Breakthrough SARS-CoV-2 infections after COVID-19 mRNA vaccination in MS patients on disease modifying therapies during the Delta and the Omicron waves in Italy

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Summary

Background In this study we aimed to monitor the risk of breakthrough SARS-CoV-2 infection in patients with MS (pwMS) under different DMTs and to identify correlates of reduced protection.

Methods This is a prospective Italian multicenter cohort study, long-term clinical follow-up of the CovaXiMS (Covid-19 vaccine in Multiple Sclerosis) study. 1855 pwMS scheduled for SARS-CoV-2 mRNA vaccination were enrolled and followed up to a mean time of 10 months. The cumulative incidence of breakthrough Covid-19 cases in pwMS was calculated before and after December 2021, to separate the Delta from the Omicron waves and to account for the advent of the third vaccine dose.

Findings 1705 pwMS received 2 m-RNA vaccine doses, 21/28 days apart. Of them, 1508 (88.5%) had blood assessment 4 weeks after the second vaccine dose and 1154/1266 (92%) received the third dose after a mean interval of 210 days (range 90-342 days) after the second dose. During follow-up, 131 breakthrough Covid-19 infections (33 during the Delta and 98 during the Omicron wave) were observed. The probability to be infected during the Delta wave was associated with SARS-CoV-2 antibody levels measured after 4 weeks from the second vaccine dose (HR=0.57, $p < 0.001$); the protective role of antibodies was preserved over the whole follow up (HR=0.57, 95%CI=0.43-0.75, $p < 0.001$), with a significant reduction (HR=1.40, 95%CI=1.01-1.94, p=0.04) for the Omicron cases. The third dose significantly reduced the risk of infection (HR=0.44, 95%CI=0.21-0.90,p=0.025) during the Omicron wave.

Interpretation The risk of breakthrough SARS-CoV-2 infections is mainly associated with reduced levels of the virus-specific humoral immune response.

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Keywords: Breakthrough infections; Multiple Sclerosis; COVID-19; Disease Modifying Treatments

Introduction

Evidence of the effect of vaccination against SARS-CoV-2, mainly on virus-specific serological responses[1–3] and, to a minor extent, on antigen-specific T cell response[4,5] in patients with MS (pwMS) treated with disease-modifying therapies (DMTs) is rapidly accumulating. There is wide consensus that the use of anti-CD20 monoclonal antibodies and fingolimod are associated with the lowest serum SARS-CoV-2 antibody concentrations, following the SARS-CoV-2 vaccines, whereas virus-specific humoral immune responses in pwMS on all the other DMTs, or untreated, were high and did not differ significantly from healthy controls.[1–4] On the other hand, there is also growing evidence that vaccinated pwMS treated with anti-CD20 generated robust virus specific CD4 and CD8 T cell responses,[4–5] while these are slightly reduced in fingolimod treated patients.[5] Indeed, Covid-19 vaccination is less immunogenic in immunocompromising conditions[6] and there is evidence that both serum SARS-CoV-2 antibody levels and the degree of vaccine-induced protection from Covid-19 decline with time since vaccination.[7,8] Thus, it is mandatory to monitor the incidence of breakthrough infections in pwMS, to better understand the role of humoral and cellular response to SARS-CoV-2 vaccination in preventing Covid-19 and its consequences. A preliminary follow-up study of 344 fully vaccinated pwMS on DMT reported 13 breakthrough infections, 10 of which were in patients under anti-CD20 therapy and the remaining 3 on fingolimod.[9]

We planned a clinical follow-up of the pwMS enrolled in the CovaXiMS (Covid-19 vaccine in Multiple Sclerosis),[1] a prospective multicenter cohort study enrolling pwMS vaccinated against Covid-19, in whom SARS-CoV-2 antibodies were measured before the first and 4 weeks after the second vaccine dose. Aim of the study was to evaluate the incidence of breakthrough infections in relation to baseline characteristics and vaccination-elicited antibody levels, and to identify possible correlates of a reduced protection against the disease and its severe form. As often happened during the pandemic, we observed unforeseen events during the follow up of our study, namely, the recommendation of a third booster vaccine dose for pwMS and the surge of the new

[1] These authors contributed equally to this work.

Research in Context

Evidence before this study

We searched PubMed (from year 2020) for cohort observational studies assessing the effect of SARS-Cov-2 vaccine in patients with MS under DMTs. (Search terms: "Multiple Sclerosis and SARS-Cov-2 Vaccine" in the title or abstract). We retrieved 30 papers. Most of them evaluated the antibody levels developed after anti- SARS-Cov-2 vaccination in patients with MS on different DMTs. The results are very consistent, showing that humoral vaccine responses were significantly impaired by anti-CD20 monoclonal antibody therapies and by fingolimod (Achiron et al, Ther Adv Neurol Disord. 2021, Sormani et al, Ebiomedicine 2021, Tortorella et al, Neurology 2021, Tallantyre EC et al, Ann Neurol 2022). The third vaccine dose was associated with modestly increased levels of anti−SARS-CoV-2 spike RBD IgG antibodies in patients with reduced protective humoral immunity before reimmunization (Koenig et al, JAMA Neur 2022, Achtnichts L et al, Vaccines 2022). On the other hand, several studies showed that vaccinated pwMS treated with anti-CD20 generated robust virus specific CD4 and CD8 T cell responses (Apostolidis SA. Nat Med. 2021) while these are slightly reduced in fingolimod treated patients (Tortorella C, Neurology 2022). Thus, it is mandatory to monitor the incidence of breakthrough infections in pwMS, to better understand the role of humoral and cellular response to SARS-CoV-2 vaccination in preventing Covid-19 and its consequences. A preliminary follow-up study of 344 fully vaccinated pwMS on DMT reported 13 breakthrough infections, 10 of which were in patients under anti-CD20 therapy and the remaining 3 on fingolimod (Rose DR et al, Mult Scler J Exp Transl Clin. 2021).

