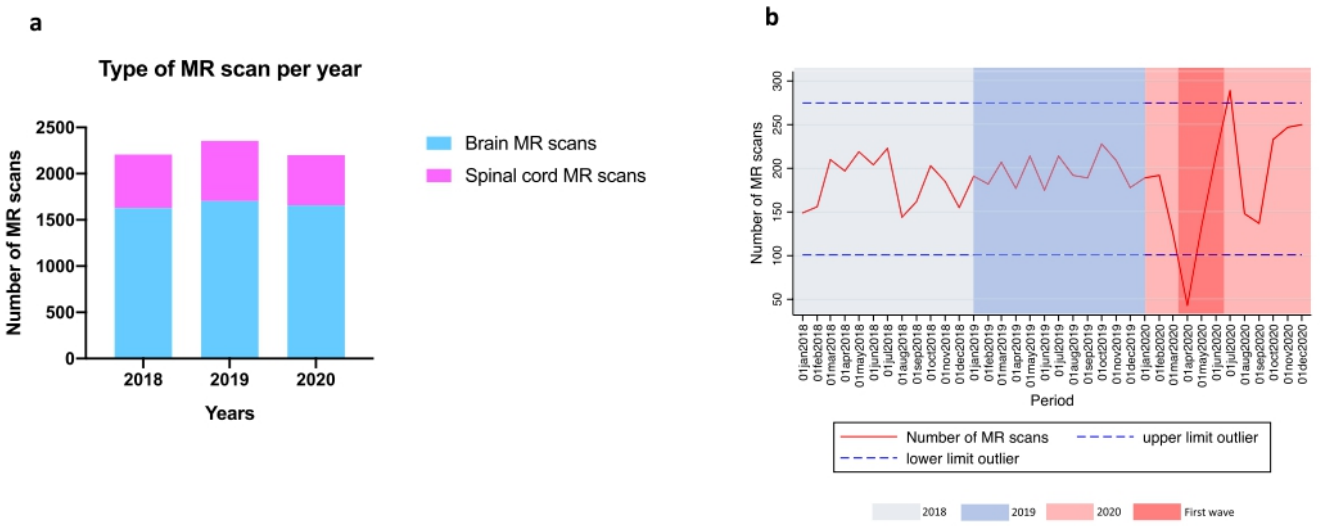

Fig. 2 Distribution of magnetic resonance scans, according to 2020 year and control periods. **a** Total number of magnetic resonance scans in 2020, and control periods 2018 and 2019. **b** Longitudinal dynamics of the monthly number of magnetic resonances during the study period. *Dash lines represent ±2 standard deviations the mean number of magnetic resonance scans during the whole study period

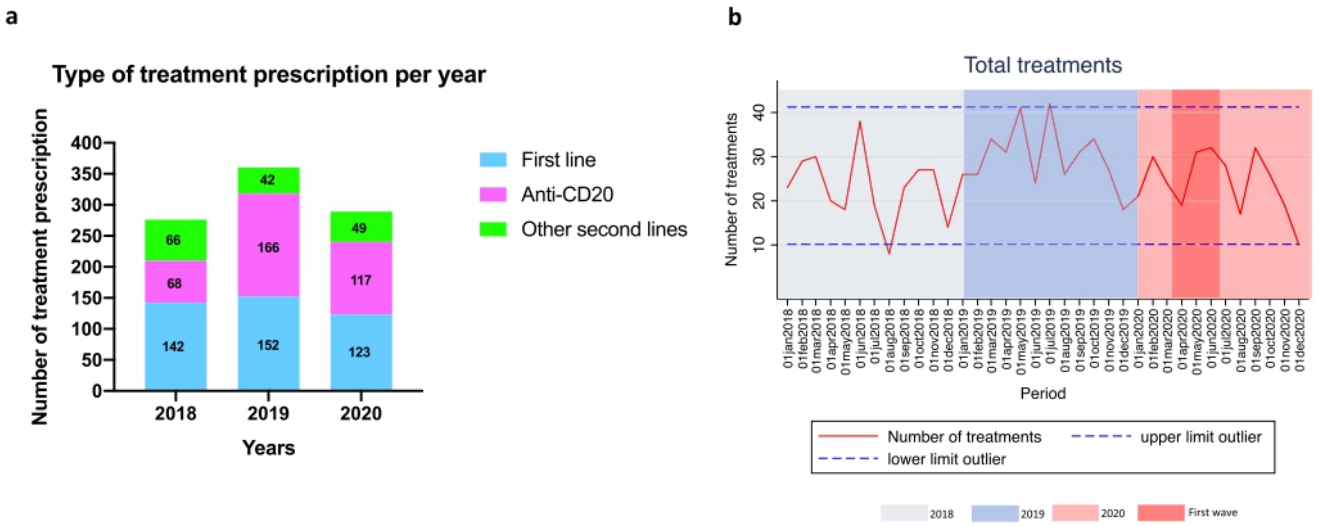

Fig. 3 Distribution of new treatment prescriptions, according to 2020 and control periods. **a** Total number of new treatment prescriptions in 2020, and control periods 2018 and 2019. **b** Longitudinal dynamics of the monthly number of new treatment prescriptions during the study period. *Dash lines represent ±2 standard deviations the mean number new treatment prescriptions during the whole study period

but no differences with 2020 were observed ($p = 0.805$). Number treatment prescriptions are depicted in Table 1. When assessing treatment prescription dynamics, the monthly number of treatment prescriptions remained stable, with the exception of months including "holiday periods" (Fig. 3b). The monthly number of first-line treatment prescriptions remained stable throughout the study period (Fig. 4a). Anti-CD20 therapy prescriptions showed a continuous increase during 2019 to reach *outlier* value in October 2019 ($n = 25$; 146.4% increase compared to the mean of monthly anti-CD20 treatment prescriptions of the whole study period); thereafter a subsequent reduction was observed in 2020 and the first five months of 2021 (Fig. 4b). A decrease in the number of anti-CD20 treatment prescriptions was observed during the "first wave" ($n = 3$ prescriptions in April; 69.4% decrease compared to the mean of monthly treatment prescriptions of the whole study period), without reaching *outlier* values. The other second-line treatment prescriptions reached *outlier* values in June ($n = 10$) and October 2018 ($n = 9$), and remained stable during 2019 and 2020 (Fig. 4c). Finally, natalizumab experienced an incremental number of new

Table 1 Number of monthly prescriptions of different therapy groups throughout the study period

Therapy	2018	2019	2020	2021[f]	Whole study period[g]
First line[a]	11.8 (5.3)	12.7 (4.67)	10.3 (3.91)	11.2 (4.02)	11.6 (4.64)
Anti-CD20[b]	5.7 (2.39)	13.8 (6.70)	9.8 (4.47)	6.4 (3.71)	9.8 (5.80)
Other second line[c]	5.5 (2.64)	3.5 (1.51)	4.1 (1.92)	5.6 (2.07)	4.4 (2.19)
Natalizumab[d]	1.1 (0.99)	1.0 (1.13)	1.8 (1.27)	2.6 (1.82)	1.3 (1.17)
All therapies[e]	23.0 (7.97)	30 (6.98)	24.1 (6.99)	23.2 (45)	25.7 (7.77)

