Predictors for insufficient SARS-CoV-2 vaccination response upon treatment in multiple sclerosis

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Summary

Background Disease-modifying therapies (DMT) for multiple sclerosis (MS) influence SARS-CoV-2 vaccination response, which might have implications for vaccination regimens in individual patients. Expanding the knowledge of predictors for an insufficient vaccination response as a surrogate for protection against severe disease courses of infection in people with MS (pwMS) under DMT is of great importance in identifying high-risk populations.

Methods Cross-sectional analysis of vaccination titre and its modifiers, in a prospective real-world cohort of 386 individuals (285 pwMS and 101 healthy controls) by two independent immunoassays between October 2021 and June 2022.

Findings In our cohort, no difference in vaccination antibody level was evident between healthy controls (HC) and untreated pwMS. In pwMS lymphocyte levels, times vaccinated and DMT influence SARS-CoV-2 titre following vaccination. Those treated with selective sphingosine-1-phosphate receptor modulators (S1P) showed comparable vaccination titres to untreated; higher CD8 T cell levels prior to vaccination in B cell-depleted patients resulted in increased anti-spike SARS-CoV2 antibody levels.

Interpretation PwMS under DMT with anti-CD20 treatment, in particular those with decreased CD8 levels before vaccination, as well as non-selective S1P but not selective S1P are at increased risk for insufficient SARS-CoV-2 vaccination response. This argues for a close monitoring of anti-spike antibodies in order to customize individual vaccination regimens within these patients.

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Keywords: SARS-CoV-2 vaccination titre; Disease-modifying therapy; Anti-CD20; S1P-Modulators

Introduction

National authorities and expert consortia recommend vaccination against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in all people with multiple sclerosis (pwMS) to prevent severe lung disease with the need for long-term ventilation and potentially life-threatening complications. Reports on safety of the BNT162b2 Covid-19 vaccine (Pifzer-BioNTech) revealed equal rates of relapse activity in vaccinated compared to non-vaccinated patients, arguing against multiple sclerosis disease reactivation due to post-vaccination pathogenic immune response.[1] Nonetheless, response to vaccination as measured by anti-spike SARS-CoV-2 antibodies might be insufficient under specific immunosuppressive disease-modifying therapies (DMT).[2] Although both mRNA (BNT162b2 and mRNA-1273) and vector vaccines (ChAdOx1, Ad26.COV2.S) were reported safe in multiple sclerosis, recent studies suggest

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Research in context

Evidence before this study

Although vaccines against SARS-CoV-2 have been reported safe and are recommended for people with multiple sclerosis (pwMS), recent studies have suggested a reduced antibody development under disease-modifying therapies (DMT). Association of breakthrough infections with low vaccination titres and treatment with specific DMT has been shown. Therefore, predictors of a reduced vaccination response are necessary to identify risk groups and prevent detrimental effects of SARS-CoV-2 infections.

Added value of this study

In pwMS, anti-spike SARS-CoV-2 antibodies were reduced under DMT with non-selective sphingosine-1-phosphate modulator (S1P) and anti-CD20 treatment, whereas lymphocyte counts and number of vaccinations increased antibody levels. In particular, selectivity of S1P favoured increased antibody levels independent of absolute lymphocyte counts. Similarly, CD8 T cell levels before vaccination predicted antibody response under anti-CD20 treatment.

Implications of all the available evidence

This study identifies predictors of vaccination response. For those patients receiving B cell-depletion and revealing low CD8 levels or treated with non-selective sphingosine-1-phosphate receptor modulators but not selective S1P, monitoring of vaccination response and adaption of vaccination and treatment regime is highly relevant.

a reduced antibody development against SARS-CoV-2 after vaccination in pwMS.[3–6] In general, breakthrough infections were associated with low vaccination titres and treatment with the non-selective sphingosine-1-phosphate modulator (ns-S1P) fingolimod or with CD20 antibodies (anti-CD20).[7,8]

Here, in a real-world scenario, we measured the antibody response (anti-spike SARS-CoV-2) via two independent immunoassays following vaccination in pwMS under DMT, in order to identify predictors of insufficient vaccination response.

Methods

Sample acquisition

Serum antibody levels in 285 pwMS (196 females and 89 males, as self-reported by study participants; detailed demographics in Table 1) were measured in the Department of Neurology at the University Medical Centre Mainz (Germany) from October 2021 to June 2022 as part of the standard laboratory examination; vaccination was performed off-site according to the recommendations of the national vaccination consortium in Germany (STIKO). At the beginning of data acquisition, two vaccinations with an mRNA vaccine or the vector vaccine ChAdOx1, or one vaccination with the vector vaccine Ad26.COV2.S was recommended. From November 2021 on, a booster vaccination was recommended. Clinical features as well as immune status including lymphocyte composition were extracted from standardised routine investigations. We also enrolled 101 age- and sex-balanced healthy volunteers (HC). DMT was grouped as platform (interferon, glatiramer acetate, dimethyl fumarate, teriflunomide), S1P (selective [s-S1P] and non-selective), anti-CD20 (ocrelizumab, rituximab) and other highly effective (natalizumab, alemtuzumab). All pwMS included in the study must have been vaccinated against SARS-CoV-2 with one of the named agents, must have been older than 18 years and treated with one of the named DMT or not treated. Individual samples lacked information on type or timing of vaccination; these were excluded from regression analysis and specific sub analyses, but included within the overall analyses.

