Review

Multiple sclerosis disease-modifying therapies and COVID-19 vaccines: a practical review and meta-analysis

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ABSTRACT

Studies among people with multiple sclerosis (pwMS) receiving disease-modifying therapies (DMTs) have provided adequate evidence for an appraisal of COVID-19 vaccination policies among them. To synthesise the available evidence addressing the effect of MS DMTs on COVID-19 vaccines' immunogenicity and effectiveness, following the Cochrane guidelines, we systematically reviewed all observational studies available in MEDLINE, Scopus, Web of Science, MedRxiv and Google Scholar from January 2021 to January 2022 and extracted their relevant data. Immunogenicity data were then synthesised in a quantitative, and other data in a qualitative manner. Evidence from 28 studies suggests extensively lower B-cell responses in sphingosine-1-phosphate receptor modulator (S1PRM) treated and anti-CD20 (aCD20) treated, and lower T-cell responses in interferon-treated, S1PRM-treated and cladribine-treated pwMS—although most T cell evidence currently comprises of low or very low certainty. With every 10-week increase in aCD20-to-vaccine period, a 1.94-fold (95% CI 1.57 to 2.41, p<0.00001) increase in the odds of seroconversion was observed. Furthermore, the evidence points out that B-cell-depleting therapies may accelerate postvaccination humoral waning, and boosters' immunogenicity is predictable with the same factors affecting the initial vaccination cycle. Four real-world studies further indicate that the comparative incidence/severity of breakthrough COVID-19 has been higher among the pwMS treated with S1PRM and aCD20—unlike the ones treated with other DMTs. S1PRM and aCD20 therapies were the only DMTs reducing the real-world effectiveness of COVID-19 vaccination among pwMS. Hence, it could be concluded that optimisation of humoral immunogenicity and ensuring its durability are the necessities of an effective COVID-19 vaccination policy among pwMS who receive DMTs.

INTRODUCTION

Global mass vaccination has been the most prominent effort of humanity to end the COVID-19 pandemic. Currently, many vaccines have been developed, all with reasonable safety and efficacy profiles (more information available at: https://COVID-19.trackvaccines.org/agency/who).

Among the people with multiple sclerosis (pwMS) who receive disease-modifying therapies (DMTs), the effectiveness of COVID-19 vaccination was thought to be affected—based on previous knowledge of the DMTs' immunomodulatory mechanisms of action and preliminary real-world evidence. Based on the same evidence, expert panels issued adjusted vaccination guidelines for pwMS, which mostly concerned people on sphingosine 1-phosphate receptor modulators (S1PRM), anti-CD20 therapies (aCD20), and other B-cell depleting therapies (BCDT).[1–4]

Later on, administration of booster doses of COVID-19 vaccines was recommended due to observation of waning humoral immunity[5] and clinical effectiveness[6–8] over time. Among the pwMS, homologous booster doses were also used to immunise the ones who did not seroconvert following their first vaccination cycle.

Now, as several studies around the globe addressed the mentioned issues, the available real-world data is adequate for an appraisal of the initial vaccination policies of pwMS, and valuable for development of more effective ones in the future. Hence, in this systematic review and meta-analysis study, we aimed to gather and synthesise the available data and investigate the effect of DMTs on B-cell and T-cell immunogenicity and effectiveness of COVID-19 vaccines among pwMS, ultimately providing an evidence base for future research, policy making and management of pwMS receiving DMTs.

METHODS

In accordance with the Cochrane guidelines, we systematically reviewed and meta-analysed the available observational studies with the following formulation of objectives:

- Population: pwMS.
- Exposure: DMTs.
- Comparison: Being unexposed to DMTs (UX)
- Primary outcomes:
  - Vaccine-induced B-cell and T-cell responses.
  - Vaccine effectiveness.

We hereby reported and discussed the mentioned study's results in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement (available from: http://www.prisma-statement.org). Due to restrictions in word

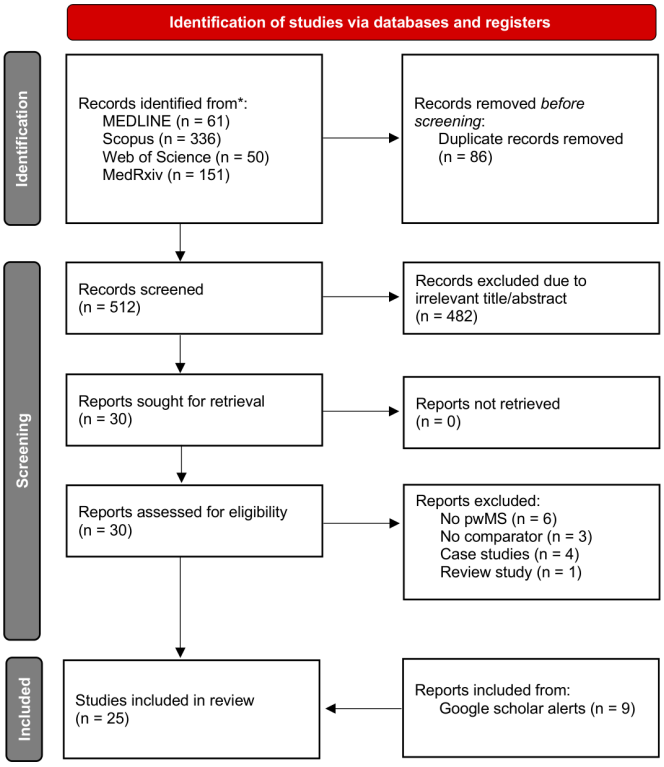

Figure 1 PRISMA flow diagram. PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses; pwMS, people with multiple sclerosis.

count, please find the further details of the methods from online supplemental material.

RESULTS AND DISCUSSION

Overall, data from 28 studies (5025 pwMS and 1635 healthy controls) were synthesised (figure 1, table 1). One study[9] was excluded despite containing eligible participants because their data could not be extracted. The studies differed in outcome measurement methods, settings, number of participants and the administered vaccines. The assessed vaccines used either mRNA (BNT162b2, mRNA-1273), adenoviral vector (AV) (Ad26.COV2.S, ChAdOx1) or inactivated (CoronaVac, BBIBP-CorV) platforms. Other prominent points of heterogeneity were the used assays, the number/types of assessed DMTs, the usage of different comparators (eg, healthy participants, pwMS on no DMT), and the time points of obtaining samples from participants.

The results of individual studies, heterogeneity tests, forest and funnel plots, and the detailed rationale behind each quality assessment—based on National Institutes of Health tools—is accessible from online supplemental file 2. The pooled measures are summarised in both table 2 and figure 2. The certainty of evidence, assessed using the Grades of Recommendations, Assessment, Development and Evaluation approach, is presented in the following paragraphs, and along its explicit rationale in table 2.

Effect of DMTs on COVID-19 vaccines' immunogenicity

Interferons

Based on evidence with moderate certainty, interferons (IFNs) do not decrease the odds of seroconversion (OR (95% CI): 0.84 (0.38 to 1.83), p=0.66) (online supplemental figure 1). All pwMS on IFN in four studies[10–13] seroconverted; they showed similar postvaccination antibody concentrations with the UX people, except in one study, in which they showed significantly higher concentrations of anti-Spike (S) receptor binding domain IgG compared with healthy controls.[13] The authors suggested that IFN-beta 1 a therapy may promote the postvaccination antibody responses in pwMS, however, this finding was not observed in other studies.

One study[14] pointed that the extent of interferon-gamma release responses to the S antigen is lower in samples from IFN-treated pwMS compared with healthy controls (OR (95% CI): 0.02 (0.00 to 0.28), p<0.01), suggesting with very-low certainty that IFNs blunt the vaccine's T-cell immunogenicity. The investigators attributed the blunt to both CD4 +and CD8+subpopulations based on a flow cytometric analysis.[14] Further replication is warranted to add to the certainty of this finding.

Glatiramer acetate

Evidence suggests with moderate certainty that glatiramer acetate (GA) does not affect the odds of post-vaccination seroconversion (OR (95% CI): 0.87 (0.31 to 2.42), p=0.79) (online supplemental figure 2). GA-treated pwMS who remained seronegative after vaccination were only present in two studies[15 16] and all received inactivated vaccination; however, their postvaccination antibody concentrations were similar with UX people in those studies.

Activation-induced marker (AIM) assay in one study with limited sample size[17] (very low-certainty evidence) suggested that GA affects vaccine immunogenicity neither in CD4+ (OR not measurable) nor CD8+ (OR (95% CI): 0.62 (0.04 to 9.00), p=0.72) subpopulations of T-cells. Furthermore, it is worth mentioning that adequate interferon-gamma release responses were present after SARS-CoV-2 infection in GA-treated people,[18] suggesting favourable T-cell responses.

Dimethyl fumarate

Evidence with moderate certainty did not confirm any effect of dimethyl fumarate (DMF) on odds of postvaccination seroconversion (OR (95% CI): 1.98 (0.96 o 4.09), p=0.07) (online supplemental figure 3). All of the pwMS on DMF in seven studies[10 12 13 16 17 19 20] seroconverted following mRNA or inactivated vaccination; postvaccination antibody concentrations of whom were similar to UX people.

Evidence on T-cell responses among DMF-treated pwMS was limited to one study with a limited sample size[17] (very-low-certainty evidence), which by using AIM assays, did not confirm any effect of DMF on the vaccine-induced responses of CD4+ (OR not measurable) and CD8+ (OR (95% CI): 3.78 (0.18 to 78.38), p=0.39) subpopulations of T-cells. Interferon-gamma release responses were sufficient in DMF-treated pwMS after SARS-CoV-2 *infection*, suggesting adequate T-cell response.[18]

Teriflunomide

A slightly negative effect of teriflunomide (TERI) was observed on post-vaccination seroconversion odds (OR (95% CI): 0.38 (0.16 to 0.90), p=0.03) (online supplemental figure 4); this finding is composed of very low certainty due to inadequate number of studies and considerable heterogeneity. The cause of heterogeneity was suspected to lie within usage of different vaccine platforms; however, as a limited number of mRNA vaccine studies had measurable relative effects, their pooled measures resulted in a wide confidence limit, and the difference between the inactivated and mRNA measures did not reach