Added value of this study

This study reports on the clinical follow up after vaccination against Sars-Cov-2 in a large sample of patients with MS treated with all the DMTs, showing that the risk of breakthrough SARS-CoV-2 infections is mainly associated with reduced levels of the virus-specific humoral immune response, that the third dose helps reducing the risk and that the probability of being infected by the Omicron variant is less affected by vaccination.

Implications of all evidence available

This finding can help to decide the vaccination strategy in patients with MS under specific DMTs.

Omicron variant. These events complicated the study of the impact of antibody levels after the second vaccination dose and are anyway relevant factors to be studied; therefore, we adapted our pre-planned analysis adding information on the Omicron infections and adjusting the analysis with time-dependent covariates.

Methods

Subjects

This was a clinical follow-up of an observational multicenter prospective study conducted in 35 Italian MS centers on pwMS undergoing the SARS-CoV-2 vaccination. Consecutive adult pwMS, with or without a previous SARS-CoV-2 infection, who were scheduled for SARS-CoV-2 vaccination, were included in the study. mRNA vaccines BNT162b2 (Pfizer Inc, and BioNTech), or mRNA-1273 (Moderna Tx, Inc) as per clinical practice and regional indications were allowed. Patients who agreed to provide a first blood test sample just before the vaccination and a second drawing one month after the last dose were enrolled in the study. Patients were then followed up, with monthly phone calls and visits as per clinical practice, and any new SARS-CoV-2 infection was recorded in a dedicated Case Report Form (CRF). Additional details are reported in a previous publication.[1]

Ethics

The study was done in compliance with the principles of the Declaration of Helsinki. The protocol was approved by the regional (CER Liguria: 5/2021 - DB id 11169- 21/01/2021) and the centralized national ethical committee AIFA/Spallanzani (Parere n 351, 2020/21). Written informed consent was obtained from all participants before starting any study procedures.

Primary outcome: breakthrough infection

The primary objective of this analysis was to quantify the incidence of breakthrough SARS-CoV-2 infection among the vaccinated pwMS included in the CovaXiMS study. These conditions entail a PCR-confirmed swab, and a time lag of at least 14 days from the second vaccination dose.

The analysis was presented as split into two time periods: the first period run from March 4, 2021 to December 15, 2021, and included the pre-planned analysis on the association between the antibody levels developed after the second vaccine dose and the risk of a breakthrough infection during follow up. At the beginning of November 2021, a third vaccine dose was proposed to pwMS who have received the second dose since more than 4 months. We therefore censored the follow up time at the third dose date for those who were free from infection at that time. Moreover, in late December the Omicron variant became predominant in Italy (on December 23, 2021, the percentage of Omicron infections was estimated to be 28% - https://www.iss.it/primo-piano, accessed on December 25, 2021). We set December 15 as the cutoff date, considering breakthrough infections before that date as Delta variant infections and after that date as Omicron variant infections. The analysis in this second period took also into

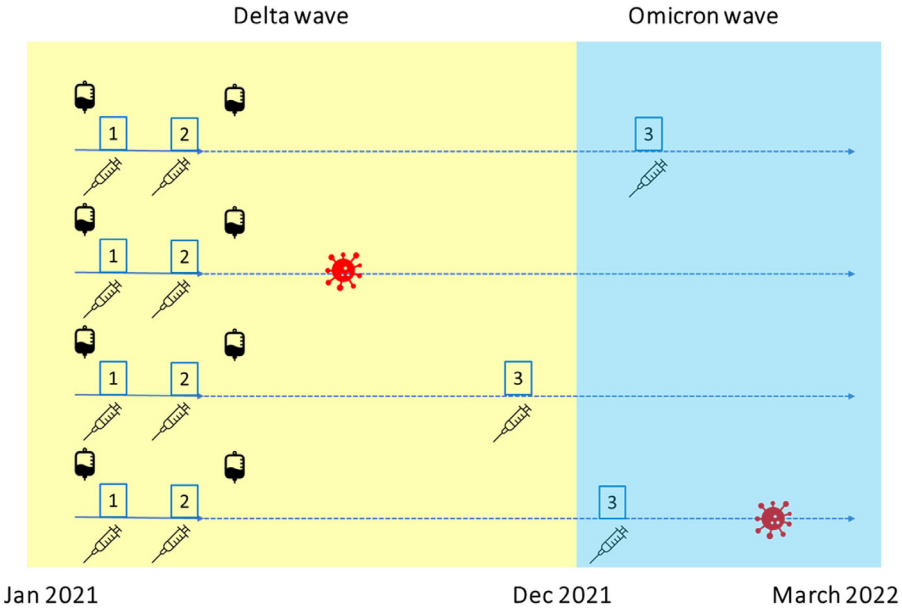

Figure 1. A schematic view of the events in the observation period. The first part of the study was pre-panned with blood samples taken after the second vaccine dose and a clinical follow up, up to the third vaccine dose, a Covid infection or the last follow up date, whichever came first (Delta wave period, yellow box). During the conduction of the study a third vaccine dose was recommended for patients with MS (starting since November 2021). The Omicron wave (blue box) arrived in Italy at mid-December.

account the third dose (but we did not collect the blood samples after the third dose, since it was not planned at the stage of study design and protocol approval).

SARS-CoV-2 antibody measurement

High-affinity pan-Ig antibodies to SARS-CoV-2 were measured by a centralized laboratory with a double-antigen sandwich-based electrochemiluminescence immunoassay (Elecsys®, Roche Diagnostics Ltd, Switzerland). Receptor-binding domain (RBD) antibodies were quantitatively measured to evaluate the humoral immune response to the two RBD-coding mRNA vaccines. RBD antibodies have been shown to positively correlate with SARS-CoV-2 neutralizing antibodies on neutralization assays.[10] Serum samples were shipped in dried ice by the centers and stored at -20°C until analysis.