Antibody measurement

SARS-CoV-2 IgG and IgM against spike (quantitative and qualitative) and nucleocapsid protein (qualitative) were detected with an electrochemiluminescence immunoassay (Elecsys Anti-SARS-CoV-2-S, Roche, Switzerland, catalogue number 09289267190) and a chemiluminescent microparticle immunoassay (Abbott Laboratories, USA, catalogue number 6R86-22, 6R87-22, 6S60-22) according to the manufacturer's protocol. Quantitative analysis with the electrochemiluminescence immunoassay (Roche) also included anti-spike IgA. As antibodies against the receptor-binding domain of the spike protein represent vaccination response, this work focusses on these. Serology tests were performed within 5 h after blood collection by the Department of Transfusion Medicine. Blinding was achieved by distributing the responsibility for recruiting, sample acquisition, measurement and statistical analysis to separate people.

Statistical analysis

Statistical analysis was performed using Graph Pad Prism 8 and SPSS 27. After distribution analysis for normality of the values (Shapiro–Wilk), we performed analysis of variance (ANOVA), t-test, Mann–Whitney U test or Kruskal–Wallis test as appropriate, always corrected for multiple comparison by Tukey or Dunn's test. Differences in distribution of nominal variables (e.g., sex, non-responder vs. responder) were assessed using a chi^2 test. p Values < 0.05 were considered statistically significant. For measuring rank correlation, Spearman's test was performed.

pwMS	n = 285 (%)	Mean (SD)
Age		42.02 (11.68)
Sex (self-reported)		
Female	196 (68.8)	
Male	89 (31.2)	
MS phenotype		
RRMS	242 (84.9)	
SPMS	25 (8.8)	
PPMS	15 (5.3)	
CIS	3 (1.1)	
Time lag (days)		105.04 (62.26)
Previous SARS-CoV-2 infection	13 (4.6)	
Previous SARS-CoV-2 infection status unknown	2 (0.7)	
Therapy		
Interferon	6 (2.1)	
Glatiramer acetate	10 (3.5)	
Dimethyl fumarate	74 (26.0)	
Teriflunomide	15 (5.3)	
ns-S1P	28 (9.8)	
s-S1P	16 (5.7)	
Natalizumab	41 (14.4)	
Ocrelizumab	52 (18.3)	
Rituximab	11 (3.9)	
None	32 (11.2)	
Times vaccinated		
1 vaccination received	15 (5.3)	
2 vaccinations received	151 (53.0)	
3 vaccinations received	95 (33.3)	
4 vaccinations received	5 (1.8)	
vaccination status unknown	19 (6.7)	
Vaccine type		
BNT162b2	183 (64.2)	
mRNA-1273	23 (8.1)	
Vector	8 (2.9)	
mRNA combi	27 (9.5)	
Vector-mRNA-combi	24 (8.4)	
Vaccine type unknown	20 (7.0)	

Time lag represents time elapsed between titre acquisition and last vaccination (days). RRMS, relapsing-remitting MS, SPMS, secondary progressive MS, PPMS, primary progressive MS, CIS, clinically isolated syndrome.

Table 1: **Baseline demographic and clinical characteristics of study participants.**

A multiple regression model was used to evaluate the impact of various independent variables on the vaccination titre. To ensure linearity in the dependent variable, the logarithm base 10 (log10) was used.[3,9] To identify specific co-founding effects, only pwMS vaccinated with mRNA vaccines were included in this analysis (Supplementary Table S1). Therapy subgroups were inserted subsequently into the same linear regression model previously performed with DMT (Supplementary Table S2). We assessed secondary outcomes in subgroups only including those treated with S1P (Supplementary Table S3) or anti-CD20 (Supplementary Tables S4 and S5). All values were referenced to untreated and BNT162b2-vaccinated, s-S1P to ns-S1P and rituximab to ocrelizumab within the respective model. The regression model on anti-CD20 did not include age, sex or time lag as also proven insignificant before[3] (Figs. 2c and 3c). We used the same model on CD19, CD8 and CD4 values prior to vaccination and at time of vaccination titre retrieval by subsequent calculation. Since none of the people for whom data on lymphocyte composition before vaccination was available had experienced a SARS-CoV-2 infection prior to titre measurement, this effect was only included in the model of values at time of vaccination titre retrieval.

Details of statistical analysis are provided in the Supplement (Supplementary Tables).

Ethics

The study was performed in compliance with the Declaration of Helsinki and was approved by the local ethics committee (2019-14758_1); participants gave written informed consent.

Role of the funding source

The funding source had no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the paper for publication.

Results

In our prospective cohort, DMT in pwMS was as follows: 32 untreated (none), 105 platform therapy, 44 S1P (28 fingolimod, 7 ozanimod, 9 siponimod), 63 anti-CD20 and 41 other highly effective therapies (Fig. 1 and Table 1). DMT in pwMS receiving mRNA vaccinations is detailed in Fig. 1 and Supplementary Table S6. Healthy controls were comparable in age and sex, which was confirmed by Mann–Whitney U test ($p = 0.07$) and chi^2 test ($p = 0.79$). Demographics of healthy controls including statistics can be found in Supplementary Tables S7 and S8.