Table 1 Characteristics of included studies

Study (location)	Sample size (pwMS, HC)	Vaccine (platform)	B-cell assay (method, manufacturer)	T-cell assay (method, manufacturer)	Study quality*
Achiron *et al*, (Israel) [19 77 78]	503 (414, 89)	BNT162b2 (mRNA)	Serum anti-S1 IgG (ELISA, EUROIMMUN) PBMC S-induced IgG-secreting B-cells (FluoroSpot, Mabtech)	PBMC S-induced IFN-g-, IL-2-positive T-cells (FluoroSpot, Mabtech)	Good
Ali *et al*, (USA)[58]	53 (46,† 7)	BNT162b2 (mRNA) mRNA-1273 (mRNA)	Serum Anti-RBD IgG (CLIA, Siemens)	NA	Fair
Apostolidis *et al*, (USA)[39]	30 (20, 10)	BNT162b2 (mRNA) mRNA-1273 (mRNA)	Serum anti-S IgG (ELISA, NA) Serum anti-RBD IgG (ELISA, NA) PBMC S-induced IgG-secreting B-cells (Cell Probe, NA)	PBMC S-induced activation marker-positive T-cells (AIM, NA)	Fair
Bigaut *et al*, (France)[9]	28 (28, 0)	BNT162b2 (mRNA) mRNA-1273 (mRNA)	Serum anti-RBD IgG (CMIA, Abbot; ECLIA, Roche)	NA	Fair
Brill *et al*, (Israel)[40]	112 (72, 40)	BNT162b2 (mRNA)	Serum anti-S IgG (CLIA, DiaSorin) Serum anti-RBD IgG (CMIA, Abbot)	PBMC S/N-induced IFN-g-positive T-cells (ELISpot, Oxford Immunotec)	Fair
Capone *et al*, (Italy)[10]	140 (140, 0)	BNT162b2 (mRNA)	Serum anti-RBD IgG (CMIA, Abbot)	NA	Good
Capuano *et al*, (Italy)[30]	57 (26, 31)	BNT162b2 (mRNA)	Serum anti-S IgG (CLIA, DiaSorin)	NA	Good
Disanto *et al*, (Switzerland)[21]	116 (116, 0)	BNT162b2 (mRNA) mRNA-1273 (mRNA)	Serum anti-RBD IgG (CMIA, Abbot)	NA	Good
Etemadifar *et al*, (Iran)[15]	358 (144, 214)	BBIBP-CorV (Inactivated)	Serum anti-S IgG (ELISA, Pishtazteb)	NA	Good
Gadani *et al*, (USA)[11]	101 (101, 0)	BNT162b2 (mRNA) mRNA-1273 (mRNA) Ad26.COV2.S (AV)	Serum anti-S1 IgG (ELISA, EUROIMMUN)	PBMC S-induced IFN-g-positive T-cells (FluoroSpot, Mabtech)	Fair
Gallo *et al*, (Italy)[79]	59 (4, 55)	BNT162b2 (mRNA)	Serum anti-S IgG (CLIA, DiaSorin)	NA	Fair
Giossi *et al*, (Italy)[12]	312 (39, 273)	BNT162b2 (mRNA)	Serum anti-RBD IgG (CMIA, Abbot)	NA	Fair
Katz *et al*, (USA)[43]	48 (48, 0)	BNT162b2 (mRNA) mRNA-1273 (mRNA) Ad26.COV2.S (AV)	Serum anti-RBD IgG (ECLIA, Roche)	Rearranged TCR gene sequences (M-PCR, Adaptive Biotechnologies)	Fair
König *et al*, (Norway)[23]	1155 (528, 627)	BNT162b2 (mRNA) mRNA-1273 (mRNA) ChAdOx1 (AV)	PBMC S-induced IgG-secreting B-cells (BBFCA, NA) PBMC RBD-induced IgG-secreting B-cells (BBFCA, NA)	NA	Fair
Madelon *et al*, (Switzerland)[41]	48 (26, 22)	BNT162b2 (mRNA) mRNA-1273 (mRNA)	Serum anti-RBD IgG (ECLIA, Roche)	PBMC S-induced activation marker-positive T-cells (AIM, NA)	Fair
Maniscalco *et al*, (Italy)[13]	149 (125, 24)	BNT162b2 (mRNA)	Serum anti-RBD IgG (ECLIA, Roche)	NA	Good
Ozakbas *et al*, (Turkey)[16]	591 (547, 44)	BNT162b2 (mRNA) CoronaVac (Inactivated)	Serum anti-RBD IgG (CMIA, Abbot)	NA	Good
Pitzalis *et al*, (Italy)[22]	975 (912, 63)	BNT162b2 (mRNA)	Serum anti-RBD IgG (ECLIA, Roche)	NA	Fair
Pompsch *et al*, (Germany)[42]	30 (10, 20)	BNT162b2 (mRNA)	Serum anti-S1 IgG (ELISA, EUROIMMUN)	S/N/M-induced IFN-g-positive T-cells (ELISpot, Miltenyi Biotec)	Fair
Sabatino *et al*, (USA)[17]	80 (67, 13)	BNT162b2 (mRNA) mRNA-1273 (mRNA) Ad26.COV2.S (AV)	PBMC S-induced IgG-secreting B-cells (BBFCA, NA) PBMC RBD-induced IgG-secreting B-cells (BBFCA, NA)	PBMC S-induced activated T-cells (AIM; ICS; pMHC, NA)	Fair
Sormani *et al*, (Italy)[20 57]	780 (780, 0)	BNT162b2 (mRNA) mRNA-1273 (mRNA)	Serum anti-RBD IgG (ECLIA, Roche)	NA	Good
Tallantyre *et al*, (UK)[24]	473 (473, 0)	BNT162b2 (mRNA) ChAdOx1 (AV) Ad26.COV2.S (AV)	Serum anti-RBD IgG (ELISA, Kantaro Biosciences; GloBody, NA)	PBMC S/N/M-induced IFN-g-positive T-cells (ELISpot, ImmunoServ Ltd.)	Fair
Tortorella *et al*, (Italy)[14]	186 (108, 78)	BNT162b2 (mRNA) mRNA-1273 (mRNA)	Serum anti-RBD IgG (NR) Neutralising antibodies (MNA, NA)	Whole blood S-induced IFN-g (ELISA, ProteinSimple)	Fair
Türkoğlu *et al*, (Turkey)[25]	59 (34, 25)	CoronaVac (Inactivated)	Serum anti-S1 IgG (ELISA, EUROIMMUN)	NA	Fair
Van Kempen *et al*, (Netherlands)[80]	87 (87, 0)	mRNA-1273 (mRNA)	Serum anti-RBD IgG (ELISA, NR)	NA	Fair
Studies with no comparators					
Guerrieri *et al*, (Italy)[81]	32 (32, 0)	BNT162b2 (mRNA) mRNA-1273 (mRNA)	Various assays	NA	NA
Novak *et al*, (Denmark, USA)[82]	60 (60, 0)	mRNA vaccines	Serum anti-RBD IgG (CMIA, Abbot)	NA	NA
Grothe *et al*, (Germany)[35]	38 (38, 0)	BNT162b2 (mRNA) mRNA-1273 (mRNA) ChAdOx1 (AV)	Serum anti-S IgG (CLIA, DiaSorin)	NA	NA

*Assessed with National Institutes of Health quality assessment tools (The rationale of judgements are presented in an online supplemental file 2; The used criteria are available at: https://www.nhlbi.nih.gov/health-topics/study-quality-assessment-tools). Good means least risk of bias; Fair, low risk of bias and Poor, moderate/high risk of bias.
†Including two participants with neuromyelitis optica and two with optic neuritis.
AIM, activation-induced marker; AV, adenoviral vector; BBFCA, bead-based flowcytometry assay; CLIA, chemiluminescent immunoassay; CMIA, chemiluminescence microparticle immunoassay; ECLIA, electrochemiluminescence immunoassay; HC, healthy controls; ICS, intracellular cytokine staining; IFN-g, interferon-gamma; IL, interleukin; M, membrane protein; MNA, microneutralisation assay; M-PCR, multiplex PCR; mRNA, messenger RNA; N, nucleocapsid protein; NA, not applicable; NR, not reported; PBMC, peripheral blood mononuclear cells; pMHC, peptide major histocompatibility complex; pwMS, people with multiple sclerosis; RBD, receptor-binding domain; S, spike protein.

statistical significance (χ^2=2.91, p=0.09). Nevertheless, considering that all of the TERI-treated pwMS seroconverted after mRNA vaccination,[10 12 16 19–22] the suggested blunt may not be generalisable to the mRNA-vaccinated pwMS receiving TERI. Furthermore, in comparison to UX people, TERI-treated pwMS had lower postvaccine antibody concentrations—regardless of the vaccine platform; this difference reached statistical significance only in one study.[22] TERI's mechanism of action—which

Table 2 Summary of findings

	Post-vac outcomes							
	B-cell response		T-cell response					
	Seroconversion		IFN-g release		CD4 +AIM+		CD8 +AIM+	
Factors (ref)	OR (95% CI)	P value (certainty*)	OR (95% CI)	P value (certainty*)	OR (95% CI)	P value (certainty*)	OR (95% CI)	P value (certainty*)
DMT (UX)								
-IFN	0.84 (0.38 to 1.83)	0.66 Moderate[1,2]	**0.02 (0.00 to 0.14)**	**<0.01 very low[2,4,5]**	Ni		Ni	
-GA	0.87 (0.31 to 2.42)	0.79 Moderate[1,2]	Ni		NE		0.62 (0.04 to 9.00)	0.72 very low[1,2,4,5]
-DMF	1.98 (0.96 to 4.09)	0.07 Moderate[1,2]	Ni		NE		3.78 (0.18 to 78.38)	0.39 very low[1,2,4,5]
-TERI	**0.38 (0.16 to 0.90)**	**0.03 very low[2,3]**	Ni		Ni		Ni	
-S1PRM	**0.04 (0.03 to 0.06)**	**<0.00001 High[1]**	**0.04 (0.02 to 0.07)**	**<0.00001 Low[1,2,3]**	**0.01 (0.00 to 0.18)**	**0.001 very low[2,4,5]**	0.95 (0.08 to 10.71)	0.97 very low[1,2,4,5]
-NTZ	0.53 (0.24 to 1.18)	0.12 Low[1,2,4]	1.00 (0.20 to 5.12)	1.00 very low[1,2,4,5]	NE		3.95 (0.23 to 69.44)	0.35 very low[1,2,4,5]
-CLAD	0.41 (0.15 to 1.11)	0.08 Low[1,2,5]	**0.01 (0.00 to 0.04)**	**<0.00001 Low[1,2,5]**	Ni		Ni	
-ALEM	**0.32 (0.10 to 0.96)**	**0.04 Very Low[2,3]**	Ni		Ni		Ni	
-aCD20	**0.05 (0.04 to 0.06)**	**<0.00001 High[1]**	1.12 (0.62 to 2.05)	0.70 Moderate[1,3]	1.13 (0.17 to 7.61)	0.90 Moderate[1,2]	2.54 (0.89 to 7.27)	0.08 Moderate[1,5]
aCD20 infusion-to-vac (per 10 weeks)	**1.94 (1.57 to 2.41)**	**<0.00001 High[1]**	Ni		Ni		Ni	

Results with p<0.05 are bolded.
*Based on GRADE approach (Baseline: moderate due to observational nature of studies; ▲upgrade; ▼downgrade): 1. ▲Result in line with quantitative analyses in individual studies; 2. ▼Low count of studies with measurable relative effect; 3. ▼ Inconsistency; 4. ▼Risk of missing results and 5. ▼Imprecision.
aCD20, anti-CD20 therapies; AIM, activation-induced marker; ALEM, alemtuzumab; CLAD, cladribine; DMF, dimethyl fumarate; DMT, disease-modifying therapy; GA, glatiramer acetate; GRADE, Grades of Recommendations, Assessment, Development and Evaluation; IFN, interferons; IFN-g, interferon-gamma; NE, not estimable; Ni, no information; NTZ, natalizumab; ref, reference; S1PRM, sphingosine-1-phosphate receptor modulators; TERI, teriflunomide; UX, unexposed; vac, vaccination.

involves inhibition of rapidly dividing cells, including activated B-cells—may explain this observation.

Furthermore, no study was found addressing the vaccine-induced T-cell responses in TERI-treated pwMS; this is therefore encouraged in future studies.