DMT groups

Patients were grouped in 12 groups, according to the therapy they were taken when vaccinated. The 12 groups included a group of untreated subjects, a group in interferon, glatiramer-acetate, dimethyl-fumarate, teriflunomide, natalizumab, fingolimod, ocrelizumab, rituximab, alemtuzumab, cladribine and other drugs.

Statistical analysis

The statistical analysis was run in two separate sections: the first analysis ("Delta") was pre-planned and included the follow up of patients from the second vaccine dose to the cut-off date (December 15, 2021). The second analysis ("Omicron") was adapted to include two time-dependent unplanned events occurring during follow up: the introduction of a third vaccine dose and the switch to the Omicron variant (Figure 1). Two different statistical approaches were applied.

In the first analysis, the cumulative incidence of breakthrough infections was calculated from the date of the second vaccine dose to the date of breakthrough infection. For those who did not have the event, time was censored on December 15, 2021, or at the date of the third dose, whichever came first. The cumulative incidence of "Delta" breakthrough infections in the different DMT groups was reported by Kaplan-Meier survival curves. A multivariable Cox model was used to evaluate the impact of DMT class and antibody levels developed 4 weeks after the second vaccination dose on the risk of a breakthrough infection after adjusting for age, sex, EDSS level, previous Covid-19 infection (detected by the positivity for N antibodies) and vaccine type. The proportional hazard assumption was checked by a global test based Schoenfeld residuals. The antibody levels were divided by 0,972 to report them in standard BAU units[10] and then transformed on a Log10 scale, to normalize their distribution and according to previous literature.[1] A ROC curve (run on the subgroup of patients with at least 6 months of follow-up) was used to assess the best antibody level cut-off indicating a protective level against the "Delta" breakthrough infections.

An analysis over an extended follow-up (cut-off date March 25, 2022) was run on all the recorded breakthrough infections, by a multivariable Cox model including the third vaccine dose and the variant (Delta vs Omicron) as time dependent variables, to minimize issues related to immortal time bias. The third vaccine dose was a time-dependent binary variable set to "No" up to the date of vaccination and set to "Yes" after the third vaccine date. The variant was a time-dependent binary variable set to "Delta" before December 15, 2021 and set to "Omicron" after December 15, 2021. We included in the model two interaction terms: the antibody level by third dose interaction (assessing whether the effect of antibody level on the risk of infection was modified by receiving the third dose) and the antibody level by variant interaction (assessing whether the effect of antibody level on the risk of infection was modified by the Omicron variant).

The characteristic of patients who had a breakthrough infection were compared between the Delta vs the Omicron group by the Mann-Whitney U test and the Chi-square test. Kaplan-Meier (KM) survival curves were used to display the cumulative probability to have a breakthrough infection in the two (Delta and Omicron) periods. In the KM curve reporting the impact of the third dose, a landmark method was used to correct for the immortal time bias (a patient must survive long enough free from the infection to receive the third dose). The landmark was set to December 15, 2021 (excluding all the breakthrough infection before that date and setting those patients who have received the third dose after the landmark time as not receiving the third dose).

Characteristics	Overall (N = 1705)
Age, years (mean, SD)	46.1 (12.45)
Females (n, %)	1161 (68.1%)
BMI, kg/m^2 (mean, SD)	24.1 (3.65)
MS phenotype (n, %)	
Relapsing remitting	1420 (83.3%)
Secondary progressive	158 (9.3%)
Primary progressive	127 (7.4%)
MS disease duration, years (median, IQR)	10.0 [5.0 – 15.6]
EDSS (median, IQR)	2.0 [1.0 – 4.0]
MS treatment (n, %)	
Ocrelizumab	272 (16.0%)
Dimethyl fumarate	267 (15.7%)
Natalizumab	199 (11.7%)
Interferon	192 (11.3%)
Fingolimod	173 (10.1%)
Teriflunomide	102 (6.0%)
Glatiramer acetate	102 (6.0%)
Cladribine	51 (3.0%)
Rituximab	48 (2.8%)
Alemtuzumab	23 (1.3%)
Other	26 (1.5%)
Untreated	250 (14.7%)
Vaccine product (n, %)	
mRNA BNT162b2	1391 (81.6%)
mRNA-1273	314 (18.4%)
Patients with a previous Covid-19 infection (n, %)	218 (12.8%)

***Table 1*: Baseline characteristics of patients with multiple sclerosis who received two vaccine doses.**
MS=Multiple Sclerosis, BMI=Body Mass Index, SD=Standard Deviation, IQR=Interquartile Range, EDSS=Expanded Disability Status Scale.

Role of the funders
The study was supported by FISM - Fondazione Italiana Sclerosi Multipla – cod. 2021/Special-Multi/001 and financed or co-financed with the '5 per mille' public funding. The had no role in the study design, data collection, data analysis, interpretation or writing the report.

Results
Data were collected between March 4, 2021, and December 15, 2021 in the pre-panned analysis and the follow up was then extended to March, 25 2022. At the time of the analysis 1914 pwMS have been invited to participate in the study. Of them 209 refused to participate: 29 (14%) declined the vaccination and 180 (86%) did not want to come for the blood sampling. Among the 1705 included pwMS (89%) who had a full vaccination cycle (2 vaccine doses, 21/28 days apart), 82% received the BNT162b2 vaccine and 18% the mRNA-1273 vaccine. Of them, 1551 (92%) had blood assessment 4 weeks after the second vaccine dose. We collected data on the third dose on 1256 patients included in the study (74%). Among them 1154 (92%) received the third dose after a mean interval of 210 days (range 90-342 days) after the second dose, while 112 (8%) did not receive it at the last follow up date. The patients' characteristics and the number of vaccinated patients in each DMT group are reported in Table 1. 218 (12.8%) patients were positive for N antibodies and had a previous natural Covid-19 infection. Figure 1 reports a scheme summarizing the timing of vaccination doses in the Delta and the Omicron periods.