SARS-CoV-2 antibody levels in the two independent immunoassays were closely correlated (Supplementary Fig. S1). Therefore, we focus here on combined spike antibodies (IgG, IgM and IgA) detected by the electrochemiluminescence immunoassay (Roche) due to its proven high specificity and sensitivity.[10]

No difference in vaccination titre could be found between the different clinical courses of multiple sclerosis (clinically isolated syndrome, relapsing-remitting, primary or secondary progressive) nor an influence of age (not shown). HC and untreated pwMS exhibited similar SARS-CoV-2 antibodies; pwMS under DMT displayed significantly lower levels compared to HC

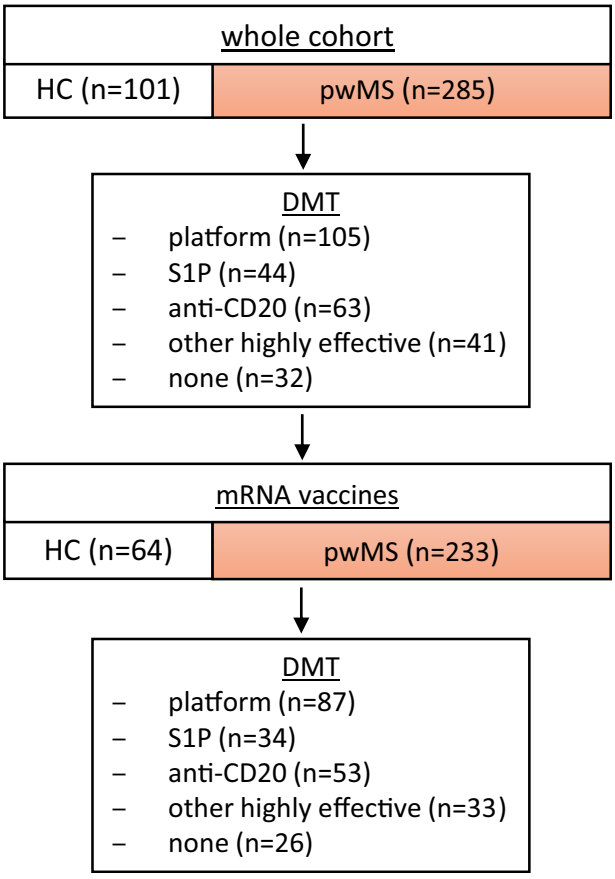

Fig. 1: **Flow diagram of cohort characteristics.** The real-world cohort of 386 participants included 101 healthy controls (HC) and 285 people with multiple sclerosis (pwMS). Of these, 105 were treated with platform therapies, 44 with sphingosine-1-phosphate receptor modulator (S1P), 63 with CD20 antibody (anti-CD20), 41 with other highly effective therapies, and 32 were untreated (none). Of the whole cohort, 64 HC and 233 pwMS received mRNA vaccines; DMT type in these patients is provided.

(difference of medians –0.2350, p = 0.0208, not shown). This observation was confirmed in a linear regression model, demonstrating a reduced linear regression coefficient B for SARS-CoV-2 antibodies in pwMS under DMT compared to no therapy (B = –0.576, 95% CI [–0.675, –0.477], p < 0.001; Supplementary Table S1). With regard to different DMT, vaccination titre was particularly reduced under S1P and anti-CD20 (Kruskal–Wallis test; mean rank difference –52.78 and –120.6, p = 0.0217 and p < 0.0001, respectively), whereas platform and other highly effective therapies showed similar levels as HC (Fig. 2a). This finding was confirmed by categorising titres into non-responder (<0.8 U/ml), responder (0.8–5000 U/ml) and high-responder (>5000 U/ml); non-responders were only present in the S1P (6.8%) and anti-CD20 (52.38%) groups (chi^2 p < 0.001) (Fig. 2b).

To investigate factors influencing anti-spike SARS-CoV-2 levels, we performed multiple linear regression including patients vaccinated with mRNA vaccines (Fig. 2c). Antibody level was positively influenced by the number of vaccinations received (B = 0.488, 95% CI [0.188–0.563], p = 0.003) and lymphocyte count (B = 0.375, 95% CI [0.165–0.811], p < 0.001; Supplementary Table S1). A negative impact was induced by DMT (B = –0.576, 95% CI [–0.675, –0.477], p < 0.001), in particular by B cell depletion (B = –2.589, 95% CI [–3.148, –2.029], p < 0.001) and S1P modulation (B = –0.820, 95% CI [–1.454, –0.186], p = 0.011; Supplementary Table S2).

Lymphocyte counts correlated with the vaccination titre in the whole cohort of pwMS (Spearman correlation r = 0.1817, p = 0.0046) (Supplementary Fig. S2). In addition, lymphocyte counts were significantly decreased by S1P (Kruskal–Wallis test; mean difference 136.7, p < 0.0001) and anti-CD20 (Kruskal–Wallis test; mean difference 53.10, p = 0.0246) (Fig. 2d).

Of the S1P-treated pwMS, only patients treated with ns-S1P displayed significantly lower titres in comparison to untreated pwMS and HC vaccinated with mRNA vaccines (Fig. 3a) (Kruskal–Wallis test; mean difference: –39.54 and –29.87, p = 0.001 and p = 0.0056,