Sphingosine-1-phosphate receptor modulators

S1PRM extensively decrease the odds of post-vaccine seroconversion (OR (95% CI): 0.04 (0.03 to 0.06), p<0.00001) (online supplemental figure 5), according to evidence with high certainty. In all of the included studies,[10 11 13–16 19–25] the S1PRM-treated pwMS had significantly lower concentrations of post-vaccine antibodies when compared with UX people; the effect measures were, however, heterogenous. Therefore, evidence indicates with moderate certainty that pwMS on S1PRM are 25 times (95% CI: 16.66 to 33.33) less likely to show anti-S1, and 8.33 times (95% CI: 3.70 to 20) less likely to show anti-S seroconversion following COVID-19 vaccination (χ^2=7.24, p<0.01). In contrast to the healthy population, evidence suggests with low certainty—due to limited count of inactivated vaccine studies—that inactivated-vaccinated S1PRM-treated pwMS are more likely to seroconvert compared with the mRNA-vaccinated and AV-vaccinated ones (χ^2=11.97, p<0.001). Although theoretically reasonable,[26] this has been contradicted in head-to-head

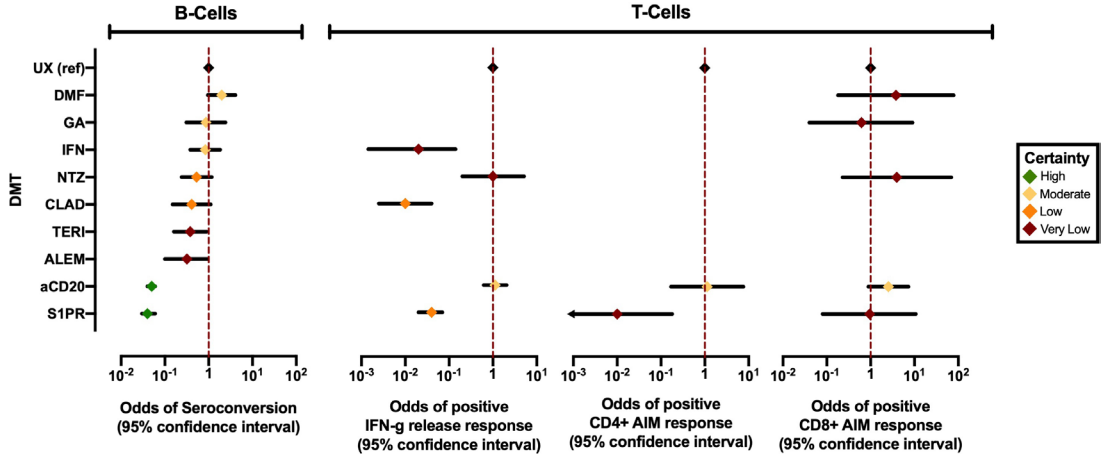

Figure 2 Summary forest plot of the pooled results. AIM, activation-induced marker; aCD20, anti-CD20 therapies; ALEM, alemtuzumab; CLAD, cladribine; DMF, dimethyl fumarate; GA, glatiramer acetate; IFN, interferons; IFN-g: interferon-gamma; NTZ, natalizumab; ref, reference; S1PR, sphingosine-1-phosphate receptor modulators; TERI, teriflunomide; UX, unexposed.

mRNA-AV[24] and mRNA-inactivated[16] immunogenicity comparisons. Hence, more replication of inactivated/mRNA/AV comparisons among S1PRM-treated pwMS is encouraged.

Furthermore, interferon-gamma release assays in two studies[14 19] suggested with low certainty that S1PRM blunt vaccine-induced T-cell responses (OR (95% CI): 0.04 (0.02 to 0.07), p<0.00001) (online supplemental figure 6). AIM assay in another study[17] confirmed this blunt in the CD4+ (OR (95% CI): 0.01 (0.00 to 0.18), p=0.001), but not in the CD8+ (OR (95% CI): 0.95 (0.08 to 10.71), p=0.97) subpopulations of T-cells.

Additionally, among the pwMS on S1PRM who failed to seroconvert following their initial vaccination cycle, one study showed that administration of booster doses increased anti-S1 antibody concentrations, but promoted seroconversion only in 2/29 (7%).[27]

S1PRM inhibit the trafficking of lymphocytes and restricts them to lymphatics; this causes peripheral lymphopenia in pwMS on S1PRM, which may explain the lower T-cell reactivity observed in their peripheral blood samples. In addition, an inhibitive effect of S1PRM on T-cell activation has previously been documented.[28] Blunted humoral responses could also be explained by the former reason (lymph-restricting effect of S1PRM); lymph flows one-way from peripheral to central areas, therefore, lymph-trapped lymphocytes in S1PRM-treated pwMS are unable to traffic to peripheral areas, and hence, are not properly exposed to the immunising materials of the vaccines. This may sound more reasonable when considering the immunising effect of systemic SARS-CoV-2 infection among S1PRM-treated pwMS.[26 29] Another hypothesis is that S1PRM interact directly with the lipid nanoparticles used in mRNA vaccines,[26] however, this hypothesis could not explain humoral blunts after other vaccine platforms.

Natalizumab

Natalizumab (NTZ) did not affect the post-vaccine seroconversion odds significantly (OR (95% CI): 0.53 (0.24 to 1.18), p=0.12) (online supplemental figure 7), suggests evidence with low certainty. All NTZ-treated pwMS in seven studies seroconverted following vaccination[10 11 17 19 20 22 30]; while being similar in others, one of the studies[22] showed a significantly lower post-vaccine antibody concentration in pwMS on NTZ compared with UX people.

Neither interferon-gamma release[11] nor AIM[17] assays suggested a blunting effect of NTZ on vaccine-induced T-cell responses (very-low-certainty evidence).

NTZ, an anti-α4-integrin monoclonal antibody, implements its effect by inhibiting lymphocyte extravasation; unlike S1PRM, it does not trap the lymphocytes in the lymphatic system—that is, it does not cause peripheral lymphopenia. Although their trafficking abilities are inhibited, the preserved presence of lymphocytes in blood flow—which, unlike the lymph flow, can be from central to peripheral areas as well—may be the reason NTZ does not blunt vaccination-induced immunisation as much as S1PRM (another class of lymphocyte trafficking inhibitor DMTs).

Cladribine

With the current guidelines, cladribine (CLAD) minimally affects the odds of postvaccine seroconversion (OR (95% CI): 0.41 (0.15 to 1.11), p=0.08) (online supplemental figure 8); however, the certainty of this finding is low due to limited number of studies with measurable relative effect and imprecision of the pooled measure. All of the available studies have measured anti-S1 IgG, and no evidence was found regarding anti-S seroconversion. In five studies,[10 13 14 16 20] all CLAD-treated pwMS seroconverted following vaccination with similar concentrations of postvaccination antibodies with the UX people. Assessment of vaccination-induced T-cell response was limited to one study[14] (very-low-certainty evidence); samples from vaccinated CLAD-treated pwMS in that study showed significantly lower extents of S-induced interferon-gamma release response compared with the UX people (OR (95% CI): 0.01 (0.00 to 0.04), p<0.00001).

Compared with its effect on the T-cell lineage, CLAD's effect on the B-cells is more extensive but less durable.[31–34] As interpreted, this has been translated into proper humoral despite blunted cellular responses to COVID-19 vaccination in CLAD-treated pwMS. Furthermore, the time since the last CLAD dose theoretically affects humoral responses; This was suggested especially by Achiron *et al* study[19] but did not reach statistical significance, and was not confirmed by other studies.[20 22 35] It seems the implemented guidelines[1–4] have suggested an adequate time window between CLAD administration and vaccination to prevent blunted humoral responses, making the probable effect unmeasurable. Additionally, although CLAD depletes the memory B-cells,[36] a preliminary study suggested its subsequent doses will not alter pre-existing humoral memory.[37] Still, there is limited evidence that the longevity of vaccine-induced humoral responses is lower in pwMS on CLAD[19]; this should be interpreted with caution as more time is generally required for an accurate assessment of longer-term responses. Replicative studies measuring the immunity waning speed in these pwMS after COVID-19 vaccination are, therefore, warranted to determine whether they require personalised booster schedules.

Alemtuzumab

With statistical significance but by a low extent, alemtuzumab (ALEM) was shown to negatively impact post-vaccine seroconversion odds (OR (95% CI): 0.32 (0.10 to 0.96), p=0.04) (online supplemental figure 9); although, due to limited number of studies and imprecision of the pooled measures, the certainty of this evidence is considered very low. All studies measured anti-S1 IgG responses, except for one which assessed anti-S seroconversion; its data could not be extracted.[23] The ALEM-treated pwMS showed 100% seroconversion rates in three studies[10 20 22] with similar concentrations of post-vaccination antibodies to the UX people.

Vaccination-induced T-cell responses were not assessed in any of the included studies, still, ALEM's durable effect of T-cell lineage[38] (similar to CLAD) suggests that it has blunting effect on vaccine-induced T-cell immunity.

ALEM, an anti-CD52 monoclonal antibody, is known to significantly deplete B- and T-cells shortly after administration. ALEM's short-term effect on B-cell and T-cell dynamics is relatively similar to CLAD.[34 38] Hence, although the time from the last ALEM infusion affects seroconversion,[19] this effect is currently not measurable as the implemented guidelines[1–4] seem to have recommended an adequate time window between ALEM infusion and vaccination. Similar to other BCDT, further studies measuring comparative immunity waning speeds in ALEM-treated pwMS are needed to determine whether they require more frequent boosters.

Anti-CD20 therapies

The aCD20 cause an extensive blunt on the vaccine-induced seroconversion rates (OR (95% CI): 0.05 (0.04 to 0.06), p<0.00001) (online supplemental figure 10). The certainty of

this finding is considered high as it was suggested by all studies; however, the studies had heterogenous effect measures. Therefore, it could be indicated with moderate certainty that with the current guidelines, pwMS on aCD20 are 20 times (95% CI: 16.66 to 25) less likely to seroconvert for anti-S1, and 12.5 times (95% CI: 7.69 to 20) less likely to seroconvert for anti-S antibodies (χ^2=2.76, p=0.10) following COVID-19 vaccination. Furthermore, evidence indicates with high certainty that every 10 week delay in subsequent aCD20 infusion is associated with a 1.94-time (95% CI: 1.57 to 2.41, p<0.00001) increase in seroconversion odds of aCD20-treated pwMS (online supplemental figure 11). A possible publication bias may be present regarding this measure, as indicated by asymmetric funnel plot (online supplemental figure 11).

Regarding the T-cell responses, aCD20 affected neither the interferon-gamma release responses (OR (95% CI): 1.12 (0.62 to 2.05), p=0.70) (online supplemental figure 12), nor the AIM responses of CD8+ (OR (95% CI): 2.54 (0.89 to 7.27), p=0.08) (online supplemental figure 13) and CD4+ (OR (95% CI): 1.13 (0.17 to 7.61), p=0.90) T-cell subpopulations. Quantitative analyses in most studies[11 14 17 24 39–42] were in line with the dichotomised evidence. Multiplex PCR assay in one study[43] indicated positive adaptive T-cell responses among 100% of postvaccine seronegative aCD20-treated pwMS.

Furthermore, the preliminary evidence indicates significant decline in seropositivity rates of pwMS on aCD20 6 months after their second dose.[44 45] Homologous mRNA boosters in pwMS on aCD20 promoted T-cell responses,[46] while humoral responses were still heavily dependent on the serostatus following the first vaccination cycle, and the B-cell dynamics at the time of booster administration.[27 45–47] In other words, among the aCD20-treated pwMS not seroconverting after their initial vaccination cycle, the booster doses promoted humoral immunisation only if their B-cells were reconstituted. Studies among people on aCD20 with diseases other than MS[48 49] support the same conclusion.

As the B-cells are the main expressors of the CD20 marker, the anti-CD20 monoclonal antibodies primarily affect the B-cell compartment of the immune system—although some recent literature suggests that their effectiveness in MS may stem from their effect on subsets of 'rogue' CD20+ T cells.[50] Similar to pwMS on other DMTs, the COVID-19 vaccines' immunogenicity among pwMS on aCD20 could be considered a translation of the previously-determined B-cell and T-cell dynamics in them,[34 51] based on which the current guidelines recommended a 12-to-36-week window between aCD20 infusion and COVID-19 vaccination.[1–4] However, the presented evidence suggests that the mentioned interval, although increases the odds, will not be adequate to reverse the humoral blunts in pwMS on aCD20 (figure 3). The alterations in the dynamics of B-cells in people receiving aCD20 last for years according to the unpublished results from the NCT00676715 phase-II extension trial,[52] suggesting durable, long-lasting benefits of aCD20 without subsequent dosing.[53] However, this durable effect of aCD20 has shown to be able to alter vaccine immunogenicity for as long as 3 years, as observed in people with haematological malignancies.[54] Hence, prior B-cell profiling and post-vaccination serological screening may be the necessities of an effective personalised vaccination strategy in pwMS who received aCD20 at any time point within 3 years.