Pre-planned analysis: "Delta" breakthrough infections
Overall, we observed 33 Covid-19 "Delta" breakthrough infections during follow-up reported on average 125 days after the second dose (range, 18-230). Table 2 reports the description of these cases: 16 pwMS were on anti-CD20, (14 on ocrelizumab and 2 on rituximab), 6 on fingolimod, and 11 on other DMTs. We did not collect information on recent steroid use. The 8-month cumulative incidence of "Delta" breakthrough infection was 4.3% (SE, 0.5%) (Figure 2, yellow box). The

	Delta variant (n=33)	Omicron variant (n=98)	p
Age, years (mean, SD)	44.0 (10.74)	42.2 (11.50)	0.42
Females (n, %)	18 (54.5%)	73 (74.5%)	0.031*
BMI, kg/m^2 (mean, SD)	24.0 (3.13)	23.9 (3.96)	0.49
MS phenotype (n, %)			
Relapsing Remitting	26 (78.8%)	88 (89.8%)	0.19
Primary Progressive	6 (18.2%)	7 (7.1%)	
Secondary Progressive	1 (3.0%)	3 (3.1%)	
MS disease duration, years (median, IQR)	11.1 (7.43)	9.9 (6.68)	0.56
EDSS (median, IQR)	2.0 [1.5 - 4.0]	1.5 [1.0 - 3.0]	0.13
MS treatment (n, %)			
Ocrelizumab/Rituximab	16 (48.5%)	36 (36.7%)	0.06
Fingolimod	6 (18.2%)	8 (8.2%)	
Other	11 (33.3%)	54 (55.1%)	
Vaccine product (n, %)			
mRNA-1273	6 (18.2%)	21 (21.4%)	0.69
mRNA BNT162b2	27 (81.8%)	77 (78.6%)	
SARS-CoV-2 antibody levels 4 weeks after the 2nd vaccine dose (median, IQR)	15.2 [0.0 - 559.2]	406.1 [2.1 - 1689.3]	0.15

***Table 2*: Characteristics of "Delta" and "Omicron" cases.**
MS=Multiple Sclerosis, BMI=Body Mass Index, SD=Standard Deviation, IQR=Interquartile Range, EDSS=Expanded Disability Status Scale.

incidence was higher in pwMS on ocrelizumab (7.2%, SE=1.9%), fingolimod (3.5%, SE=1.6%), rituximab (3.9%, SE=2.7%), as compared to dimethyl-fumarate (2.3%, SE=0.9%), teriflunomide (1.0%, SE=1.0%), and untreated patients (2.0%, SE, 0.6%), and it was zero for all the other therapies. The log-rank test for heterogeneity among DMTs was highly significant (p=0.001). Figure 3 (panel a) reports the KM curves with DMTs

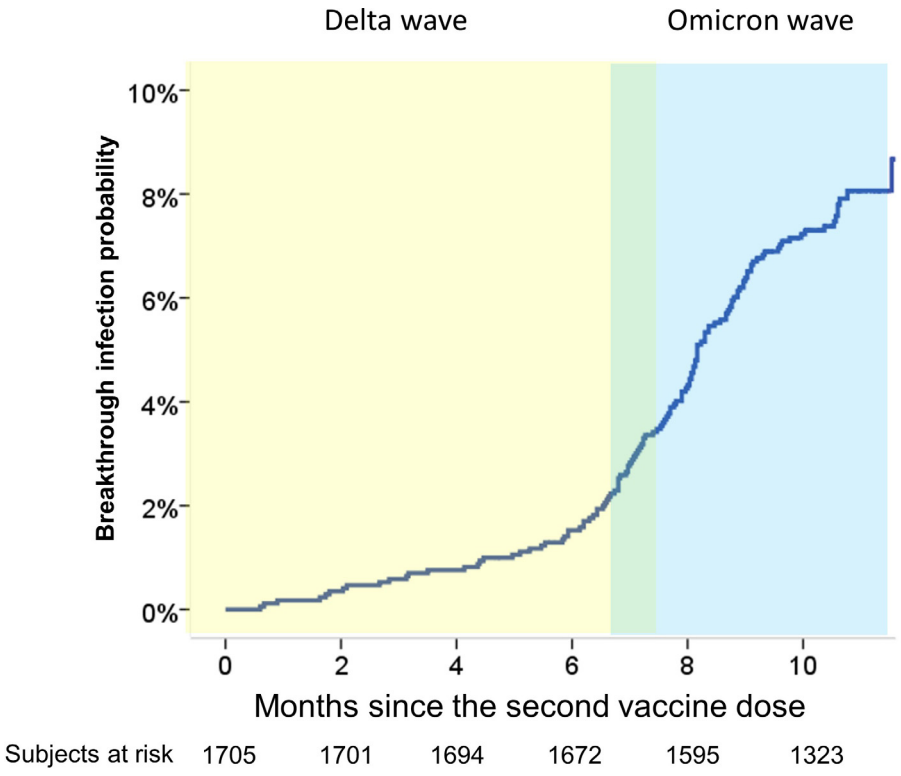

Figure 2. Cumulative probability of breakthrough infection in the two periods. The yellow box reports infections during the Delta wave, the blue box infections during the Omicron wave.