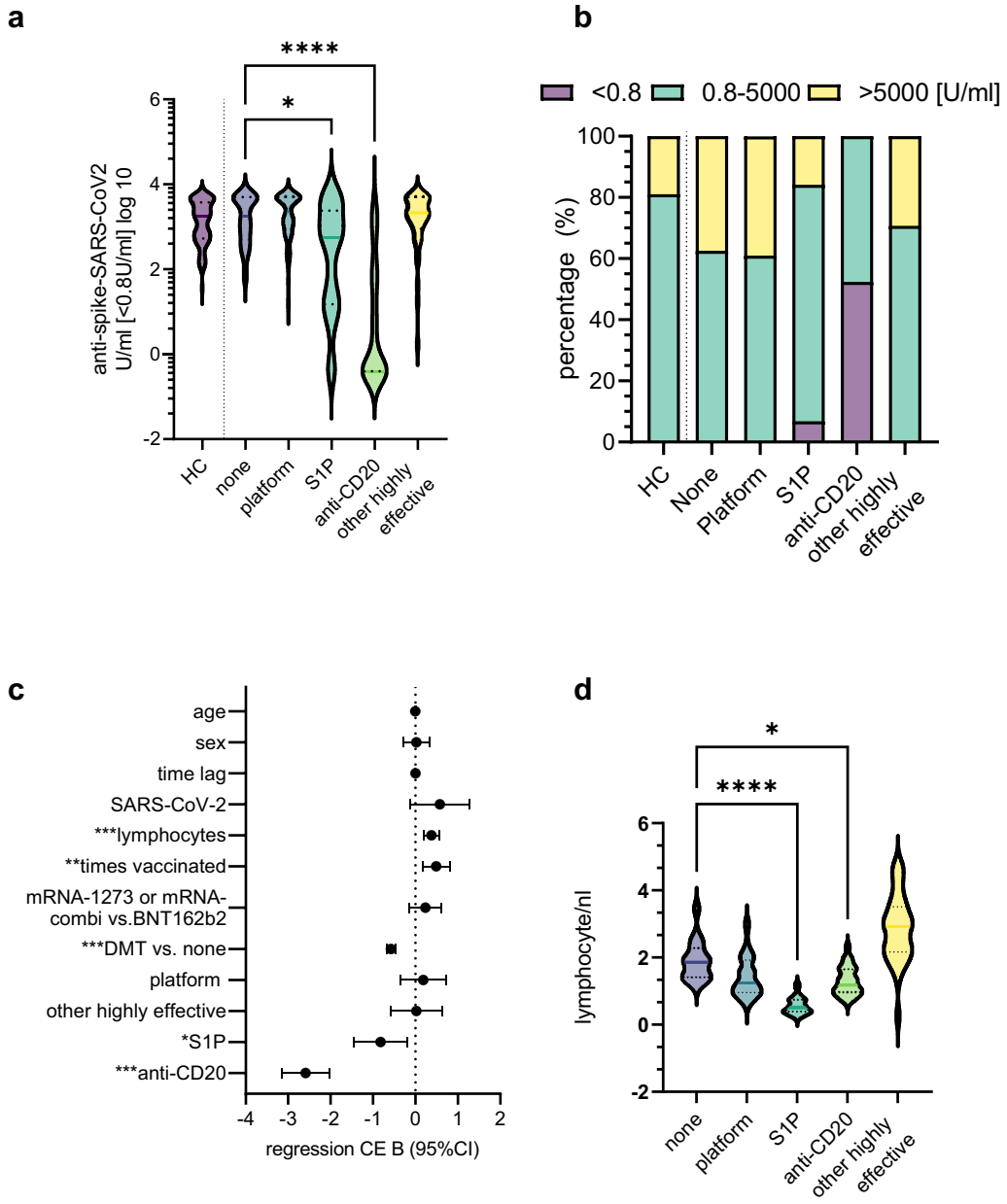

***Fig. 2:* Anti-spike SARS-CoV-2 vaccination titres in pwMS are influenced by DMT, lymphocytes and times vaccinated.** (a) Antibody levels (log10) are especially decreased in patients treated with S1P and anti-CD20 compared to untreated patients (none). Whole cohort, n = 386 (HC n = 101, pwMS n = 285, respective DMT type counts provided in Fig. 1) (Kruskal–Wallis test: overall comparison $p < 0.0001$; corrected by multiple comparison). (b) Distribution of titre divided into non-responder (<0.8 U/ml, purple), responder (0.8–5000 U/ml, light green) and high responder (>5000 U/ml, yellow) in relation to DMT. Non-responders are present in subgroups treated with S1P or anti-CD20. Whole cohort (chi^2 test: $p < 0.001$, n = 285). (c) After correction, titre (log10) was still significantly influenced by lymphocyte count, number of vaccinations, and DMT (primarily S1P and anti-CD20). Forest plot displaying regression coefficients (CE) B (dot) with 95% confidence interval (95% CI, whiskers) of linear regression model performed on pwMS vaccinated with mRNA vaccines and anti-spike-SARS-CoV-2 as dependent variable, n = 199. (d) Lymphocytes (counts per nl) are significantly decreased by treatment with S1P or anti-CD20 (Kruskal–Wallis test: overall $p < 0.00001$, corrected by multiple comparison, n = 241). *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