Vaccine types

Based on phase-III data, the efficacy profiles of different available COVID-19 vaccine types (ie, mRNA, AV, inactivated and protein based) seems to correlate to their anti-S/S1 humoral immunogenicity—both in healthy people[55 56] and pwMS.[57] For the pwMS and in terms of efficacy, head-to-head comparisons of the available COVID-19 vaccines reveals the superiority of mRNA-1237 over BNT162b2 (both mRNA-based)[14 20 58]—probably due to higher concentrations of active material—BNT162b2 and mRNA-1237 (mRNA) over ChAdOx1 and Ad26.COV2.S (AV),[11 17 24 43] and BNT162b2 (mRNA) over CoronaVac (inactivated)[16]—although humoral immunisation did not differ significantly in pwMS on aCD20 receiving BNT162b2 and CoronaVac in Ozakbas *et al*[16] study. The choice of a specific vaccine type among pwMS is encouraged and should be based on an individualised risk/benefit assessment with careful consideration of their COVID-19 risk factor profile,[59 60] their DMT and the availability/cost-effectiveness of the vaccine.[61 62]

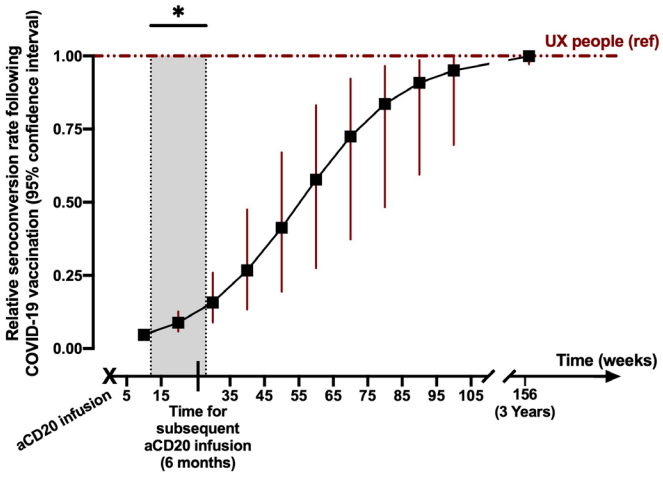

Figure 3 Schematic curve showing the association of post-vaccination seroconversion rates with time since last anti-CD20 infusion. *Current guideline recommendations on minimum delay of vaccination after anti-CD20 infusion. UX, unexposed.

Moving into the clinic

While the serum anti-S/S1 assays are deemed predictive of its neutralising activity,[63 64] and the serum neutralising activity predictive of the clinical protection against symptomatic SARS-CoV-2 infection,[65] pwMS with adequate humoral responses—mostly on DMTs other than aCD20 and S1PRM—would theoretically show adequate protection against SARS-CoV-2. However, in the absence of humoral immunisation, it is doubted whether the adaptive T-cell responses could provide adequate protection. Currently one real-world study confirms the predictive effect of seroconversion on post-vaccination COVID-19 incidence and severity,[57] and four studies from UK, Italy and Iran indicate rising comparative incidence and severity of COVID-19 among pwMS on S1PRM and aCD20 following mass vaccination of pwMS.[57 66–68] The registry-based UK study[66] marks a rise in COVID-19 comparative incidence among aCD20-reated and S1PRM-treated pwMS after the date the mass vaccination of pwMS was started in the area—although the study did not provide any information on the vaccination status of its individual participants. The Italian and Iranian studies[57 67 68]—which to the best of our knowledge, were not peer-reviewed up to the time of the present study—followed up cohorts of vaccinated pwMS receiving different DMTs; they noted significant rises in both incidence and severity of breakthrough COVID-19 in aCD20-treated and S1PRM-treated pwMS compared with

the others. Based on these studies, the less-extensive humoral blunts in pwMS on TERI, ALEM and possibly CLAD, do not seem to have had any significant effect on vaccine effectiveness. Regarding the protective effect of adaptive T-cell responses, currently, the only clue lies within the Iranian study[67]—although it has not been confirmed by a larger Italian study[68] and should be considered with excessive caution due to the current limitation of evidence; it showed that among the vaccinated pwMS, the ones on aCD20 experienced comparable incidence and severity of COVID-19 with those on fingolimod (an S1PRM). Unlike the S1PRM-treated pwMS, the aCD20-treated are known to be prone to a severer course of COVID-19 before vaccination[59 69]; robust postvaccination T-cell responses in the aCD20-treated pwMS—which is absent in the S1PRM-treated ones—may therefore, explain the unexpected comparable severity of breakthrough COVID-19 among them after vaccination. Still, it should be emphasised that the protective effect of adaptive T-cell responses in the absence of antibodies could neither be confirmed nor measured until further comparative evidence of real-world incidence and severity of COVID-19 among vaccinated aCD20-treated and unvaccinated pwMS becomes available.

CONCLUSION

The presented analyses highlight and corroborate the relevance for an optimal treatment strategy in pwMS before COVID-19 vaccination. It was demonstrated that the current vaccination strategy has failed to promote adequate humoral immunity in aCD20-treated and S1PRM-treated pwMS, which is being translated into low clinical effectiveness of COVID-19 vaccines among them—despite adequate T-cell responses in the ones on aCD20. Their susceptibility to worse COVID-19 outcomes, and the dependency of COVID-19 vaccines' humoral immunogenicity to the B-cell dynamics at the time of administration—and therefore, the timing of aCD20 infusion—stress the importance of personalising vaccination strategies for pwMS on aCD20 with respect to their B-cell profiles and aCD20 infusion timings. Theoretically and based on limited evidence, mode of action and administration method may be important factors to consider also when vaccinating S1PRM-treated pwMS. Milder humoral and considerable T-cell response blunts—also depending on dosage timings—and higher immunity waning speeds may be present in pwMS on CLAD and ALEM. It should be emphasised that these findings currently comprise of inadequate certainty; however, subject to their confirmation by further evidence, they stress the importance of earlier booster administrations among BCDT-treated pwMS. TERI may also cause a humoral immunogenicity blunt, however, being less extensive and clinically irrelevant based on current evidence—which also comprises of low certainty—pwMS on TERI may not require countering policies other than being provided with reliable information about the importance of booster doses. Evidence to date does not indicate any significant effect of IFN, GA, DMF, and NTZ on COVID-19 vaccines' immunogenicity and effectiveness.

Additionally, similar to the healthy population,[70–76] heterologous boosters may be more immunogenic and effective for pwMS; we unfortunately could not find any evidence in this regard. Therefore, further immunogenicity and effectiveness studies are encouraged among pwMS receiving heterologous vaccination regimens.

LIMITATIONS

Some limitations account to this study. Most important of all, it is a synthesis of observational studies which often present low to moderate-level evidence. Most probably, this limitation will account to future reviews as well as randomised studies are doubted to be considered ethical at the current stage. Other limitations included restrictions in obtaining data from primary investigators, computational problems/restrictions in meta-analysis/meta-regression and lack or low certainty of evidence in some instances. Future updated reviews are encouraged to account for the mentioned limitations.

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Multiple sclerosis disease-modifying therapies and COVID-19 vaccines: A practical review and meta-analysis: Methods and Supplementary Figures

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1. Methods

We hereby report our systematic review and meta-analysis study methods in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (available from: http://www.prisma-statement.org).

1.1. The Search

Based on the objectives of the review, a comprehensive search of the MEDLINE, Scopus, and Web of Science was performed with consideration of their specific vocabulary and indexing approaches. To consider the unpublished data, the medRxiv preprint server and Google Scholar were also searched as secondary sources. The main backbone of the search consisted of, but was not limited to the following keywords: "COVID-19 OR SARS-CoV-2 OR coronavirus", "vaccine OR vaccination", "multiple sclerosis OR MS", and "disease modifying therapies OR disease modifying drugs OR DMT OR DMD". As the COVID-19 vaccines were available after early 2021, the search was restricted to the studies after January 2021 and was conducted on November 7, 2021. Furthermore, a Google Scholar weekly alert was set, enabling us to screen the new results after the initial search date. The last screening of the new results was done in January 27, 2022.

After conducting the searches independently, two review authors used the Mendeley application for duplicate removal and screening of the titles and the abstracts of the results one by one. The possibly eligible studies were sought for retrieval of the full texts. Any errata or other linked citations were also retrieved. The reference lists of the possibly eligible studies were also scanned for more possibly eligible studies. All of the search results were archived in a reference manager file format, including a record of the excluded studies along with the reasons for exclusion.

1.2. Eligibility

The exclusion of studies from syntheses was based on the following criteria and priorities:

1. Not a primary investigation;

2. Retracted/withdrawn;
3. No eligible participants;
4. No eligible exposures; and
5. No eligible comparators.

Nine group of DMTs were considered as eligible exposures and receiving no DMTs – either pwMS receiving no DMTs or healthy participants – was considered as the eligible comparators. For the further syntheses addressing the effects of dosing intervals among pwMS on BCDT, the fifth criterion was ignored. No restriction was set for language or sample size of the studies.

The eligibility criteria for the participants were defined as:

1. No history/evidence of previous COVID-19; and
2. No history of corticosteroid administration within two months.

Three review authors independently assessed the full texts for eligibility. The papers that at least two review authors consider eligible were included in the study, and others were documented along with their reason for exclusion.

1.3. Assessment of Risk of Bias

The National Institutes of Health Study Quality Assessment Tools were used by three review authors (raters) independently to assess the risk of bias in different levels of the studies. The source of each risk-of-bias judgment of the raters, along with detailed explanations and the final results of each assessment, is presented narratively and summarized in a separate ***Supplementary File***.

1.4. Data Extraction

Considering the heterogeneity of the used assays and their measuring units – which was anticipated – in order to present more practical/translational syntheses and facilitate the data extraction and synthesis processes, the data were extracted in a dichotomized fashion based on the seropositivity cut-off indices of the assays used in the studies. Hence, the number of seropositive and total participants were extracted,

stratified by DMT exposure status, and with the unexposed (UX) people (healthy controls and/or pwMS on no DMTs) set as the comparator for all other DMTs. When piloting the data extraction process, we noticed that in most studies, the number of participants with negative post-vaccination serostatus would be zero for the UX cohorts and most DMT cohorts, i.e., the "zero" cells will cause computational problems for calculation of the effect measures. Hence, we decided to use the Peto method for estimation of odds ratio (OR) and 95% confidence interval (95%CI), which avoids the addition of a fixed continuity correction factor and has shown to feasibly provide unbiased estimations for relatively balanced cohorts [1]. When neither the specific DMT nor the UX cohorts contained seronegative participants, no relative effect measurement was possible; therefore, those studies were only reported narratively. Additionally, due to the usage of the Peto method, in order to prevent biased estimations, we decided to exclude from quantitative synthesis and present narratively the measures calculated from studies with arms containing less than five total participants and/or considerably uneven arms. It should be pointed that the mentioned studies were not excluded from the review, but only from the quantitative synthesis.

Furthermore, for extraction of further measures pertaining to the dosing intervals of infused aCD20, unstandardized beta coefficient (B) along with 95%CI was calculated manually by implementing a univariate logistic regression model on the descriptive measures presented in the study.

Another issue identified when piloting the data extraction was that some studies assessed their specified outcomes in multiple time points, including before the first dose, before the second dose, and two to six weeks after the second dose. We only extracted the measures pertaining to the latest timepoints after the second dose, limiting the probability of missing the outcomes that took more time to occur. Data extraction piloting also revealed inconsistent reporting of the effect of baseline CD19/CD20-positive B-cell counts among pwMS on BCDT, preventing us from extracting and synthesizing them.

In case the studies used more than one assay for detecting humoral responses to SARS-CoV-2 – e.g., total anti-Spike (S) IgG, anti-S1 subunit IgG assays –including anti-

receptor-binding domain (RBD) assays, anti-nucleocapsid (N) IgG, or other immunoglobulins than IgG, results were extracted regarding all assays, separately. The anti-N IgG-positive participants who received mRNA vaccines were excluded from synthesis as it indicated previous COVID-19 contraction.