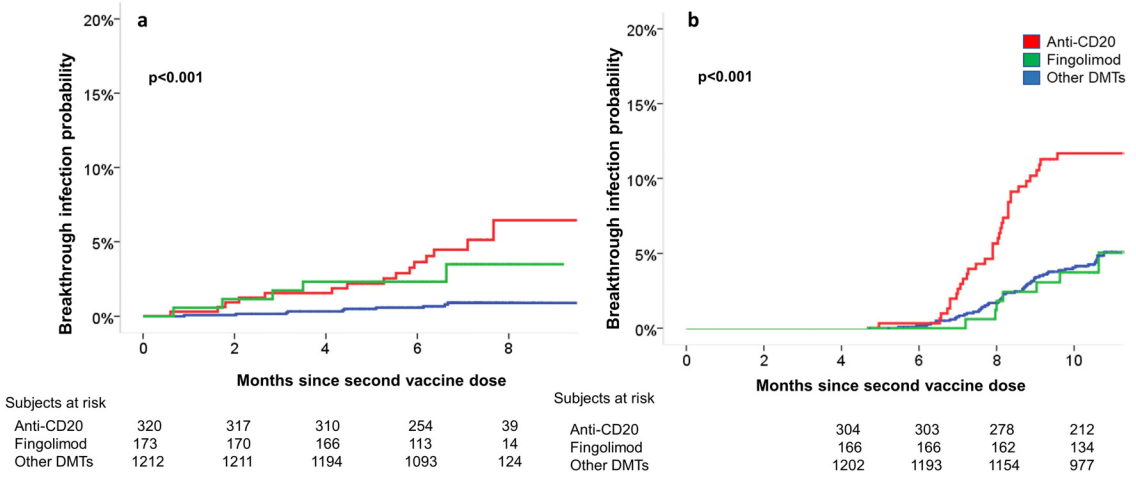

Figure 3. Kaplan Meier survival curves displaying the cumulative probability of a breakthrough infection during the Delta wave (March 4 to December 15, 2021) (panel a), and during the Omicron wave (December 15, 2021, to March 25, 2022) (panel b) in groups defined by patients treated by anti-CD20 drugs (ocrelizumab or rituximab), by fingolimod and other therapies. P-values according to the log-rank test.

grouped as anti-CD20 (ocrelizumab and rituximab) vs fingolimod vs other drugs. The mean time between the vaccination and the last infusion for patients in ocrelizumab was 136 days (range, 30-664).

The proportional hazard assumption was not violated (global test chi square=3.81, 5 DF, p=0.70). At multivariable analysis (Table 3) the only significant factor associated to the risk of breakthrough infection was the antibody level after the second dose, with an HR of 0.51 (95%CI=0.38-0.69, $p < 0.001$). This value indicates that the risk of breakthrough infection was reduced by 49% every X10 increase in the antibody level. The ROC curve applied to patients with at least 6 months of follow-up (n=1543, 99%) indicated a value of log antibody level of 2.82 (659 BAU/mL) as the best cutoff discriminating those at a higher risk of infection (AUC=0.71; sensitivity, 85%, specificity, 58%, Figure 4). Figure 5 reports the Delta breakthrough infections in each DMT group and according to antibody levels.

Extended follow-up: "Omicron" breakthrough infections

The incidence of breakthrough infection rapidly increased during the "Omicron" wave (Figure 2, blue panel) and we observed 98 breakthrough infections between December 15, 2021 and March 25, 2022. Table 2 reports the characteristics of the Omicron cases: they were more female (74.5%) than for the Delta variants (54.5%, p=0.03); also, in the Omicron vs the Delta period there was a decrease of the relative percentage of anti-CD20 (from 48.5% to 36.7%) and fingolimod cases (from 18.2% to 8.2%). Table 4 reports the result of the multivariable time-dependent Cox model run on the extended follow up. After adjusting for age, sex, EDSS and vaccine type, the antibody level after the second vaccine dose was still a strong predictor of the risk of breakthrough infections during follow up (HR=0.57, 95%CI=0.44-0.73, $p < 0.001$); patients who received the third dose had a 56% reduction in the risk of

	Multivariable Analysis* n=1508	
Variable	**HR (95% C.I.)**	**p**
Antibody levels BAU/mL (log10)	0.51 (0.38-0.69)	<0.001
Age (years)	1.00 (0.96-1.04)	0.94
Sex (Female vs Male)	0.58 (0.26-1.28)	0.18
EDSS (1 point score)	0.94 (0.76-1.17)	0.59
Vaccine (mRNA-1273 vs mRNA BNT162b2)	0.86 (0.29-2.52)	0.94
Previous Covid-19 infection (yes vs no)	0.98 (0.24-4.23)	0.98

***Table 3*: Multivariable Cox regression model evaluating risk factors for breakthrough SARS-CoV-2 infections.**
HR=Hazard Ratio, C.I.=Confidence Interval, EDSS=Expanded Disability Status Scale.

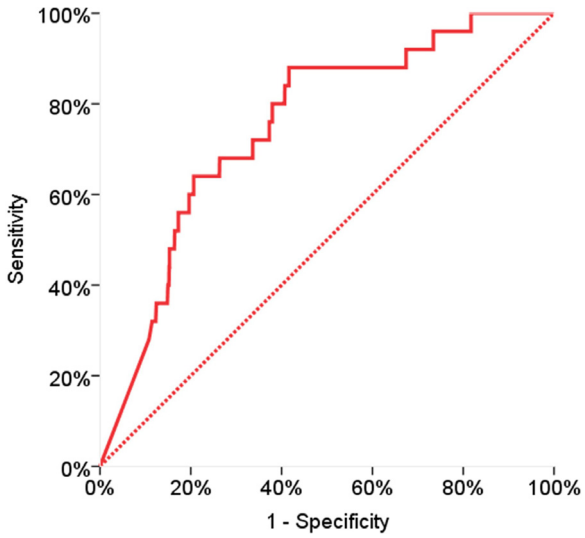

Figure 4. ROC curve reporting sensitivity and specificity of antibody levels after the second vaccination dose to identify a higher risk of breakthrough infection.