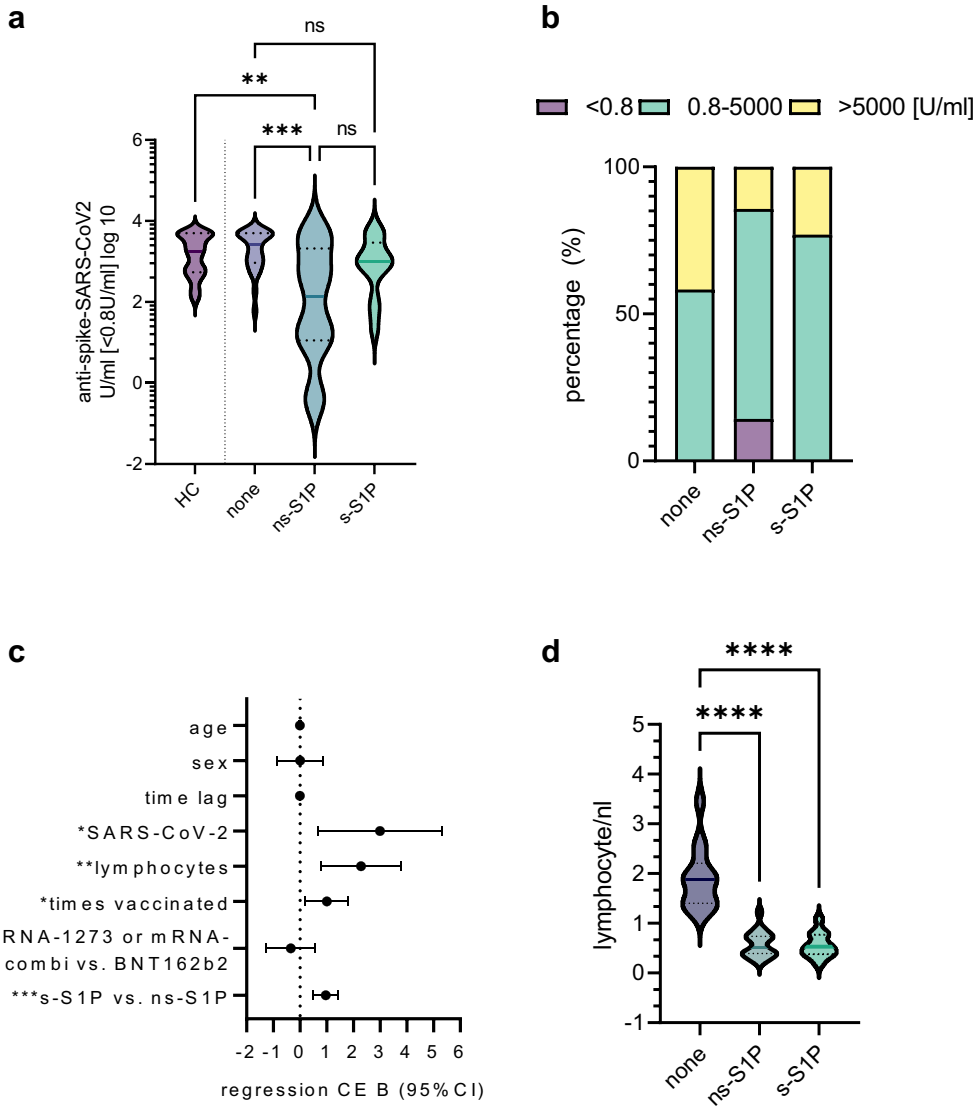

***Fig. 3:* Effect of selective and non-selective S1P modulation on vaccination response.** (a) Anti-spike SARS-CoV-2 (log10) vaccination response was significantly reduced in pwMS treated with ns-S1P compared to HC and untreated pwMS (none). Kruskal–Wallis test: p = 0.0012 (for overall difference), corrected by multiple comparison, HC: n = 64, pwMS: n = 60 (none n = 26, S1P n = 34). (b) Percentages of non-responders (titre <0.8 U/ml, purple), responders (0.8–5000 U/ml, light green) and high responders (>5000 U/ml, yellow), in patients without treatment (none) and after treatment with ns-S1P or s-S1P. Non-responders are exclusively present in MS patients treated with ns-S1P, chi^2 test: p = 0.049. (c) Anti-spike-SARS-CoV-2 levels (log10) are increased in MS patients after treatment with s-S1P compared to ns-S1P. Previous SARS-CoV-2 infection, higher absolute lymphocytes and number of vaccine dosages are all associated with higher titres. Regression coefficient B (dot) with 95% confidence interval (95% CI, whisker) from linear regression model in S1P-treated patients. Values displayed are from the mRNA vaccines cohort only, n = 33. (d) Lymphocytes (counts per nl) were significantly decreased after treatment with both ns-S1P and s-S1P. Kruskal–Wallis test: p < 0.0001, corrected by multiple comparison, n = 46 (none n = 13, ns-S1P n = 20, s-S1P n = 13). *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

respectively). Under s-S1P, antibody levels were not significantly lower; there were no non-responders in this subgroup (Fig. 3b, chi^2 p = 0.049). This was confirmed in the linear regression model (Fig. 3c) with selective in reference to non-selective S1P having a significant positive effect on vaccination titre (B = 0.968, 95% CI [0.509–1.425], p < 0.001; Supplementary Table S3). However, in comparison to untreated pwMS, lymphocyte counts were similarly reduced in s-S1P (mean difference: 22.81) and ns-S1P (mean difference: 22.95, both p < 0.0001, both Kruskal–Wallis test) (Fig. 3d). In addition, previous SARS-CoV-2 infection (B = 2.994,