All reported values are: mean (standard deviation)

[a]First-line therapies include interferon, acetate glatiramer, teriflunomide, and dimethyl fumarate

[b]Anti-CD20 therapies include rituximab and ocrelizumab

[c]Other second-line therapies include natalizumab, fingolimod, cladribine, and alemtuzumab

[d]Only natalizumab prescriptions

[e]All therapies include first-line, anti-CD20 and other second-line therapies

[f]Includes data from January 1 to May 31, 2021

[g]Includes data from 2018, 2019, and 2020

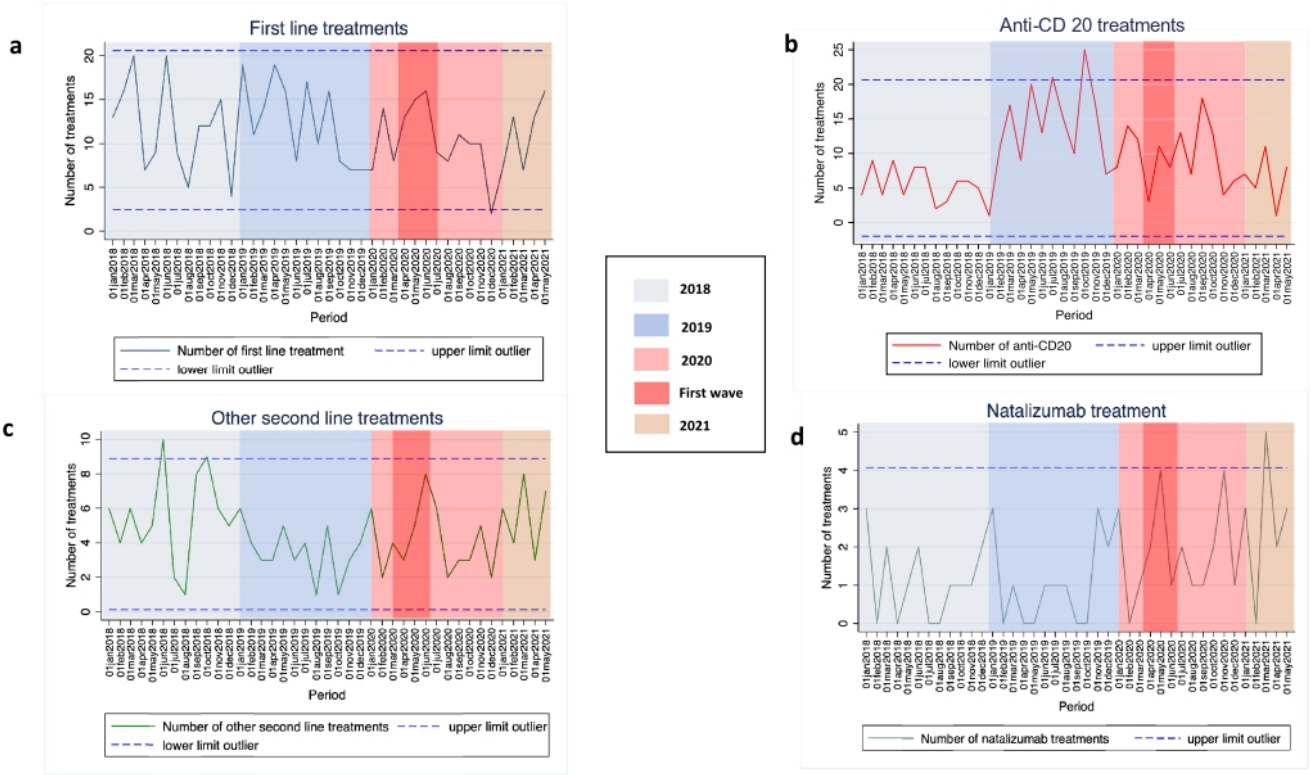

Fig. 4 Longitudinal dynamics of new treatment prescription, according to type of treatment prescription. **a** First-line treatments. **b** Anti-CD20 drugs. **c** Other second-line treatment. **d** Natalizumab treatment. *In figure c, other second-line treatment comprises natalizumab, fingolimod, cladribine and alemtuzumab. **Dash lines represent ± 2 standard deviations the mean number of new treatment prescriptions during the whole study period

prescriptions from the beginning of the COVID-19 pandemic to the end of the study period (Table 1); the monthly number of natalizumab prescriptions increased to reach *outlier* value during the "first wave" ($n = 4$), in November 2020 ($n = 4$), and in March 2021 ($n = 5$) (Fig. 4d). All other

second-line treatment prescriptions displayed stable patterns (data not shown).

Discussion

COVID-19 pandemic has hardly stricken formal operational plans in many health centres, leading to a negative impact in patient care. With the present study, we evaluate standards of care in a single MS centre for the first time, during the first year of COVID-19 pandemic. This may serve as a useful learning process for future pandemics and other potential local or global severe disruptions of usual operations. Overall, clinical and radiological activity was maintained as a result of successful adaptations to face the pandemic [18]. However, treatment strategies were subject to some variations which likely reflect a "change of concept" when treating MS patients during the COVID-19 pandemic.

The total number of clinical visits performed during 2020 remained stable in comparison with control periods, as the slight decrease observed during the "first wave" was rapidly corrected during the following months, thanks, at least in part, to rapid adoption of teleconsultation. Telemedicine had already been endorsed in terms of feasibility, cost-effectivity and patient satisfaction before the COVID-19 pandemic [21–24]. Interestingly, recent European and USA surveys have revealed that 73% of MS centres adopted telemedicine and one-third used telemedicine to provide over 75% of the clinical care during the first months of the pandemic, respectively [25, 26]. These figures clearly highlight the impressive capability of many MS centres to develop rapid structural adaptations. At our centre, up to a third of the whole outpatient clinical visits was performed via telemedicine by the end of 2020. This lower figure of telemedicine adoption in our centre may be explained by that the fact that, even though 30 out of 79 (38%) of Cemcat employees were either COVID-19-infected or quarantined in 2020, only 3 (3.8%) of them were physicians; in this way, resorting to telemedicine was only driven by patient needs and not due to low availability of physicians on site. In addition, Cemcat facilities are located in a stand-alone building, with its own route of access, and away from other clinical facilities in the Hospital campus; such location may have decreased the perception of contagion risk, thus favoring face-to-face visits. Altogether, both the prompt adaptation from face-to-face towards telemedicine visits as well as the centre architectural particularities were essential points to keep clinical requirements during the COVID-19 pandemic.