infections (HR=0.44, 95%CI=0.21-0.90, p=0.025, Figure 6); the Omicron variant increased by about 6 times the risk of infection (HR=6.31, 95%CI=2.55-15.49, $p < 0.001$). The interaction analysis revealed no significant impact of the third dose on the protective role of antibody levels, while the significant interaction between antibody level and Omicron variant (HR=1.44, 95% CI=1.04-1.99, p=0.03) indicated that the protective role of the antibody level is reduced by the Omicron variant by 40%. Figure 3 (panel b) reports the risk of infection in the Delta vs the Omicron period according to DMT (anti-CD20 vs fingolimod vs other DMTs).

Hospitalization risk

Four patients were hospitalized. Three of them in the Delta period, (an over 60-year-old woman on ocrelizumab with a very low antibody titer (5.2 BAU/mL), an over 40-year-old woman in ocrelizumab with no antibody detected and an over 40-year-old man on teriflunomide with medium antibody titer (630 BAU/mL)) and one of them in the Omicron period (an over 40-year-old woman on ocrelizumab with a very low antibody titer (4.9 BAU/mL) who had received also the booster dose 50 days before the infection). All of them recovered without the need for supplemental oxygen. Overall, the

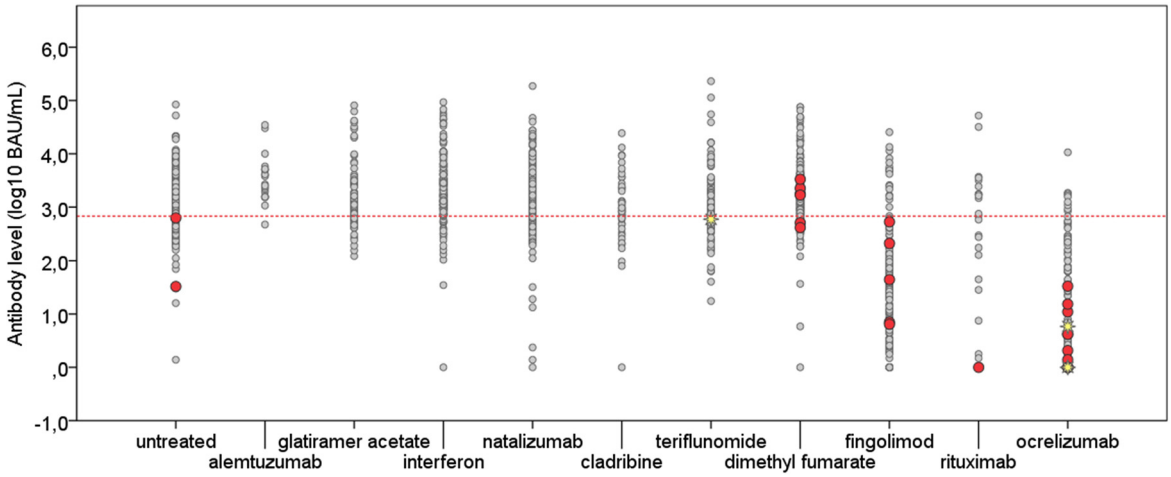

Figure 5. Covid-19 cases (red dots) and severe Covid-19 cases (hospitalized) (yellow stars) according to disease modifying therapy and antibody level. The red dotted line represents the antibody level cut-off better discriminating patients at risk of breakthrough infection (627 BAU/mL).

Variables	HR	p value
Antibody levels BAU/mL (log10)	0.57 (0.44-0.73)	<0.001
Age (years)	0.99 (0.97-1.00)	0.11
Sex (Female vs Male)	1.04 (0.69-1.56)	0.84
Vaccine (mRNA-1273 vs mRNA BNT162b2)	0.65 (0.24-1.73)	0.39
EDSS (1 EDSS point)	0.94 (0.84-1.05)	0.26
Previous Covid-19 infection (yes vs no)	0.63 (0.31-1.26)	0.19
Third dose (yes vs no)	0.44 (0.21-0.90)	0.025
Variant (Omicron vs Delta)	6.31 (2.55-15.49)	<0.001
Third dose by antibody level interaction	0.84 (0.63-1.12)	0.24
Omicron variant by antibody level interaction	1.44 (1.04-1.99)	0.028

***Table 4*: Time dependent multivariable Cox regression model evaluating risk factors for breakthrough SARS-CoV-2 infections including the third vaccine dose and the new Omicron variant.**
HR=Hazard Ratio, C.I.=Confidence Interval, EDSS=Expanded Disability Status Scale.

hospitalization rate was 1.2% (95%CI=0%-6.5%) in pwMS treated with DMTs other than ocrelizumab, reduced by 90% as compared to the hospitalization rate reported in Italy in the pre-vaccination era in the same group of patients (that was 11.9%).[11] The hospitalization rate was 5.8% (95%CI=1.2%-16.0%) in pwMS treated with anti-CD20, reduced by 70% as compared to the pre-vaccination rate (19.5%).[11]

Discussion

The risk of both contracting SARS-CoV-2 infection and of not responding to Covid-19 vaccines is higher in pwMS on anti-CD20 monoclonal antibodies or fingolimod. Vaccine-induced protection from the disease is expected to waning with time since vaccination[7,8] and different levels of immunity impact on susceptibility to breakthrough infections. In this study we assessed the incidence of breakthrough infections in a large Italian cohort of patients fully vaccinated with mRNA vaccines. The cumulative incidence of breakthrough infection over a follow-up of 8 months was 4.5%, with some heterogeneities among groups treated with different DMTs. A Cox model, including the antibody level as a continuous variable and adjusted for the main baseline covariates, revealed that lower SARS-CoV-2 antibody