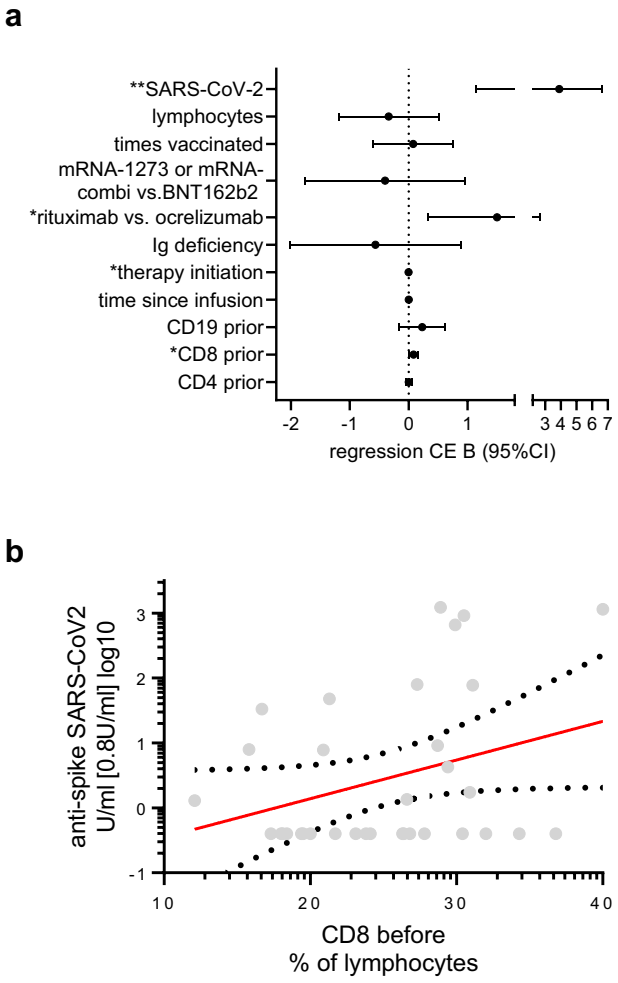

***Fig. 4:* Anti-spike SARS-CoV-2 titres in pwMS are influenced by absolute CD8 count prior vaccination.** (a) Display of regression coefficient B (dot) with 95% CI (whisker) of linear regression model in the mRNA vaccines cohort. Mean time since infusion was 154 days (95% CI [77, 231]). The same linear regression model was performed with CD19, CD4 and CD8 values at time of titre determination (n = 42) and prior vaccination (n = 32). (b) Correlation of percentage of CD8 cells prior to vaccination with antibody titre (log10). Linear fit (red, $r^2 = 0.15$) to individual values from mRNA vaccines cohort (n = 38) (grey) is shown with 95% CI (dotted black); Spearman correlation: r = 0.33, p = 0.043. *p < 0.05, **p < 0.01.

95% CI [0.669–5.320], p = 0.014), lymphocyte count (B = 2.283, 95% CI [0.778–3.788], p = 0.005) and number of vaccinations (B = 1.0, 95% CI [0.195–1.805], p = 0.017) independently influenced vaccination titre (Fig. 3c, Supplementary Table S5). In an additional sensitivity analysis, including duration of therapy had no impact on vaccination titre (Supplementary Table S9).

Within the anti-CD20 subgroup, CD8 levels at time of sampling (B = 0.082, 95% CI [0.025–0.138], p = 0.006; Supplementary Table S4) as well as prior vaccination (B = 0.084, 95% CI [0.012–0.156], p = 0.024; Supplementary Table S5) positively influenced (Fig. 4a) and positively correlated with antibody levels (Fig. 4b) (Spearman correlation r = 0.33, p = 0.043). Previous SARS-CoV-2 infection (B = 3.899, 95% CI [1.144–6.655], p = 0.007), time since therapy initiation (B = –0.001, 95% CI [–0.002, 0.000], p = 0.03) and treatment with rituximab (B = 1.5, 95% CI [0.332–2.668], p = 0.013) were all associated with increased anti-spike SARS-CoV-2 levels (Fig. 4a, Supplementary Table S4). Mean time since last infusion was 154 days (95% CI [77–231 days]).

After two vaccinations, antibody levels were significantly higher upon receiving mRNA-1273 twice in comparison to receiving BNT162b2 twice (Kruskal–Wallis test; mean difference: 41.15, p = 0.0005), receiving vector vaccines twice (Kruskal–Wallis test; mean difference: 70.17, p = 0.0003), or receiving a combination of vector and mRNA vaccines (Kruskal–Wallis test; mean difference 48.06, p = 0.0058)

(Supplementary Fig. S3a). This effect diminished with the third vaccination (Supplementary Fig. S3b).

Discussion

Functional sphingosine 1-phosphate receptor inhibition leads to a retention of lymphocytes in lymphoid tissue and has clinical benefit in pwMS and other autoimmune diseases such as inflammatory bowel disease. A number of different agents are currently available: fingolimod was the first widely used S1P inhibitor for active relapsing-remitting multiple sclerosis, while ozanimod and ponesimod are new generation S1P inhibitors for this indication. Siponimod, in contrast, is used in later phases of the disease for patients with active secondary progressive multiple sclerosis. The main differences in these substances besides receptor subtype selectivity are CNS penetration and half-time of lymphocyte recurrence. Interestingly, this work reports that the selectivity of S1P influences vaccination response. People treated with ns-S1P, functionally antagonising sphingosine-1-phosphate-receptor 1, 3, 4 and 5, presented a lower antibody level. In contrast, vaccination response in people receiving new generation S1P, selectively acting on S1P receptors 1 and 5, was comparable to those untreated. We observed a dependency of vaccination response on lymphocyte count in the complete cohort of pwMS including the subgroup treated with ns-S1P, as previously suggested,[3] but the vaccination response under s-S1P was preserved despite lymphocyte counts comparable to ns-S1P. A recent meta-analysis confirming the risk of impaired serological response upon SARS-CoV-2 vaccination in S1P- (mainly fingolimod) and anti-CD20-treated pwMS did not analyse ns-S1P separately.[2] In our real-world cohort, we did not find support for the previously suggested negative correlation between humoral response after SARS-CoV-2 vaccination and duration of S1P treatment.[2,11,12]

S1P receptor 1, which is influenced by both ns-S1P and s-S1P, is mainly responsible for lymphocyte maturation and egression from the lymphoid tissue, thereby inducing the positive effect on multiple sclerosis activity.[13–15] S1P receptors 3 and 4, which are not influenced by s-S1P, are reported to be expressed by B cells, whereas S1P receptor 4 is expressed on T cells and dendritic cells as well. S1P receptor 3 was shown to regulate migration and endocytosis of mature dendritic cells in mice.[16] This influence on antigen presentation and thereby activation of the innate immune system might explain the preserved vaccination response in pwMS treated with s-S1P modulators. In addition, the large difference in half-life from 6 to 9 days for ns-S1P to 19–30 h for s-S1P could explain observed effects.