Radiology departments have been greatly impacted by COVID-19. Preliminary data from Yale New Haven Hospital, USA, revealed volume imaging drops greater than 50% [27]. Another more recent international survey has shown that urgent MR scans were the only test allowed in 58% of centres, 17% of centres suspended or postponed radiological activity, and only 19% maintained usual activity [25]. At our centre, radiological activity suffered a 73% abrupt reduction during the first two months of "the first wave" compared with overall activity in 2020, but showed a sharp recovery immediately after. The radiology department went through a deep reorganization to guarantee a low transmission risk which directly determined radiological dynamics during the first months of pandemic: redeployment of technicians and radiologist to cover COVID-19 activity, more extended intervals between and longer duration of MR scans due to hygienic measures and, finally, an increase of examinations associated with COVID-19-related neurological disorders [28]. The overall stability of radiological activity observed across periods suggests that the initial activity decreased activity was not a consequence of variations in MR scan requests, but rather from rescheduling of non-urgent studies. Moreover, MR scan requests from primary care medicine were referred to other external centres, thus freeing slots to focus on radiological requests from our own centre, which greatly helped keeping the same levels of activity. Overall, the maintenance of the radiological activity after the first months of the pandemic indicates the adoption of first measures to be a key learning point to face the current or future pandemics.

MS therapy approach during the COVID-19 pandemic has been and still remains a challenge for MS neurologists trying to balance benefits and risks. Prescription of lympho-depleting agents in MS has been a matter of discussion due to the association with COVID-19 susceptibility and outcome risks [7, 29, 30]. Indeed, a survey has recently reported that 23% of centres avoided such therapies, whereas 8% postponed any type of DMD in treatment naïve-patients during the first months of the pandemic [25]. Description of treatment strategies performed during the first year of the pandemic might be of interest to conduct an interim assessment of previous recommendations at a time when the effects of DMD on COVID-19 susceptibility and outcomes were still unknown [18]. At our centre, treatment prescriptions during 2020 were lower than 2019, but similar to 2018. Whether COVID-19 pandemic was the only reason for such decrease might be difficult to ascertain since treatment prescription patterns depend on several un-controlled temporally related variables: number of patients derived to the MS centre, changes in treatment guidelines, number of on-going clinical trials or a lower rate of treatment-switches related to a wider use of highly effective drugs, among others. Since both the total number of yearly visits and radiological tests were unaffected in 2020 but treatment prescriptions modified, it needs to be considered that the rapid and wide adoption of telemedicine might have modified treatment prescription by physicians, by a number of different reasons (i.e., missing relapses and progression events or delaying final

decision till next face-to-face visit). A different relationship between neurologist and patient, a lack of direct physical examination or even the absence of important non-verbal information are some of the consequences of a sudden shift from in-person visits to telemedicine [31]. In fact, telemedicine may be better suited to other neurological diseases, such as epilepsy or headache, where the neurological examination is not a key point for decision-making [31]. This should not be interpreted as a claim against telemedicine in MS, but rather suggests that telemedicine could be more beneficial in patients with stable disease forms and lower chances of treatment switch.

A non-pandemic-related decrease in prescription of anti-CD20 drugs during 2020 compared to 2019 may have also had an impact on the overall number of treatment prescriptions. Anti-CD20 drugs were approved to treat progressive MS forms by the end of 2018, producing a high number of prescriptions during 2019 compared to 2018. It is likely that important proportions of suitable patients for anti-CD20 drugs were treated during the first year after its approval, leaving therefore a small proportion of candidates to be treated during 2020. Obviously, a second potential cause for such decrease is the neurologist reluctance to prescribe anti-CD20 drugs due to the undesirable effects in the pandemic context [18, 32]. Whether the COVID-19 vaccine will modify anti-CD20 prescription patterns is still unknown, although previous experience suggests a decremental humoral response to vaccines in anti-CD20 MS treated patients when compared to other DMD [33]. To this regard, most recent information points to an attenuated humoral response to COVID-19 vaccine in a non-negligible proportion of anti-CD 20 MS patients [34, 35]. An interesting finding corroborating a "change of concept" on treatment strategies during the pandemic comes from the higher number natalizumab prescriptions once the pandemic hit MS patients, in comparison to previous years. These data present natalizumab to be a comfortable and safety option for patients with a highly active disease that may had otherwise been proposed to initiate anti-CD20 or other lymphocyte-depleting drugs.

Because a pandemic cannot be foreseen, the nature of the present study entails those limitations associated with a retrospective design. In addition, some comments deserve to be added. First, the activity registered in a given health centre usually does not follow a linear pattern, but depends on un-controlled elements (i.e., disease incidence, hospital eligibility, course of the disease, etc.), which makes difficult to measure the direct impact of a pandemic on the clinical activity routine. As a proxy to identify pandemic-derived associations, *outliers* were defined as well as the inclusion of control periods before and after 2020, providing reasoned statements to consider these *outliers* as a direct consequence of the pandemic. Second, whether treatment prescriptions changes are a direct consequence of the COVID-19 pandemic or due to temporal fluctuations associated to intrinsic prescriptive patterns might be difficult to ascertain. Third, patients included in on-going clinical trials were not analyzed and, therefore, depicted figures do not show the whole spectrum of DMD used at our centre. Finally, the low sample sizes in some specific treatments prevented us to perform statistical comparisons. However, longitudinal descriptive analysis allowed us to show clear trends for anti-CD20 drugs or natalizumab.

Conclusion

Overall, the present study shows a proper adaptation of the clinical activity, even at a very early starting point of the pandemic by switching the type of visit from face-to-face towards telemedicine. Radiological activity was difficult to maintain during the first wave of pandemic, but showed an early recovery and further consolidation. Finally, overall treatment prescription suffered a slight quantitative decrease during 2020 together with substantial qualitative changes in prescription of specific treatments, although temporal fluctuations in prescription patterns may have affected these findings.

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Declarations

Conflicts of interest AC–C has received grant from Instituto de Salud Carlos III, Spain; JR19/00007; AZ has received travel expenses for scientific meetings from Biogen-Idec and Novartis, speaking honoraria from Eisai and a study grant from Novartis; JR has received speaking honoraria and personal compensation for participating on Advisory Boards from Almirall, Bayer Schering Healthcare, Biogen-Idec, Genzyme; GA has received compensation for consulting services or participation in advisory boards from Sanofi, Merck and Roche; research support from Novartis; travel expenses for scientific meetings from Novartis, Roche, Stendhal and ECTRIMS; speaking honoraria from Sanofi and Merck; and is a member of the International Women in Multiple Sclerosis (iWiMS) network executive committee; SO has received compensation for consulting services from Biogen-Idec and Genzyme, and research support from Novartis; PT was an ECTRIMS clinical fellowship awardee in 2019–2020; has received travel expenses for scientific meetings from Roche; SC-R was an ECTRIMS clinical fellowship awardee 2019–2020; has received travel expenses for scientific meetings from Biogen-Idec and Genzyme; compensation for consulting services or participation in advisory boards from Roche and Novartis; and speaking honoraria from Novartis. MC reports no disclosures. CE reports no disclosures; MR reports no disclosures; PC has received travel expenses from Biogen. PC's yearly salary is supported by a grant from Biogen to Fundació privada Cemcat towards statistical analysis; BR has received honoraria for consulting services from Wellspect; LM has received honoraria for consulting services and speaking honoraria from Roche and Novartis; AVJ has received compensation for consulting services and speaking honoraria from Novartis, Roche, Teva, Mylan, Biogen and Genzyme-Sanofi. CT is