1.5. Syntheses

After assessing heterogeneity using Cochran's Q and I^2 tests, the extracted results were pooled using the Peto fixed-effects model. The funnel plots were assessed for small study effects and possible publication bias. When the adequate number of studies were available, we adopted an assay-specific fashion in the synthesis, i.e., measures of anti-S1 (including RBD) IgG, anti-S (including trimeric S), interferon-gamma release, CD4+ and CD8+ activation-induced marker (AIM), and multiplex polymerase chain reaction (M-PCR) assays were pooled and presented separately; otherwise, the T-cell assays were presented narratively, and the B-cell assays were pooled all together.

The results of pooled analyses were presented in an extended forest plot, funnel plots, and all outcomes which could not be entered into the pooled analysis were presented narratively. A meta-regression analysis was planned. However, it was not conducted due to several limitations, including our restrictions in obtaining data from the primary investigators. Hence, the possible reasons behind heterogeneity were only discussed narratively. We also found a few studies assessing the responses to boosters and narratively synthesized them, although this was not planned in our initial protocol.

Sensitivity analyses by performing all analyses on the subgroup of outcomes from the studies with "good" quality (least risk of bias) was done but was not presented as it did not show any change in any analysis, except for lower statistical power. A meta-analysis while adjusting the study weights based on the sensitivity of the used assays was initially planned; however, as all the studies used assays with more than 90% sensitivity, it was not conducted. The GRADE approach was used by two review authors to assess the certainty of the evidence. The baseline certainty of evidence was considered as moderate –due to observational nature of the synthesized studies, however, they were upgraded to high in case they were completely supported by non-dichotomized analysis in the individual studies. The judgments and arguments for down- or upgrading using

GRADE was presented and justified to ensure transparency. The final assessment of certainty was presented in Table 2 (summary of findings), along with the effect measures and CI of the outcomes.

1.6. Software

The Mendeley Desktop software version 1.19.8 for MacOS (Mendeley Ltd.) was used for management and screening of the studies. The Review Manager (RevMan) software version 5.4.1 for MacOS (The Cochrane Collaboration) was used for data extraction and synthesis. The SPSS software version 23 for MacOS (IBM Inc.) was used for the manual analyses.

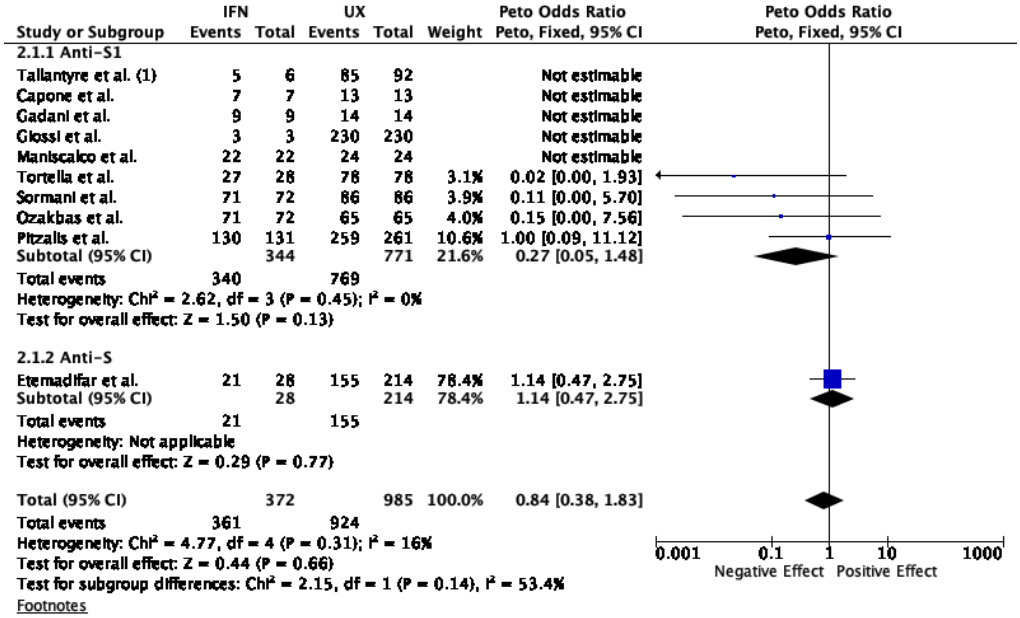
1.7. Registration and Approval

This study was registered in PROSPERO before initiation (id: CRD42021278107). No Institutional Review Board / ethics committee approvals were required for this study according to the national guidelines, as it did not involve human subjects.

1.8. Availability

All of the used materials for this review are available upon reasonable request from the corresponding review author.

147 # 2. Supplementary Figures



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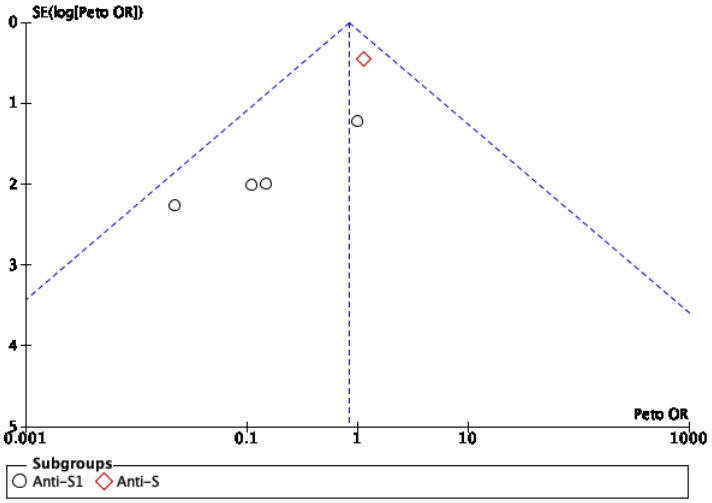

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150 *Supplementary Figure 1; Results of individual studies, heterogenicity tests, forest and funnel plots of studies*
151 *measuring humoral response in pwMS on IFN*

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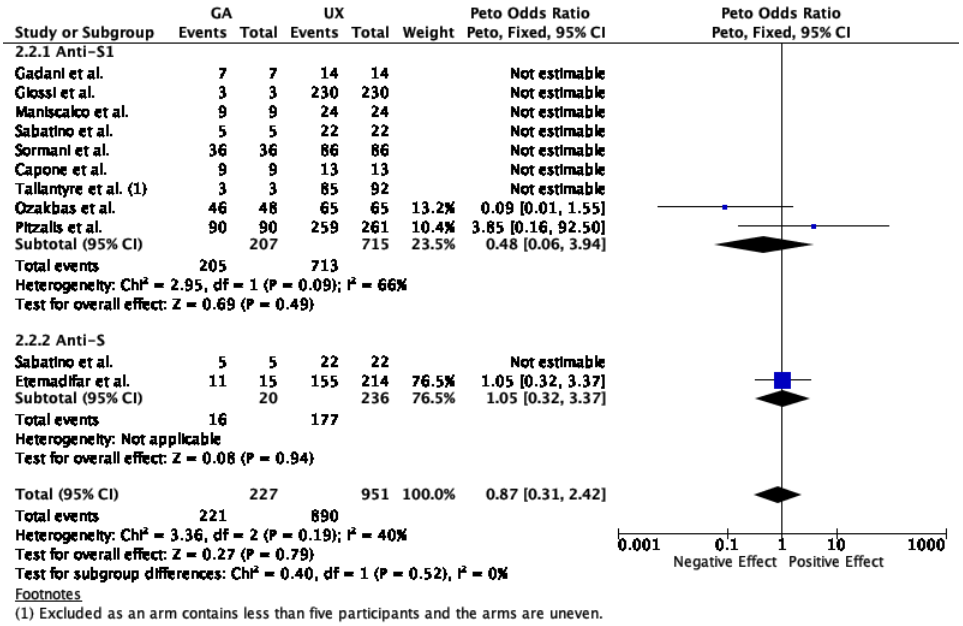
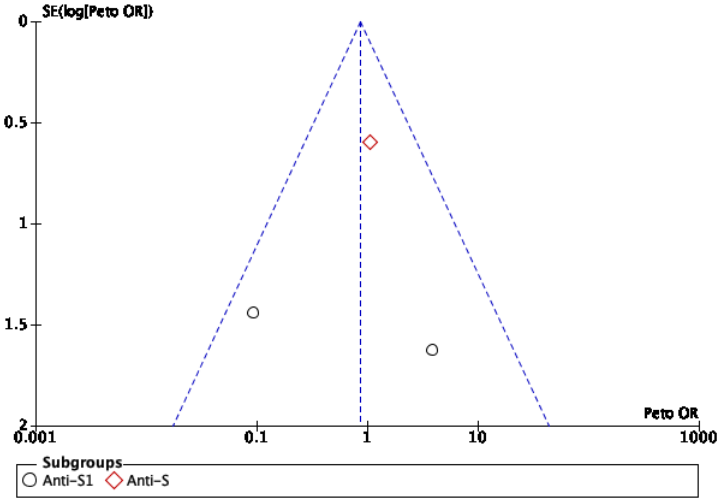
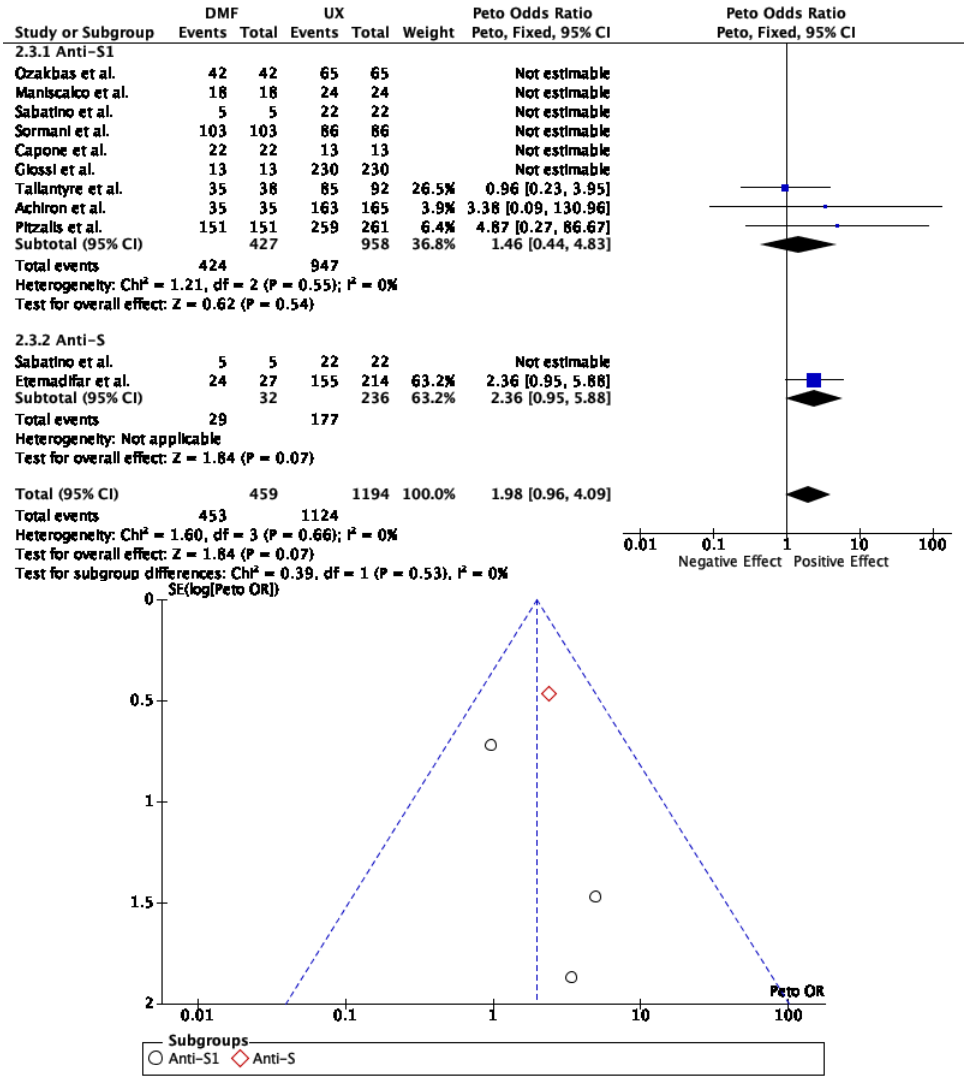
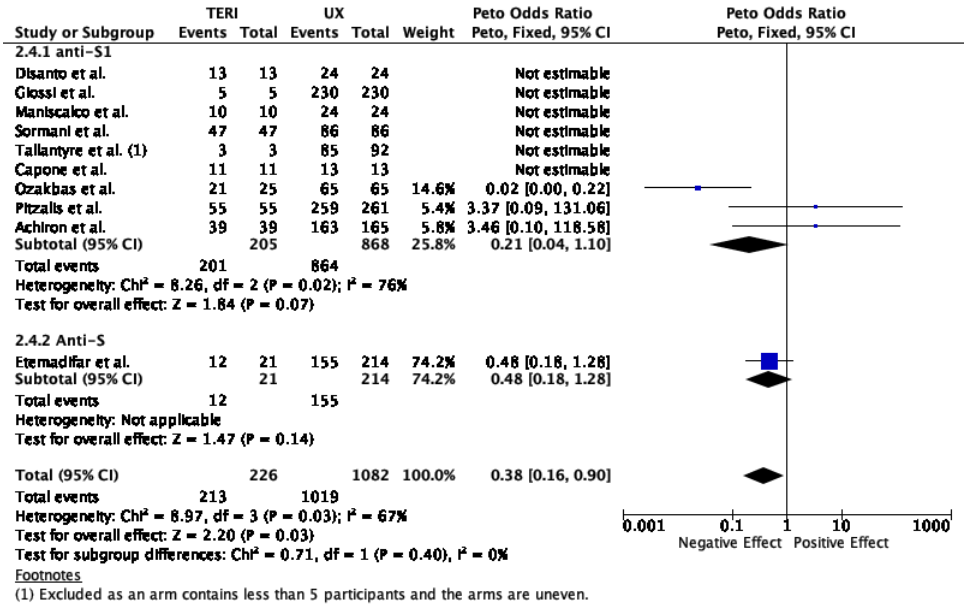