Previous studies suggested that B cell depletion increases the risk of both a weaker vaccination response and hospitalization due to SARS-CoV-2 infection.[3,17] Here, we found that CD8 levels before vaccination improved response upon SARS-CoV2 vaccination. Fast functional vaccine-specific CD8 T cell response has been shown even before neutralizing antibodies or CD4 T cell response and early CD8 T cell response in people infected with SARS-CoV-2 was revealed to be protective against severe disease courses.[18] Another study identified an important role in immune response by a specific CD8 T cell subtype (expressing surface marker CD8, CD38, and inducible T cell co-stimulator [ICOS]), that was associated with plasmablasts and SARS-CoV-2 specific immunoglobulin G formation.[19] Even though vaccination-specific CD4 and CD8 T cell induction in B cell depletion has been reported, lower levels were detected when vaccine-specific antibodies were lacking.[4,20–23] In contrast to this, two other studies report greater abundance of SARS-CoV-2 spike-specific and highly activated CD8 T cells in those B cell-depleted patients who were lacking spike antibodies.[4,11] These observations underline the complexity of the interconnection between B cell response and CD8 activation. The influence of B cells on CD8 T cells was reported to be dependent on interleukin (IL)-27 release by B cells.[21,24]

Although a clear role of CD4 T cells in promoting antibody response is known, and the above demonstrates an important role of CD8 T cells in protection against SARS-CoV-2 infection, the role of CD8 T cells in antibody development is still unclear. An influence of CD8 T cells in B cell differentiation and antibody class-switch by localising in the B cell follicle and expressing B cell co-stimulatory proteins (germinal centre localising chemokine receptor CXCR5, principal T follicular helper transcription factors Bcl6, T follicular helper effector cytokine IL-21) in infection and autoimmune disease has been proposed.[25–28] This might suggest that the role of CD8 T cells in class switching and maturation might also promote vaccination response, especially when B cell function and count is reduced in a B cell-depleted state. All of the above supports the relevance of CD8 monitoring in risk assessment for SARS-CoV-2 in pwMS receiving anti-CD20 treatment.

Time since initiation of anti-CD20 therapy negatively influenced antibody levels as suggested before.[3] Rituximab showed a positive effect on vaccination titre in comparison to ocrelizumab, suggesting an impact of infusion interval as the latter is typically administered every 6 months instead of in response to rising B cell levels. Time since last infusion was notably shorter, and reported percentage of B cells lower within our cohort compared to previous work that suggested a relationship between antibody level and infusion interval.[3,4] According to an accepted best clinical practice, patients within our cohort receive ocrelizumab in a standard interval (every 6 months), or ocrelizumab or rituximab in a B cell-dependent scheme. For the latter, infusion is scheduled when the proportion of CD19 B cells reaches 1% of lymphocytes.[29]

There are some limitations to our study. Although association of breakthrough infections with low vaccination titres has been demonstrated in SARS-CoV-2 vaccinated people, correlation of protection is still not established, which limits vaccination titre interpretation. Vaccination-specific T cell response was not measured in our real-world cohort. Due to the real-world study design, vaccination timelines were not aligned, which we addressed through correction by the regression model. Our centre is one of the leading providers and very active in treating multiple sclerosis in Germany, thereby attracting a large and mixed cohort of pwMS. Thus, the number of people with low activity multiple sclerosis or with clinically isolated syndrome receiving basic therapies such as interferon or glatiramer acetate might be underrepresented in our cohort. Moreover, even though we corrected for previous infection status and vaccination type by performing a regression model, the sample size is relatively small within each DMT subgroup, especially with only few having prior infection. PwMS were very careful and reduced contacts during the past two to three years.

Altogether, in this cohort, vaccination response in pwMS depends on DMT, lymphocyte level and times vaccinated. Decreased vaccination response in pwMS should be expected upon treatment with non-selective S1P but not selective S1P. Patients treated with anti-CD20 medication and exhibiting low CD8 counts need closest monitoring of vaccination response and individual adaption of treatment and vaccination plans.

Contributors

Muriel Schraad performed the literature research, data collection, data analysis and interpretation, writing of the first draft and figure design. Timo Uphaus verified the underlying data and performed data analysis and interpretation, writing and figure design. Stefan Runkel and Walter Hitzler performed vaccination titre assays. Stefan Bittner supervised data collection and verified the underlying data and data interpretation. Frauke Zipp developed the research idea and study concept, evaluated the data and edited the manuscript. All authors read and approved the final version of the manuscript.

Data sharing statement

The data that support the findings of this study will be available upon reasonable request to the corresponding author of the study.

Declaration of interests

The authors declare no relevant conflicts of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ebiom.2022.104411.