currently being funded by a Junior Leader La Caixa Fellowship. The fellowship code is LCF/BQ/PI20/11760008. She has also received the 2021 Fundación Merck Salud Award for the Investigation in Multiple Sclerosis (Spain). In 2015, she received an ECTRIMS Post-doctoral Research Fellowship and has received funding from the UK MS Society; IG reports no disclosures; JC reports no disclosures; MC has received compensation for consulting services and speaking honoraria from Bayer Schering Pharma, Merck Serono, Biogen-Idec, Teva Pharmaceuticals, Sanofi-Aventis and Novartis; CN has received funding for travel from Biogen-Idec and F. Hoffmann-La Roche, Ltd., and speaker honoraria from Novartis; CA reports no disclosures; MT is co-editor of Multiple Sclerosis Journal-Experimental, Translational and Clinical, and has received compensation for consulting services and speaking honoraria from Almirall, Bayer Schering Pharma, Biogen-Idec, Genzyme, Merck-Serono, Novartis, Roche, Sanofi-Aventis Viela-Bio and Teva Pharmaceuticals AR serves/ed on scientific advisory boards for Novartis, Sanofi-Genzyme, Synthetic MR, Bayer, Roche, Biogen, Icometrix and OLEA Medical, and has received speaker honoraria from Bayer, Sanofi-Genzyme, Bracco, Merck-Serono, Teva Pharmaceutical Industries Ltd, Novartis, Roche and Biogen. XM has received speaking honoraria and travel expenses for scientific meetings, has been a steering committee member of clinical trials or participated in advisory boards of clinical trials in the past 3 years with Actelion, Alexion, Bayer, Biogen, Celgene, EMD Serono, Genzyme, Immunic, Medday, Merck, Mylan, Nervgen, Novartis, Roche, Sanofi-Genzyme, Teva Pharmaceutical, TG Therapeutics, Excemed, MSIF and NMSS; JS-G has received compensation for consulting services and speaking honoraria from Almirall, Bayer, Biogen, Celgene, Sanofi, Merck, Novartis, Roche, Bial, Biopass and Teva, is member of the editorial committee of Multiple Sclerosis Journal, and director of Humoral and cellular responses to SARS-CoV-2 in convalescent COVID-19 patients with Multiple Sclerosis Revista de Neurología M.

Ethical approval The study was approved in the session Number 499 by the local Ethics Committee at Vall d'Hebron University Hospital (VHUH) in Barcelona, Spain.

Informed consent Written informed consent for participation was not required for this study.

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CORRECTION

Correction to: Impact of COVID-19 pandemic on frequency of clinical visits, performance of MRI studies, and therapeutic choices in a multiple sclerosis referral centre

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Correction to: Journal of Neurology
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The original version of this article unfortunately contained a mistake. The number 1 affiliation of the authors is missing some information.

It should say:

Centre d'Esclerosi Múltiple de Catalunya (Cemcat), Department of Neurology/Neuroimmunology, Edifici Cemcat, Vall d'Hebron Institut de Recerca, Hospital Universitari Vall d'Hebron, Departament de Medicina, Universitat Autònoma de Barcelona, Pg. Vall d'Hebron 119-129, 08035, Barcelona, Spain

Instead of:

Centre d'Esclerosi Múltiple de Catalunya (Cemcat), Department of Neurology/Neuroimmunology, Edifici Cemcat, Vall d'Hebron Institut de Recerca, Hospital Universitari Vall d'Hebron, Universitat Autònoma de Barcelona, Pg. Vall d'Hebron 119-129, 08035, Barcelona, Spain

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6. OVERALL SUMMARY OF RESULTS

The incidence analysis was performed with the results of the on-line survey. Out of the 2903 surveys sent, a total of 875 were answered with a response rate of 30.1%. Of these, 117 (13.4%) patients were excluded for not meeting the general inclusion criteria. Of the remaining cases, 48 met the definition criteria of COVID-19 and the remaining 710 were classified as non-COVID-19. The incidence of COVID-19 in our cohort was estimated at 6.3% (95% CI 4.6%–8.1%). Additionally, 45 suspected COVID-19 cases were detected through the spontaneous method. Overall, 93 suspected COVID-19 cases were identified.

The susceptibility analysis was performed only in those patients who had answered the survey (N=875). Demographic, MS characteristics and proportion of specific DMTs were similar in COVID-19 and non-COVID-19 cases. Multivariable models determined that age (odds ratio [OR] per 10 years 0.5, 95% confidence interval [CI] 0.3–0.9), contact with a confirmed case (OR 197.0, 95% CI 56.6–688.8), residence in Barcelona (OR 2.2, 95% CI 1.0–4.8), MS duration (OR per 5 years 1.4, 95% CI 1.1–1.8) and time on anti-CD20 treatment (OR per 2 years 3.5, 95% CI 1.4–8.5) were independent factors for presenting COVID-19. A sensitivity analysis of susceptibility was performed in patients with anti-CD20s therapies (n=144). In this cohort, 10 (6.9%) had suspected COVID-19 infection. Amongst anti-CD20 treated patients, demographic and MS characteristics of patients with COVID-19 were like those without COVID-19 except for the median anti-CD20 treatment duration which was slightly longer in COVID-19 cases (2.8, IQR 3.1, vs. 1.2, IQR 1.3 years, p = 0.069). Regarding laboratory findings, three COVID-19 cases had IgG hypogammaglobulinaemia whereas no non-COVID-19. The multivariable analysis showed that younger patients (OR per 10 years 0.03, 95% CI 0.1–0.9, p = 0.023) and a longer anti-CD20 treatment duration increased the risk of COVID-19 (OR per 2 years 3.4, 95% CI 1.4–8.5, p < 0.01) (**Article 1: Table 1**).

Of the 93 cases of suspected or confirmed COVID-19 in our cohort, nineteen (20.4%) were hospitalized, nine (9.7%) had a severe or critical disease course and two (2.2%) patients died. Although in the univariate analysis older patients with longer disease duration, a higher disability, a progressive form and previous comorbidities presented a higher risk of a severe COVID-19 disease, in the multivariable analysis only age remained as an independent risk factor (OR per 10 years 2.7, 95% CI 1.1–6.5) for a severe COVID-19.

Humoral and cellular responses against SARS-CoV-2 infection and vaccine and its persistence in patients with MS was evaluated in relation to DMT.