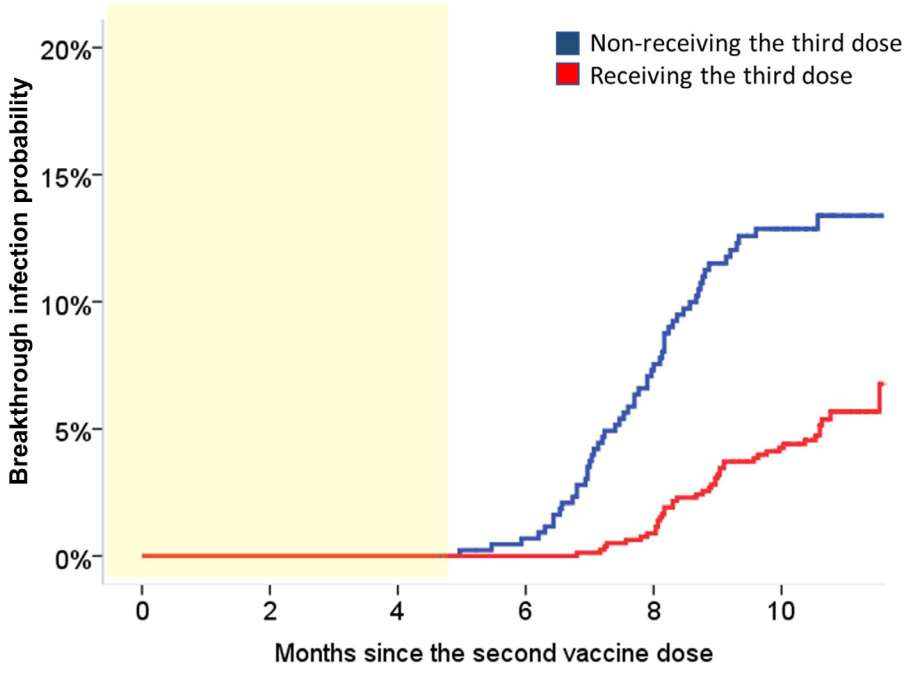

Figure 6. Kaplan Meier survival curves displaying the cumulative probability of a breakthrough infection in pwMS who received vs those who did not receive a third vaccine dose (landmark analysis, see text for details).

Patient	Age	Sex	EDSS	DMT	Time (day) since the second dose	Time (day) of the third dose	Antibody levels after the second dose (BAU/ml)
1	>65	F	4,5	Ocrelizumab	63	-	5,2
2	>40	M	3,5	Teriflunomide	132	-	630,0
3	>40	F	6,5	Ocrelizumab	186	-	0,0
4	>40	F	1,5	Ocrelizumab	218	168	4,9

***Table 5*: Characteristics of the hospitalized patients.**

levels as the only relevant risk factor for breakthrough infection risk. The risk of infection decreases of about 43% for every 10 times-fold increase in the antibody levels. In this study, SARS-CoV-2 antibody level equal to, or lower than 659 BAU/mL are associated with higher risk of infection in the subsequent 6 months. Post-vaccination neutralizing antibody titers predicted the risk of breakthrough infection in health care workers.[12] However, no neutralizing or binding antibody threshold titer identified so far can predict the degree of protection, depending on unpredictable titer changes over time and on the strength of immunity at the moment when a subject is exposed. In addition, other SARS-CoV-2 infection risk modifiers, such as each patient's safety precautions and viral load exposure amount, can impact on the risk of Covid-19 independently from antibody titers.

In December the Omicron variant became dominant in Italy and we observed a 6-fold increase in the risk of infection in pwMS who received two vaccine doses. However, a high antibody level after the second dose is still relevant to prevent the infection, even if the advantage of being vaccinated is reduced by the Omicron variant. The advent of the Omicron variant was just proceeded by the third vaccination introduction for pwMS in Italy. It seems that the booster dose is a relevant protective factor for the risk of infection, even during the Omicron wave.

On the other hand, the main goal of vaccination is not to prevent infections, but rather to prevent the severe disease. In this respect, we observed just four breakthrough infections that caused Covid-19 requiring hospitalization followed by resolution within one week. The prevalence of hospitalization in our Italian Covid-19 cohort of pwMS in the pre-vaccination era was 12.8%[11] and therefore around 16 cases were expected out of the 131 infected patients. The incidence of infections requiring hospitalization detected in our sample (3.1%) was therefore strongly reduced after vaccination. Three out of 4 hospitalizations were among patients treated with ocrelizumab (over the 48 who had a breakthrough infection). Anyway, the hospitalization rate in this group of subjects (6.3%) was largely reduced as compared to the pre-vaccination hospitalization rate we have observed in Italy in pwMS treated with ocrelizumab. This observation, even if based on a small number of cases, confirms the role of the preserved T cell response in pwMS on ocrelizumab to prevent severe infections requiring hospitalization, despite the impaired humoral response.

Analogously to the other study on anti-CD20 treated patients following Covid-19 vaccination,[13] the age factor, which typically associate with lower antibody responses to Covid-19 vaccines,[14] did not have an independent role on influencing the risk of breakthrough infections in our pwMS cohort, when we take into account the antibody levels. This phenomenon appears to be evident in the general population too.[15]

This study has some limitations. First, the power of the study is limited by the small number of cases detected during follow up. Second, incidence data of breakthrough infections in the general population, as well as those of non-breakthrough infections in unvaccinated people, were not available for comparing these rates with those of our pwMS cohort. In solid organ transplant recipients, the diminished antibody responses to SARS-CoV-2 resulted in 41-to-82-fold (depending on the statistical approach) higher risk of breakthrough infection vs general population.[17] Third, we have no data on SARS-CoV-2 molecular characterization, so our classification of Delta and Omicron variant are just based on average expectation in specific time periods. Fourth, the recruited patients and their immunotherapies may not reflect the situation in real life.