References

1 Achiron A, Dolev M, Menascu S, et al. COVID-19 vaccination in patients with multiple sclerosis: what we have learnt by February 2021. *Mult Scler*. 2021;27(6):864–870.
2 Wu X, Wang L, Shen L, Tang K. Response of COVID-19 vaccination in multiple sclerosis patients following disease-modifying therapies: a meta-analysis. *eBioMedicine*. 2022;81:104102.
3 Sormani MP, Inglese M, Schiavetti I, et al. Effect of SARS-CoV-2 mRNA vaccination in MS patients treated with disease modifying therapies. *eBioMedicine*. 2021;72:103581.
4 Apostolidis SA, Kakara M, Painter MM, et al. Cellular and humoral immune responses following SARS-CoV-2 mRNA vaccination in patients with multiple sclerosis on anti-CD20 therapy. *Nat Med*. 2021;27(11):1990–2001.
5 Achiron A, Mandel M, Dreyer-Alster S, et al. Humoral immune response to COVID-19 mRNA vaccine in patients with multiple sclerosis treated with high-efficacy disease-modifying therapies. *Ther Adv Neurol Disord*. 2021;14:17562864211012835.
6 Tortorella C, Aiello A, Gasperini C, et al. Humoral- and T-cell-specific immune responses to SARS-CoV-2 mRNA vaccination in patients with MS using different disease-modifying therapies. *Neurology*. 2022;98(5):e541–e554.
7 Sormani MP, Schiavetti I, Inglese M, et al. 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. *eBioMedicine*. 2022;80:104042.
8 Schiavetti I, Cordioli C, Stromillo ML, et al. Breakthrough SARS-CoV-2 infections in MS patients on disease-modifying therapies. *Mult Scler*. 2022;28(13):2106–2111.
9 Resman Rus K, Korva M, Knap N, Avšič Županc T, Poljak M. Performance of the rapid high-throughput automated electrochemiluminescence immunoassay targeting total antibodies to the SARS-CoV-2 spike protein receptor binding domain in comparison to the neutralization assay. *J Clin Virol*. 2021;139:104820.
10 National S-C-SAEG. Performance characteristics of five immunoassays for SARS-CoV-2: a head-to-head benchmark comparison. *Lancet Infect Dis*. 2020;20(12):1390–1400.
11 Sabatino Jr JJ, Mittl K, Rowles WM, et al. Multiple sclerosis therapies differentially affect SARS-CoV-2 vaccine-induced antibody and T cell immunity and function. *JCI Insight*. 2022;7(4):e156978.
12 Holroyd KB, Healy BC, Conway S, et al. Humoral response to COVID-19 vaccination in MS patients on disease modifying therapy: immune profiles and clinical outcomes. *Mult Scler Relat Disord*. 2022;67:104079.
13 Liu G, Burns S, Huang G, et al. The receptor S1P1 overrides regulatory T cell-mediated immune suppression through Akt-mTOR. *Nat Immunol*. 2009;10(7):769–777.
14 Muls N, Dang HA, Sindic CJ, van Pesch V. Fingolimod increases CD39-expressing regulatory T cells in multiple sclerosis patients. *PLoS One*. 2014;9(11):e113025.
15 Mandala S, Hajdu R, Bergstrom J, et al. Alteration of lymphocyte trafficking by sphingosine-1-phosphate receptor agonists. *Science*. 2002;296(5566):346–349.
16 Maeda Y, Matsuyuki H, Shimano K, Kataoka H, Sugahara K, Chiba K. Migration of CD4 T cells and dendritic cells toward sphingosine 1-phosphate (S1P) is mediated by different receptor subtypes: S1P regulates the functions of murine mature dendritic cells via S1P receptor type 3. *J Immunol*. 2007;178(6):3437–3446.
17 Simpson-Yap S, De Brouwer E, Kalincik T, et al. Associations of disease-modifying therapies with COVID-19 severity in multiple sclerosis. *Neurology*. 2021;97(19):e1870–e1885.
18 Tan AT, Linster M, Tan CW, et al. Early induction of functional SARS-CoV-2-specific T cells associates with rapid viral clearance and mild disease in COVID-19 patients. *Cell Rep*. 2021;34(6):108728.
19 Kramer KJ, Wilfong EM, Voss K, et al. Single-cell profiling of the antigen-specific response to BNT162b2 SARS-CoV-2 RNA vaccine. *Nat Commun*. 2022;13(1):3466.
20 Stefanski AL, Rincon-Arevalo H, Schrezenmeier E, et al. B cell numbers predict humoral and cellular response upon SARS-CoV-2 vaccination among patients treated with rituximab. *Arthritis Rheumatol*. 2022;74(6):934–947.
21 Graalmann T, Borst K, Manchanda H, et al. B cell depletion impairs vaccination-induced CD8(+) T cell responses in a type I interferon-dependent manner. *Ann Rheum Dis*. 2021;80(12):1537–1544.

22 Klarquist J, Cross EW, Thompson SB, et al. B cells promote CD8 T cell primary and memory responses to subunit vaccines. *Cell Rep*. 2021;36(8):109591.
23 Madelon N, Lauper K, Breville G, et al. Robust T-cell responses in anti-CD20-treated patients following COVID-19 vaccination: a prospective cohort study. *Clin Infect Dis*. 2022;75(1):e1037–e1045.
24 Klarquist J, Chitrakar A, Pennock ND, et al. Clonal expansion of vaccine-elicited T cells is independent of aerobic glycolysis. *Sci Immunol*. 2018;3(27).
25 Valentine KM, Davini D, Lawrence TJ, et al. CD8 follicular T cells promote B cell antibody class switch in autoimmune disease. *J Immunol*. 2018;201(1):31–40.
26 Wagner UG, Kurtin PJ, Wahner A, et al. The role of CD8+ CD40L+ T cells in the formation of germinal centers in rheumatoid synovitis. *J Immunol*. 1998;161(11):6390–6397.
27 He R, Hou S, Liu C, et al. Follicular CXCR5- expressing CD8(+) T cells curtail chronic viral infection. *Nature*. 2016;537(7620):412–428.
28 Kang YM, Zhang X, Wagner UG, et al. CD8 T cells are required for the formation of ectopic germinal centers in rheumatoid synovitis. *J Exp Med*. 2002;195(10):1325–1336.
29 Rolfes L, Pawlitzki M, Pfeuffer S, et al. Ocrelizumab extended interval dosing in multiple sclerosis in times of COVID-19. *Neurol Neuroimmunol Neuroinflamm*. 2021;8(5):e1035.