Humoral response after COVID-19 was evaluated in two studies. In the first study, serological tests were performed in 79 (84.9%) out of the 93 patients with suspected or confirmed COVID-19 around 3 months after COVID-19 symptom onset (median 3.12 months, IQR 2.8–3.2). Patients on anti-CD20 therapies presented a lower proportion of positive serological tests (3/19; 15.8%) than those with other DMTs (20/41; 48.8%; p = 0.045) or without DMTs (13/19; 68.4%; p = 0.003). Although a serological response was found in patients with all types of DMTs, including anti-CD20s, the proportion of positive serological tests varied depending on the DMT. In the second study, humoral response was evaluated in 145 patients with MS. 121 (83.5%) of these 145 were seropositive and this response was found from 0 to 13.1 months after COVID-19. The multivariable analysis revealed that males were more likely to become seropositive (OR=3.6, 95%CI 1.0-12.7, p<0.05), whereas MS patients under anti-CD20 therapy had a higher risk of remaining seronegative than untreated patients (OR=0.08, 95%CI 0.01-0.6, p=0.01) (**Additional material of manuscript 2: Table 1**). A sensitivity analysis was performed in anti-CD20 treated patients, in these patients only severe COVID-19 infection (OR 14.1, 95%CI 1.0-192.7, p=0.048) and a longer time between the last treatment infusion and COVID-19 disease (OR per month 1.5, 95%CI 1.0–2.2, p=0.042) were significantly associated with a higher probability of developing a humoral response after COVID-19. Antibody titers were measured in 124 patients. Anti-CD20-treated patients presented lower IgG-S and Ig-N median titers than those on other DMTs or untreated patients. Patients on fingolimod presented lower median titers of IgG-S and Ig-N than those on other DMTs, though no significant differences were found due to the small number of cases.

Cellular response after COVID-19 was analyzed in 42 convalescent COVID-19 patients selected according to DMT. Twenty-five (59.5%) presented a cellular response, which was detected 0.6 to 13.0 months after COVID-19. Cellular response was detected in patients with all types of DMT, except for glatiramer acetate (n=0/2). No differences were found in demographic and MS variables between positive and negative responders (**Additional material of manuscript 2: Table 3**). Nonetheless, all patients with severe COVID-19 presented a cellular response (p=0.018). 5 out of 7 (71.4%) anti-CD20-treated seronegative patients had a cellular response. In multivariable analysis, cellular response was decreased in progressive MS forms (OR=0.04, 95%CI 0.001-0.9, p<0.05).

Humoral response persistence after COVID-19 was analyzed in 53 patients with two serological determinations with a median follow-up of 14.2 months (**Additional material of manuscript 2: Table 4**). Forty-one (81.13%) of those presented humoral persistence. In univariable analysis, patients with humoral persistence over 6 months presented a higher

median lymphocyte count before COVID-19 than those without persistence (1715 [IQR 685] vs. 1200 [IQR 100], p<0.05). However, the multivariable analysis did not confirm this result.

457 participants from the two MS centers (Cemcat and UNIEMTG): were included in the study to analyze the immunological responses to SARS-CoV-2 vaccine. 421 patients of those presented MS and 36 other autoimmune diseases treated with anti-CD20s. The distribution of DMTs of the patients included was: 139 anti-CD20s, 38 DMF, 36 SP1RM, 32 NTZ, 31 TF and IFN, 30 CLA and ALZ, 28 without treatment and 26 GA. The 36 with other autoimmune diseases presented: 14 Neuromyelitis Optica Spectrum Disorder (NMOSD), 5 Myelin oligodendrocyte glycoprotein antibody disorders (MOGAD), 3 IgG4-RD, 3 ANCA-associated vasculitis, 2 idiopathic membranous nephropathies, 2 minimal-change disease nephropathy, 1 central nervous system vasculitis, 1 systemic lupus vasculitis, 1 myelorradiculitis, 1 Tolosa-Hunt syndrome, 1 GFAP-encephalitis and 1 Susac syndrome. Of the 457 included, 431 (94.3%) were fully vaccinated with an mRNA vaccine and the rest with adenoviral vector vaccines. 17 patients were excluded from the analysis: 12 because they presented a positive SARS-CoV-2 antibodies in the pre-vaccination sample and 5 because they presented COVID-19 post-vaccination.

Post-vaccination samples were collected in 430 of the 440 (97.7%) patients within 2.0 (standard deviation 0.8) months after the last vaccine dose. Humoral responses were detected in patients with all types of DMTs. The overall seroconversion rate was of 74.4%. However, when considering response in relation to DMTs it was of more than 92.0% in all DMTs and untreated, except for patients on anti-CD20s and S1PRMs (45.6% and 51.4%, respectively). These last groups presented lower IgG titers compared to those untreated or on other DMTs. In the multivariable analysis, negative antibodies were associated with anti-CD20s (OR=$1.7x10^4$, 95%CI 612.7-$4.5x10^5$, p<0.001), S1PRMs (OR=$2.0x10^3$, 95%CI 123.0-$3.4x10^4$, p<0.001) and longer treatment duration with any DMT (OR per year=1.5, 95%CI 1.3-1.8, p<0.001). In the sensitivity analysis performed only in anti-CD20 treated patients, only a longer treatment duration (OR per year= 1.8, 95%CI 1.3-2.5, p<0.001) was associated with negative antibodies. Additionally, IgG titers positively correlated with days elapsed between the last infusion and the first vaccine dose (r=0.2, 95%CI 0.1-0.4, p<0.001) and previous IgG immunoglobulin levels (r=0.3, 95%CI 0.1-0.5, p<0.001) and inversely correlated with treatment duration (r=-0.4, 95%CI -0.6 - -0.3, p<0.0001). Seroconversion was >80.0% from 4.5 months after the last infusion.

Cellular responses were analyzed in 141 patients and detected in 84.4% of these patients. All DMTs presented a cellular response rate of >75.0%, except for patients on S1PRMs (11%). Furthermore, 91.4% of anti-CD20-treated patients with negative antibodies had a cellular response. Patients on S1PRMs presented lower IFN-ɣ levels compared to those on other DMTs or untreated (p<0.010 for all comparisons). In the multivariable analysis, cellular response decreased in S1PRM-treated (OR=199.9, 95%CI 15.7-2.3x10^3, p<0.001) and >50-year-old patients (OR=4.2, 95%CI 1.2014.8, p=0.025).

We evaluated the impact of the COVID-19 pandemic on the number of clinical visits, magnetic resonance scans and treatment prescriptions at Cemcat. From January 2018 to May 2021, a total of 28,230 visits were carried out at the outpatient care centre (8898 in 2018, 9564 in 2019, and 9768 in 2020). The monthly mean (standard deviation [SD]) of visits performed in 2020 (814 [137.6]) was like 2018 (741 [99.7] ; p=0.153), and 2019 (797 [116.3]; p=0.747). During the COVID-19 period (2020 year), 36.3% of the activity was performed through telemedicine.