In conclusion, the results of this study suggest that the rates of breakthrough infections in pwMS on DMTs depends on the level of humoral immunity to SARS-CoV-2, which is reduced in patients under specific DMTs and that declines over time. The Omicron variant was less sensitive to the antibody levels and increased the rate of breakthrough infection risk in pwMS. Larger cohorts are needed to understand the protective role of vaccination for severe Covid-19 in pwMS under different DMTs, even if it seems that the rate of infection requiring hospitalization was largely reduced after vaccination, also in patients on anti-CD20 (with an impaired humoral response to vaccination). It resulted also that the protection from Covid-19 increases after the third booster vaccine doses. Identifying the frequency, severity, and predisposing factors of breakthrough infections in frail patients may inform how to protect them with anticipates boosting doses of vaccines. The recommended safety precautions, such as masks and distancing, should remain a mainstay to reduce the incidence of SARS-CoV-2 infection.

Contributors

All authors participated in the study planning. Maria Pia Sormani, Irene Schiavetti, Matilde Inglese, Luca Carmisciano, Alice Laroni, Caterina Lapucci, Diego Franciotta, Carlo Serrati, Marco Salvetti, Antonio Uccelli had a role in the study conceptualization and in writing the original draft. Cinzia Cordioli, Doriana Landi, Marinella Clerico, Elisabetta Signoriello, Eleonora Cocco, Jessica Frau, Ilaria Gandoglia, Tiziana Tassinari, Germana Perego, Giampaolo Brichetto, Paola Gazzola, Antonio Mannironi, Maria Laura Stromillo, Maria Teresa Ferrò, Alessia Di Sapio, Livia Pasquali, Monica Ulivelli, Fabiana Marinelli, Matteo Pizzorno, Graziella Callari, Rosa Iodice, Giuseppe Liberatore, Francesca Caleri, Anna Maria Repice, Susanna Cordera had a role in data curation and in writing, reviwing and editing the manuscript. Valeria Visconti and Diego Franciotta performed formal analyses of blood samples. Mario Alberto Battaglia had a role in project administration and in funding acquisition. Maria Pia Sormani, Irene Schiavetti, Luca Carmisciano had a role in the data curation and statistical analysis. Maria Pia Sormani and Irene Schiavetti verified the underlying data. All authors read and approved the final version of the manuscript.

Declaration of interests

Sormani MP received consulting fees from Roche, Biogen, Merck, Novartis, Sanofi, Celgene, Immunic, Geneuro, GSK, Medday; received payment or honoraria for lectures, presentations, speakers' bureaus, manuscript writing or educational events from Roche, Biogen Merck, Novartis, Sanofi, Celgene; participated on a Data Safety Monitoring Board or Advisory Board for Roche, Sanofi, Novartis, Merck. Uccelli A received grants (to his Institution) from FISM, Biogen, Roche, Alexion, Merck Serono; participated on a Data Safety Monitoring Board or Advisory Board (to his Institution) for BD, Biogen, Iqvia, Sanofi, Roche, Alexion, Bristol Myers Squibb. Ulivelli M received consulting fees from Biogen, Novartis, Serono. Caleri F received honoraria for lectures or presentation from Biogen, Merck, Teva, Novartis, Sanofi-Genzyme, Roche; received support for attending meeting and travel grant from Biogen, Merck, Teva, Novartis, Sanofi-Genzyme, Roche; received honoraria for participation on Advisory Boards from Biogen, Merck, Teva, Novartis, Sanofi-Genzyme, Roche. Cordioli C received grants or contracts from Roche, Novartis, Merck Serono, Biogen, Celgene; received consulting fees from Biogen. Inglese M received grants or contracts from FISM, INAIL, European Union. Laroni A received grants or contracts from Italian Ministry of University, Ministry of Health; received consulting fees from Merck, Biogen, Roche, Novartis, Bristol-Myers Squibb Pharma EEIG; received honoraria for lectures, presentations, speakers bureaus, manuscript writing or educational events from Mercks, Biogen, Roche, Novartis, Bristol-Myers Squibb Pharma EEIG. Salvetti M received grants or contracts from Biogen, Merck, Novartis; received payments or honoraria for lectures, presentations, speakers' bureaus, manuscript writing or educational events from Biogen, Merck, Novartis, Roche, Sanofi. Landi D received consulting fees from Merck Serono, Celgene, Bristol Myers Squibb, Roche, Novartis, TEVA; received payments or honoraria for lectures, presentations, speakers' bureaus, manuscript writing or educational events from Merck Serono, Celgene, Bristol Myers Squibb, Biogen, Roche, Novartis, Sanofi Genzyme, Mylan; received support for attending meetings and/or travel from Merck Serono, Biogen, Roche, Sanofi Genzyme, Novartis, Mylan; participated on Data Safety Monitoring Board or Advisory Board for Merck Serono, Celgene Bristol Myers Squibb, Biogen, Roche, Sanofi Genzyme. Mannironi A, Pasquali L, Ferrò MT, Liberatore G, Brichetto G, Serrati C, Marinelli F, Carmisciano L, Clerico M, Di Sapio, A, Tassinari T, Visconti V, Perego G, Pizzorno M, Callari G, Cocco E, Frau J, Gazzola P, Repice AM, Schiavetti I, Signoriello E, Stromillo ML, Cordera S, Franciotta D, Iodice R, Lapucci C, Battaglia MA, Gandoglia I have nothing to disclose.

Data sharing statement

Data can be shared by the authors upon request.

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Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.ebiom.2022.104042.

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