Supplementary Figure 2; Results of individual studies, heterogenicity tests, forest and funnel plots of studies measuring humoral response in pwMS on GA

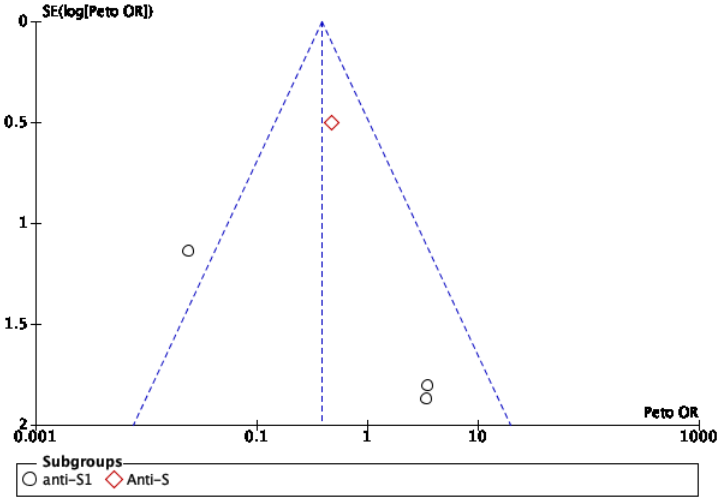


Supplementary Figure 3; Results of individual studies, heterogenicity tests, forest and funnel plots of studies measuring humoral response in pwMS on DMF

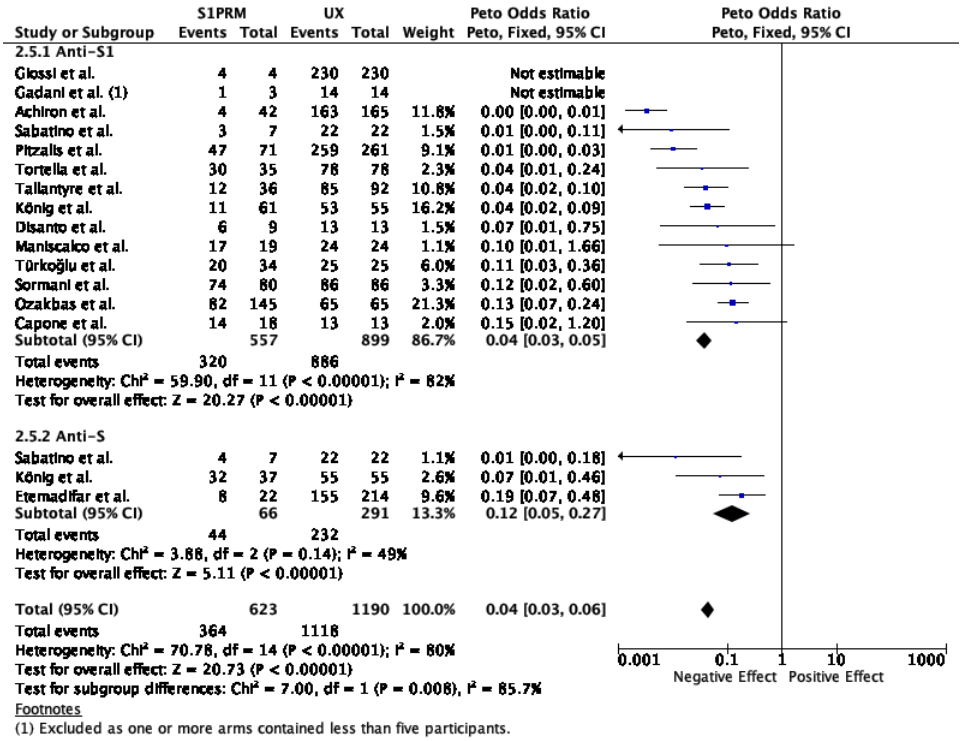


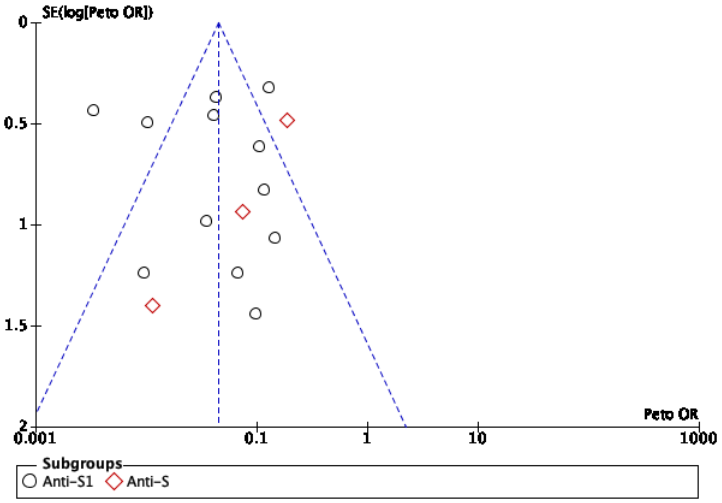
Footnotes

(1) Excluded as an arm contains less than 5 participants and the arms are uneven.

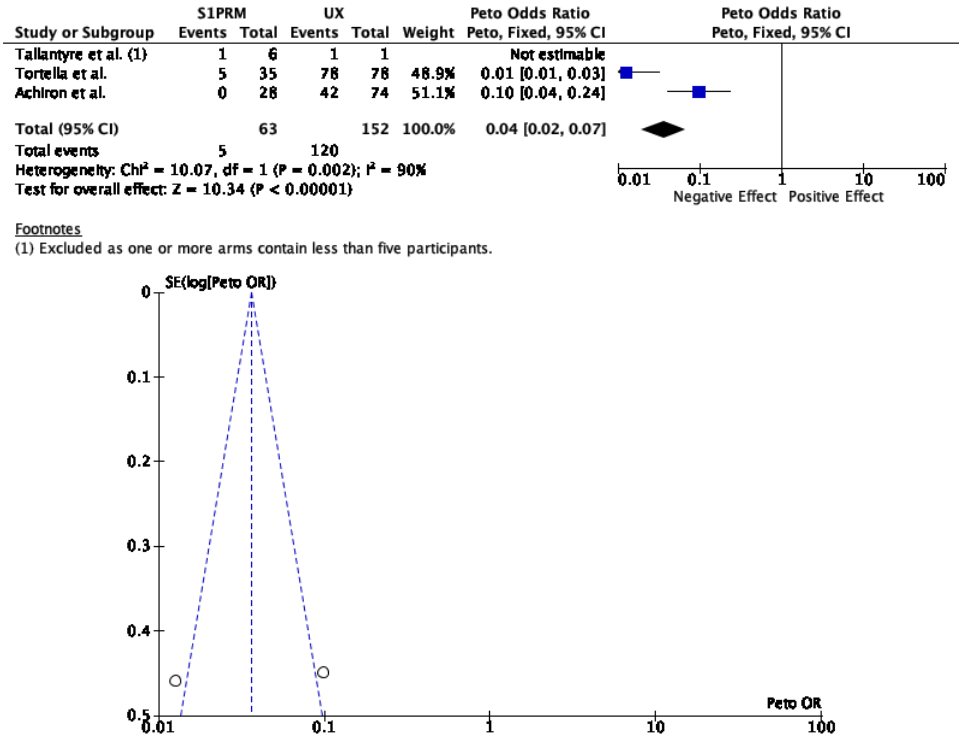


Supplementary Figure 4; Results of individual studies, heterogenicity tests, forest and funnel plots of studies measuring humoral response in pwMS on TERI

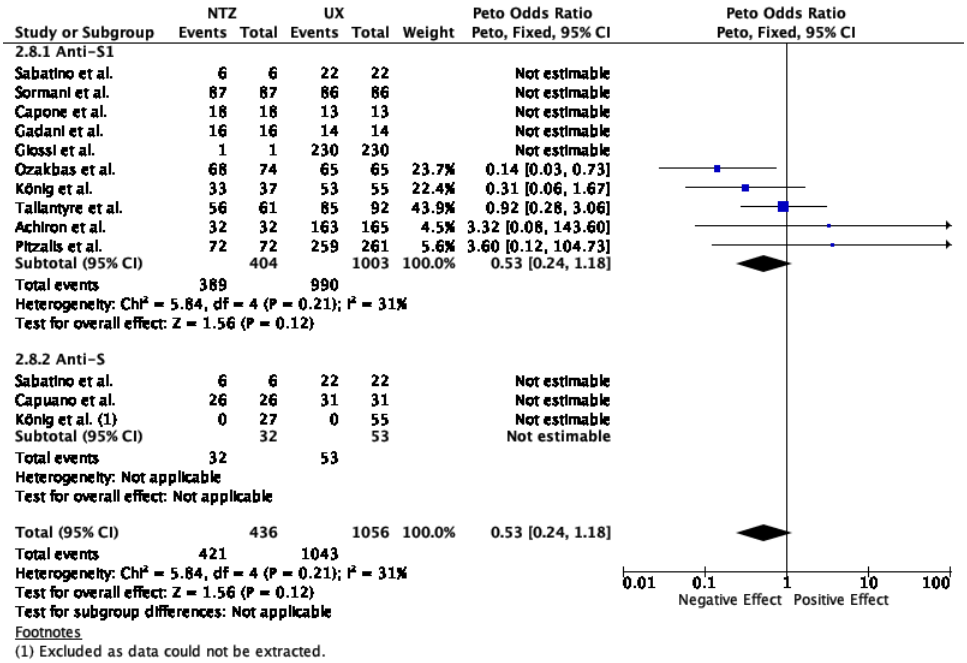


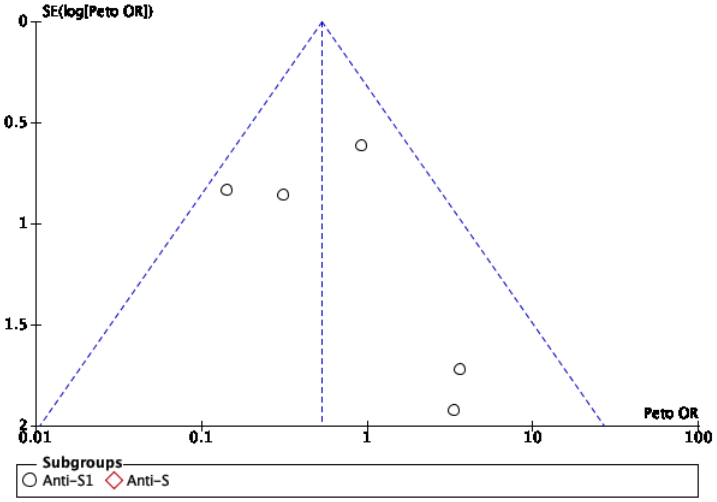


Supplementary Figure 5; Results of individual studies, heterogenicity tests, forest and funnel plots of studies measuring humoral response in pwMS on S1PRM