A total of 6765 MRI were performed during the study period (2207 during 2018, 2356 during 2019, and 2202 during 2020). The number of MRI performed dropped by 76.6% during the first wave compared to the mean monthly activity in 2020 (183.5 [68.9]), with a recovery during the subsequent two months.

In relation to treatment prescription, the total number of treatment prescriptions was 925 throughout the whole study period (276 in 2018, 360 in 2019, and 289 in 2020). The monthly mean of treatment prescriptions approved in 2020 (24.1 [7.0]) was lower than in 2019 (30 [7.0]; p=0.049), but similar to 2018 (23.8 [8.0]; p=0.727). Natalizumab prescriptions increased in the “first wave” and onwards, whereas antiCD20 prescriptions decreased during the COVID-19 period.

7. OVERALL SUMMARY OF THE DISCUSSION

In this work we evaluated COVID-19 incidence and susceptibility and severity risk factors in our cohort of pwMS and analyzed the immunological responses to SARS-CoV-2 infection and vaccine. Finally, we assessed the impact of these findings and COVID-19 pandemic in the activity of our MS center.

The **incidence** of COVID-19 amongst our cohort was estimated at 6.3% (95% CI 4.6%–8.1%), which is similar to the incidence of Catalonia when the study was performed (6.1%). Comparable studies performed in the same pandemic moment (first wave) in Barcelona and Madrid showed inconclusive results as one of them showed an increased adjusted risk of COVID-19 in MS patients compared to the general population (83) and the other showed a decreased risk (84). Reviews performed further in the pandemic have not shown an increased risk of COVID-19 in MS patients (85,86).

In our cohort, **COVID-19 susceptibility risk factors** were having been in contact with a PCR-positive person, living in Barcelona, being younger and having a longer MS disease duration, increased the risk of presenting COVID-19. As previously described in the general population, having had contact with a PCR-positive person (74,87) and living in a very populated place such as Barcelona was strongly associated with COVID-19. Although in other regions and countries the risk of SARS-CoV-2 infection and of COVID-19 severe course and hospital admission was higher in low income areas (88) no differences regarding socioeconomic status were found. However, it is possible that the annual mean wage lacks enough precision and accuracy as a socioeconomic proxy.

When analyzing the reasons why younger patients have a higher susceptibility risk it could be argued that the younger age reflects the online selection method, with young people being more prone to participate. Also, it could be because this age group has a higher level of social interaction or had to continue working during the pandemic lockdown and therefore the risk of exposure increased. In fact, younger age has also been found a susceptibility risk factor in other MS cohorts (83,89). However, these results could also be due to the reduced number of participants. In this sense, we participated in a study to assess COVID-19 susceptibility factors with two American MS centers with a larger number of responders (3028), where age did not remain an independent factor for COVID-19 susceptibility (87).

We also found that a longer MS duration increased the risk of COVID-19, which has been only described by a previous group and has not been corroborated by posterior studies (74).

In contrast with previous studies, no comorbidity was independently associated with the susceptibility to COVID-19 in contrast with previous experiences (83,90) possibly due to the low frequency of individual comorbidities in our sample.

In relation to previous laboratory data, despite reports suggesting that low vitamin D levels may play a role in COVID-19 susceptibility in the general population (91) no differences between COVID-19 and non-COVID-19 patients were found. Likewise, neither previous lymphocyte counts, nor the degree of lymphopenia increased COVID-19 risk.

In published literature, the relation between a specific DMT and an increased susceptibility risk is not well stablished. Initially it was suggested that as most DMTs do not particularly target the innate immune system, which is responsible for the initial response against infections, the majority of them may not increase the susceptibility to SARS-CoV-2 infection (92), except perhaps for non-selective cell-depleting therapies such as alemtuzumab or cladribine. In our cohort, no clear association of COVID-19 susceptibility with immunotherapy or with the use of low or high efficacy therapies was found which is consistent with some of the reported experience in MS and other autoimmune diseases (83,93,94). However, some other studies have found an increased risk in patients on anti-CD20s (74) or natalizumab (89). As both these treatments need to be administrated in the day-care hospital, it could be argued that the major exposure to a hospital environment increases the susceptibility risk of COVID-19.

In the sensitivity analysis performed in anti-CD20-treated patients, COVID-19 susceptibility was higher in patients treated for a longer period of time, independently of age, previous comorbidities and MS characteristics. Some of these patients presented low IgG levels which increase with repeated infusions. As hypogammaglobulinemia is a known factor for infection susceptibility (70,95,96), it is possible that a longer treatment duration increased COVID-19 risk.

The proportion of severe and hospitalized COVID-19 cases in our cohort was similar to that of other COVID-19 and MS cohorts (20.4% vs 19.3-24.7%) (72,97,98). Mortality rate in our cohort was 2.2% which is comparable to other MS registries and to the general population at that moment of the pandemic (2.2% vs 1.5-8%) (72,97,98). Although reports on COVID-19 mortality of MS patients are heterogenous and inconclusive, a recent study of the Italian registry demonstrated and increased death risk of MS patients compared to the general population in those patients with previous comorbidities and a higher disability (99).

The only risk factor associated to **COVID-19 severity** in our cohort is advanced age as seen in the general population and MS patients (72,73,100–103). Similar to other MS cohorts (72,73,83,101–103), pre-existing comorbidities, patients with progressive forms and a longer disease duration increased the risk of a critical disease but, in our cohort, none of them showed an independent association in the multivariable analysis. However, two larger

national and international multicenter studies were we participated confirmed these data (101,104).

In relation to DMTs, we found that those with a severe COVID-19 were either on anti-CD20 (33.3%) or untreated (66.6%). Also, that a lower proportion of patients with severe COVID-19 were on DMTs compared to mild cases (33.3% vs 81.1%). This is in line with further studies performed in MS cohorts with a larger number of patients, where untreated patients and anti-CD20-treated patients seem to have an increased risk of severe COVID-19 and interferon may play a protective role (73,99,101,102,105).

Even though lymphopenia during the infection is associated with a severe disease course (106) it was not possible to replicate those findings in our sample. Similarly, we did not find any relationship between laboratory variables and a severe COVID-19.

Immunological responses to SARS-CoV-2 infection and vaccine and its persistence in patients with MS were assessed by analyzing humoral and cellular responses. We found that patients with MS present a humoral response against **SARS-CoV-2 infection** up to 1 year after COVID-19 but that this response and its persistence is influenced by different factors. In the first article which included both confirmed and suspected COVID-19 convalescent patients with MS, we found a remarkable decrease of the rate of seropositive patients in those treated with anti-CD20s (17.6%) compared to patients treated under other DMTs (48.8%) or without treatment (68.4%). This information was confirmed in the second manuscript that only included confirmed cases. In the second study, 83.4% of patients presented antibodies against SARS-CoV-2, with a positivity rate of over 70.0% in patients with most DMTs except anti-CD20s. In line with previous reports in patients with MS, anti-CD20 therapy also decreased the median titers of antibodies against SARS-CoV-2 (107–109). As anti-CD20 therapy affects the B-cell lineage, impairing differentiation into memory B cells or plasma cells, it is not surprising then that patients with MS under such treatments fail to develop a humoral response. Our results also suggest that male sex increases the probability of seroconversion, in line with previous data published for the general population (110).

We detected cellular responses in 59.5% of patients with convalescent COVID-19. As previously described in the general population (35), the cellular response was associated with severe COVID-19 in univariable analysis. In fact, all patients with severe COVID-19 had detectable cellular responses. This suggests an increased immune response with higher viral loads and inflammatory mediators during acute infection (111). Nevertheless, we

detected a specific cellular response despite the absence of a humoral response in 5 patients given anti-CD20 therapy but not in patients on other DMTs. Some studies of SARS-CoV-2 infection and vaccination have already described specific cellular responses in the absence of humoral responses (112–114). Therefore, the cellular response might play an important role in COVID-19 recovery when humoral immunity is impaired.

Additionally, progressive phenotypes were less likely to present humoral and cellular responses in our cohort. In both cases, the decreased response may be justified by the older age of these patients or premature immunosenescence associated with progressive forms, leading to a weakened immune response (115). However, potential confounders such as anti-CD20 therapy should be ruled out in future analyzes with larger cohorts.

Humoral and cellular responses against SARS-CoV-2 after COVID-19 are detected within a few days of COVID-19 onset to up to 12 months (33,116). We were able to detect both of them up to 13.1 months after COVID-19 disease. The humoral response persisted for more than 6 months in 81.1% of patients with 2 determinations. In the general population, increased severity of COVID-19 and younger age have been associated with longer SARS-CoV-2 humoral persistence (117,118), although we did not find any association in this regard, probably because of the cohort´s small sample size.

We found that after **SARS-CoV-2 vaccination**, humoral and cellular responses are presence in patients with MS with all types of DMTs or other AIDs on anti-CD20s. However, the magnitude of these responses is modified depending on the DMT. In our cohort, the seroconversion rate after SARS-CoV-2 vaccination was up to 90% in all patients except in those receiving anti-CD20 therapies or SP1RM, which is consistent with previous literature (119–121). We confirmed this data in a large international multicenter study (122). Moreover, patients on these two DMTs presented lower IgG titers compared to those on other DMTs. The reduced humoral response with anti-CD20s therapies was expected as they impair memory B-cell production as we have already seen in the post-COVID-19 study and in other vaccines (81,109,123). In the SP1RMs case, humoral and cellular responses are probably reduced by the suppression of lymphocyte egress from lymph node, although this has been seen to reduce humoral response in vaccinated MS patients but not in COVID-19 convalescent cases (108,109,123,124). Additionally, SP1RMs have not shown to increase COVID-19 severity in comparison to anti-CD20s therapies (101). Therefore, a different immunological process may be at work in response to SARS-CoV-2 natural infection and vaccination, especially in SP1RMs treated patients (125).

The cellular response after SARS-CoV-2 vaccination appeared to be preserved under most DMTs similar to the convalescent patients (121). Conversely, in our cohort less that 12% of SP1RM-treated patients mounted a cellular response, regardless of their serostatus or lymphocyte count. Additionally, we confirmed the results seen in the post-COVID-19 study, where anti-CD20s present a high percentage of cellular response rate even in the absence of a humoral response as seen by other groups (112,113,124,127). In addition, the cellular response was weakened in patients aged >50 years which has already been described in the general popular and it is probably due to immunosenesce.(128)

In the sensitivity analysis performed in patients treated with **anti-CD20 therapies** both after SARS-CoV-2 infection and vaccine, we found that the longer the time of SARS-CoV-2 infection or vaccine after the last anti-CD20 infusion, the higher the IgG titers and the proportion of humoral response positivity. This is probably explained due to an increasing repopulation of memory B cells over the months. After vaccination, we also observed that the seroconversion rate increased up to 80.0% 4.5 months after the last anti-CD20 infusion, similar to other groups (109,112,113,124,126). Therefore, optimizing the moment of vaccine administration could potentially lead to an increased antibody response. In fact, following the results obtained in the vaccination study, we optimized the treatment and vaccination protocols so that patients received the third dose of SARS-CoV-2 vaccines 4.5 months after the last infusion in order to try to increase the immunological response to them.

Our study showed than during the COVID-19 pandemic **clinical and radiological activities** were maintained but also that some changes in the treatment prescription pattern were put in place.

The total number of clinical visits performed during 2020 remained stable in comparison with control periods, as the slight initial decrease observed during the “first wave” was rapidly corrected during the following months by implementing telemedicine. Surveys responded by MS neurologists in Europe and USA reveled that telemedicine was a well stablished strategy used by MS centers around the world to ensure health care persistence during the pandemic (129–131). However, the patient’s point of view should also be considered. In this sense, a study on how the pandemic affected patients with neurological disorders, they point out that although telemedicine was implemented they perceived an inappropriate overall care of their disease (132).

Radiological activity suffered a drastic decrease during the “first wave” of the pandemic with a 73% reduction of activity. However, after the “first wave”, the radiology department

restructured its activity to guarantee a low SARS-COV-2 transmission risk and was able to compensate for the initial reduction of MRI scans. Altogether, the adoption of new measures allowed the radiological activity of 2020 to be similar to the control periods. Similar experiences happened in other centers (129,133).

The approach to MS therapy during the COVID-19 pandemic has been and still remains a challenge for MS neurologists trying to balance benefits and risks. During the initial part of the pandemic, lympho-depleting agents such as alemtuzumab, cladribine or anti-CD20s were hypothesized to potentially increase the susceptibility and severity of COVID-19, leading to a decrease of their prescription in MS centers.(129) At our center, there was a reduction of the total number of prescriptions during 2020, although this could explained by other reasons not related to the pandemic such as number of patients derived to the MS centre, changes in treatment guidelines or number of on-going clinical trials. However, the decrease of anti-CD20 prescription as a high efficacy treatment in favor of natalizumab could be justified by the pandemic. As the pandemic went through, anti-CD20s became established factors of severe COVID-19 risk in MS patients (73,101) and were shown to decrease of humoral response to SARS-CoV-2 vaccination (112,123). Therefore, it is not surprising the neurologist's reluctance to prescribe these treatments and endorse natalizumab which has shown a low risk of severe COVID-19 and a good response to vaccines.

The **limitations** of this work include the following. The retrospective nature of all the studies except for the vaccine response study makes it prone to recall bias and missing data.

In the first article, given the high non-respondent rate in the survey and the limited access to PCR during the first-wave, it is likely that asymptomatic, mild or atypical COVID-19 cases were missed, limiting our conclusions. Nevertheless, the rate of PCR testing in our province was restricted as well, so the incidence estimates of the general population are subject to the same limitations. Moreover, this might be compensated by a potential response bias where COVID-19 cases might have been overrepresented amongst the responders of our survey. Another limitation is the small sample size of COVID-19 cases and especially of those with a severe course, probably leading to overestimation of associations and a high degree of uncertainty.