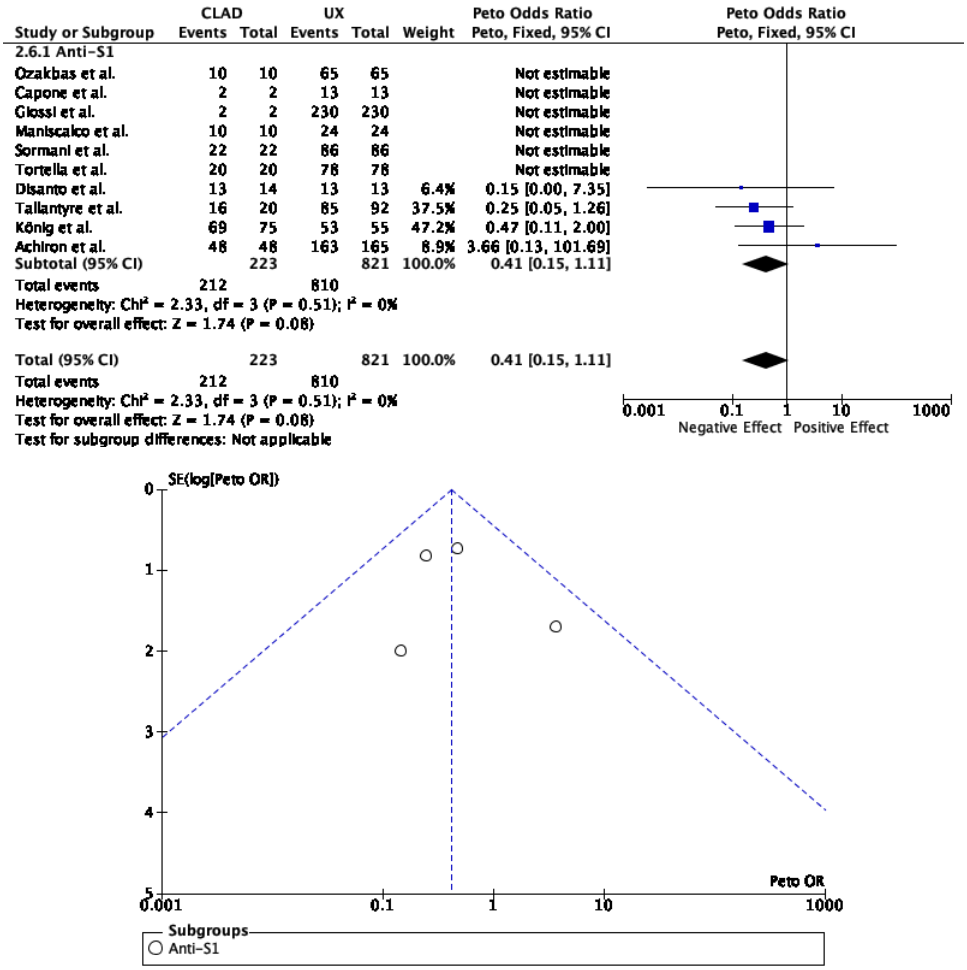


Supplementary Figure 6; Results of individual studies, heterogenicity tests, forest and funnel plots of studies measuring interferon-gamma release response in pwMS on S1PRM

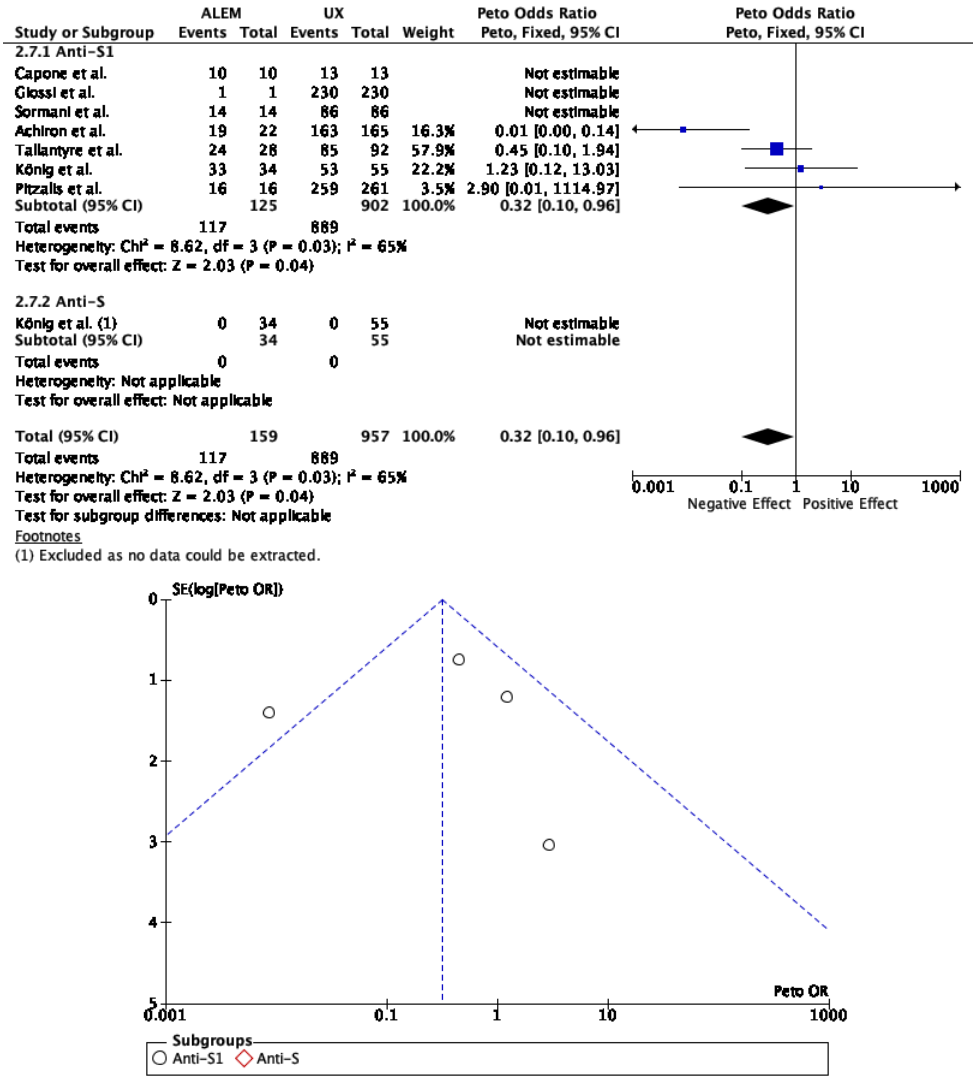




Supplementary Figure 7; Results of individual studies, heterogenicity tests, forest and funnel plots of studies measuring interferon-gamma release response in pwMS on NTZ



Supplementary Figure 8; Results of individual studies, heterogenicity tests, forest and funnel plots of studies measuring humoral response in pwMS on CLAD



Supplementary Figure 9; Results of individual studies, heterogenicity tests, forest and funnel plots of studies measuring humoral response in pwMS on ALEM

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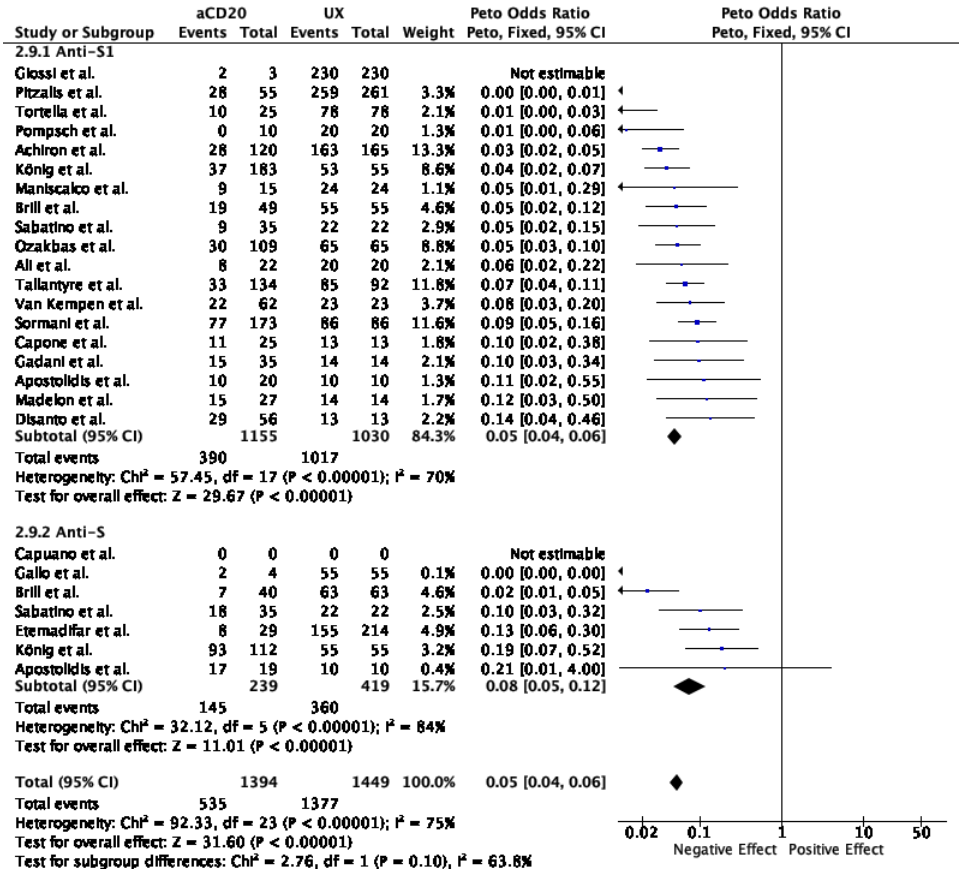

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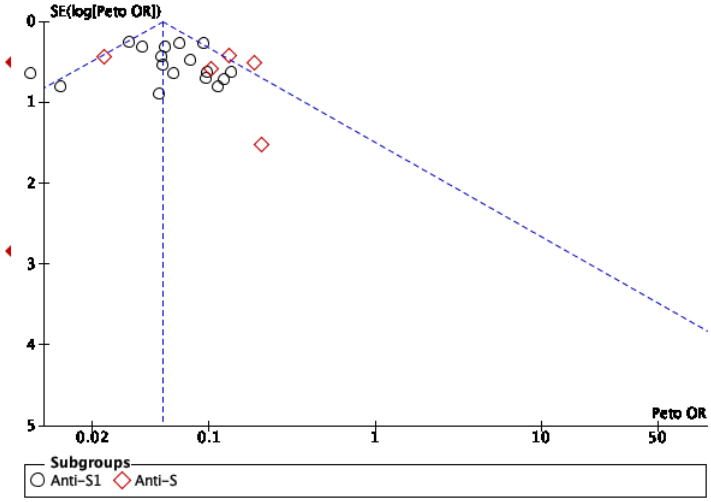