In the second article, we only included confirmed cases and during the first wave of the pandemic RT-PCR testing in our province was restricted to hospitalized patients. Therefore, it is possible that our cohort's first cases were either severe cases or patients with positive

serology performed in the convalescence phase. This might have led to an increased estimation of the positive serologic rate, as there might be an overrepresentation of patients with severe COVID-19 infection. In addition, all tests were performed according to clinical practice and not to established time points after COVID-19 diagnosis, which has led to variability in the time and frequency of testing after COVID-19, increasing the heterogeneity of the sample.

In the humoral and cellular response studies, the relatively small sample size of the studies, has probably led to an overestimation of associations and a high degree of uncertainty. In the cellular response substudy, there were few cases for each DMT, which prevented us from performing group comparisons. The collaborative studies where we participated with the COVID-19 database and with serum samples to analyze the vaccine serological response, allowed us to compensate the small individual numbers of each subgroup of patients (87,101,104,122).

Also, it should be considered that previous studies on the SARS-CoV-2 cellular response in MS after COVID-19 and after SARS-CoV-2 vaccination have used other methods, such as intracellular cytokine staining or other IGRAs, which may limit the reproducibility of our results. These results apply to patients having received two doses of vaccination. We cannot rule out that some differences could be found after further doses.

In the last article, the activity registered in a center and its prescription pattern usually does not follow a linear pattern, but depends on un-controlled elements (i.e., disease incidence, hospital eligibility, course of the disease, etc.), which makes difficult to measure the direct impact of a pandemic on the clinical activity routine and count account for some of the variability seen among the different years. As a proxy to identify pandemic-derived associations, outliers were defined as well as the inclusion of control periods before and after 2020.

All these results may apply to the first wave and to the variants that were responsible for the first and second waves. We acknowledge that different variants, further vaccine doses, increase knowledge of SARS-CoV-2 physiology or development of new therapies may elicit different results. Also, the different restrictions occurring during the different waves could further modify our results.

Altogether, we consider that the results given in this work are relevant for the MS community and can be used in a near future to optimize treatment prescription and vaccination campaigns.

8. CONCLUSIONS

1. The incidence of COVID-19 in our cohort of pwMS in Barcelona was estimated at 6.3% (95% CI 4.6%–8.1%). COVID-19 susceptibility risk factors were having been in contact with a PCR-positive person, living in Barcelona, being younger and having a longer MS disease duration increased the risk of presenting COVID-19. The only COVID-19 severity risk factor was age. No DMT were found to modify the susceptibility or severity risk.

2. 83.4% of convalescent COVID-19 MS patients presented humoral response and 59.5% cellular response from a few days up to 13.1 months after COVID-19. The humoral response decreased in patients with anti-CD20s and increased in male patients. In patients treated with anti-CD20 therapies, the longer the time between SARS-CoV-2 infection and the last anti-CD20 infusion, the higher the proportion of humoral response positivity. Cellular response decreased in MS progressive forms. No factors were associated to humoral response persistence.

3. After two doses of SARS-CoV-2 vaccination, the humoral response in pwMS and other AIDs on anti-CD20s was of 74.4% and the cellular response of 84.4%. Humoral response was decreased in patients treated with anti-CD20s therapies or S1PRMs and those with a longer treatment duration. In anti-CD20s-treated patients, the seroconversion rate increased in up to 80% after 4.5 months since last infusion. Cellular response decreased in pwMS under S1PRM treatment and in those patients over 50 years of age.

4. During the first wave of the COVID-19 pandemic, the clinical and radiological activities at the Cemcat were maintained but the number of treatment prescriptions was reduced, and the pattern modified. Specifically, there was a change in the high efficacy prescription pattern where anti-CD20s in favor of natalizumab.

9. FUTURE LINES OF RESEARCH

In the SAR-EM study, an additional time-point after the third SARS-CoV-2 vaccine dose has been collected in selected patients according to DMT (fingolimod, natalizumab and anti-CD20s). These samples will be analyzed with flow-cytometry together with the previous PBMCs samples to deeply phenotype the specific T-cell subsets, correlate them with the humoral response, evaluate its changes and its persistence over time and analyze its clinical effect in preventing or decreasing COVID-19 severity.

The COVID-19 and MS database is being updated with the intention to continue sharing its data with other national and international groups working on the same topic.

Additionally, we have the objective to expand the knowledge acquired in this study in infection risk and humoral and cellular response after SARS-CoV-2 infection and vaccine to other infections and vaccines. Considering that there is an important gap of knowledge in relation to vaccine responses according to the different DMTs, the aim is to continue evaluating the humoral and cellular response to other vaccines (hepatitis B virus, hepatitis A virus, attenuated…) to try to optimize the best vaccination strategy. Also, we would like to evaluate the different infection risk according to DMT and its risk factors.

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11. APPENDIX

11.1 On-line survey (Article 1)

1. **En las últimas 2 semanas, ha tenido alguno de los siguientes síntomas (puede marcar una o varias respuestas) y la fecha de inicio de estas:**
   - Tos continuada o persistente
     - En caso afirmativo, fecha de inicio de los síntomas: dd / mm / aaaa
   - Febrícula (temperatura 37-37,9ºC) o fiebre (temperatura ≥ 38ºC)
     - En caso afirmativo, fecha de inicio de los síntomas: dd / mm / aaaa
   - Sensación de ahogo o dificultad respiratoria
     - En caso afirmativo, fecha de inicio de los síntomas: dd / mm / aaaa
   - Disminución o pérdida total de olfato o gusto
     - En caso afirmativo, fecha de inicio de los síntomas: dd / mm / aaaa

2. **¿Se le ha diagnosticado como posible COVID-19 (Coronavirus)?** Sí/No.
   - En caso afirmativo, ¿Cuándo?" (dd / mm / aaaa)

3. **¿Se le ha confirmado el diagnóstico de COVID-19 (Coronavirus) mediante el test apropiado?** Sí/No.
   - En caso afirmativo, ¿Cuándo?" (dd / mm / aaaa)
   -

4. **¿Ha estado ingresado en un hospital debido a la enfermedad COVID-19?** Sí/No
   - En caso afirmativo, "¿Cuándo?" (dd / mm / aaaa)
   -

5. **¿Con cuántas personas convive?** (0-10)

6. **¿Alguna de las personas con las que convive ha tenido alguno de los síntomas mencionados previamente?** Sí/No
   - En caso afirmativo, ¿Cuándo?" (dd / mm / aaaa)

7. **¿Alguna de las personas con las que convive han sido diagnosticadas de COVID-19 (Coronavirus)?**
   - En caso afirmativo, ¿Cuándo?" (dd / mm / aaaa)

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