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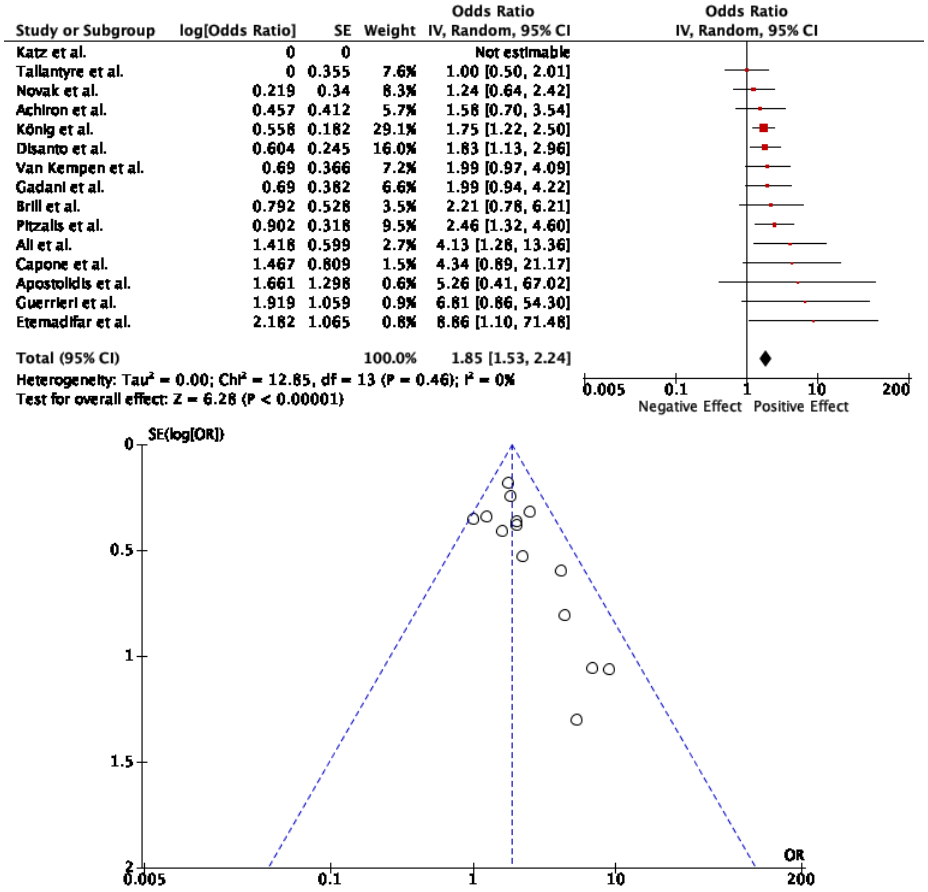
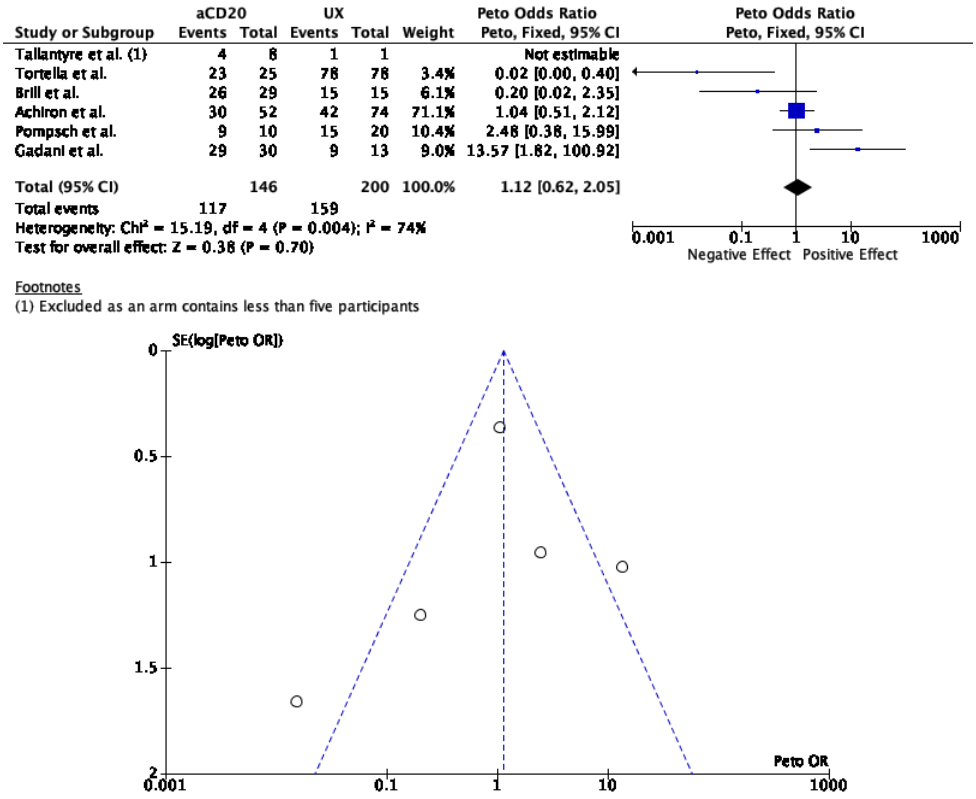
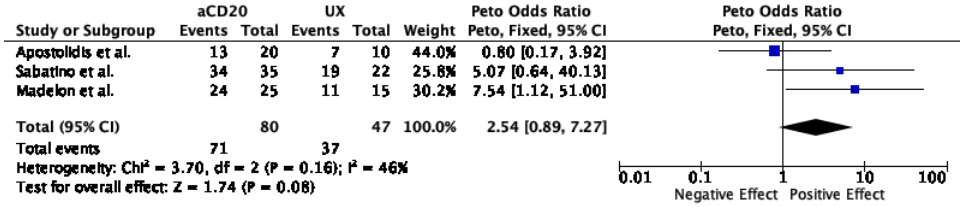
202 *Supplementary Figure 10; Results of individual studies, heterogenicity tests, forest and funnel plots of studies*
203 *measuring humoral response in pwMS on aCD20*

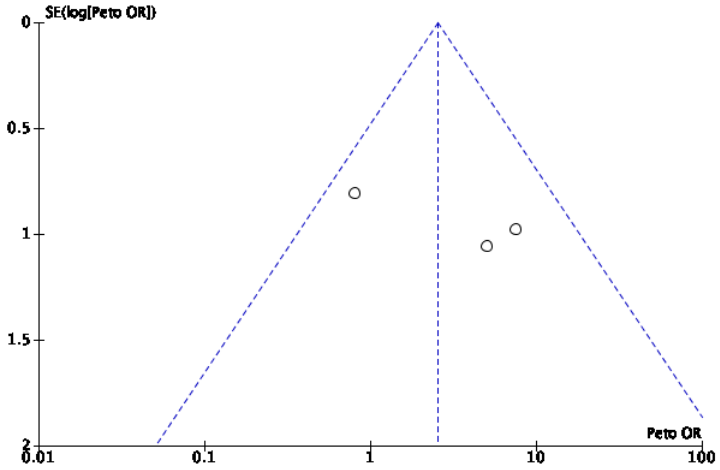


Supplementary Figure 11; Results of individual studies, heterogenicity tests, forest and funnel plots of studies measuring the effect of aCD20-to-vaccine period on humoral response



Supplementary Figure 12; Results of individual studies, heterogenicity tests, forest and funnel plots of studies measuring interferon-gamma release response in pwMS on aCD20





Supplementary Figure 13; Results of individual studies, heterogenicity tests, forest and funnel plots of studies measuring CD8+ AIM response in pwMS on aCD20

References

1. Sweeting M, Sutton A, Lambert P. What to add to nothing? Use and avoidance of continuity corrections in meta-analysis of rare events. 2002:

NIH Quality Assessment Tool for Observational Cohort and Cross-Sectional Studies available at: https://www.nhlbi.nih.gov/health-topics/study-quality-assessment-tools

Study	Criteria														Quality*
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	
Achiron et al.	Y	Y	N/R	Y	NO	Y	Y	Y	Y	N/A	Y	N/R	Y	Y	Good
Capone et al.	Y	Y	Y	Y	NO	Y	Y	Y	Y	N/A	Y	N/R	Y	Y	Good
Capuano et al.	Y	NO	Y	Y	N/R	Y	Y	Y	Y	N/A	Y	N/R	Y	Y	Good
Disanto et al.	Y	Y	N/R	Y	NO	Y	Y	Y	Y	N/A	Y	N/R	Y	Y	Good
Etemadifar et al.	Y	Y	N/R	Y	Y	Y	Y	Y	Y	N/A	Y	N/R	N/R	Y	Good
Maniscalco et al.	Y	Y	Y	Y	NO	Y	Y	Y	Y	N/A	Y	N/R	Y	Y	Good
Ozakbas et al.	Y	Y	N/R	Y	NO	Y	Y	Y	Y	N/A	Y	N/R	Y	Y	Good
Sormani et al.	Y	Y	Y	Y	Y	Y	Y	Y	Y	N/A	Y	N/R	NO	Y	Good
Ali et al.	Y	Y	N/R	N/R	NO	Y	Y	Y	Y	N/A	Y	N/R	N/R	Y	Fair
Apostolidis et al.	Y	NO	N/R	N/R	N/R	Y	Y	Y	Y	N/A	Y	Y	N/R	N/R	Fair
Bigaut et al.	Y	Y	N/R	NO	NO	Y	Y	Y	Y	N/A	NO	N/R	N/R	Y	Fair
Brill et al.	Y	Y	N/R	NO	NO	Y	Y	Y	Y	N/A	Y	N/R	N/R	Y	Fair
Gadani et al.	Y	NO	N/R	Y	N/R	Y	Y	Y	Y	N/A	Y	N/R	Y	Y	Fair
Gallo et al.	Y	Y	N/R	Y	NO	Y	NO	Y	Y	N/A	Y	N/R	Y	N/R	Fair
Giossi et al.	Y	Y	N/R	Y	NO	Y	Y	Y	Y	N/A	Y	N/R	Y	N/R	Fair
Katz et al.	Y	Y	N/R	Y	NO	Y	NO	Y	Y	N/A	Y	N/R	Y	Y	Fair
König et al.	Y	Y	N/R	Y	NO	Y	Y	Y	Y	N/A	Y	N/R	Y	N/R	Fair
Madelon et al.	Y	NO	N/R	Y	NO	Y	Y	Y	Y	N/A	Y	N/R	Y	Y	Fair
Pitzalis et al.	Y	Y	N/R	NO	NO	Y	Y	Y	Y	N/A	Y	N/R	Y	Y	Fair
Pompsch et al.	Y	NO	N/R	Y	N/R	Y	Y	Y	Y	N/A	Y	N/R	N/R	Y	Fair
Sabatino et al.	Y	NO	N/R	Y	NO	Y	Y	Y	Y	N/A	Y	N/R	Y	N/R	Fair
Tallantyre et al.	Y	NO	N/R	Y	N/R	Y	Y	Y	Y	N/A	NO	N/R	Y	Y	Fair
Tortorella et al.	Y	NO	N/R	Y	NO	Y	Y	Y	Y	N/A	Y	N/R	Y	Y	Fair
Türkoglu et al.	Y	N	N/R	Y	NO	Y	Y	Y	Y	N/A	Y	N/R	Y	Y	Fair
VanKempen et al.	Y	NO	Y	Y	NO	Y	Y	Y	Y	N/A	Y	N/R	Y	N/R	Fair

*Good: least risk of bias; Fair: low risk of bias; Poor: moderate/high risk of bias.

Study: Achiron et al. DOI: 10.1016/j.jneuroim.2021.577746

1	YES	The aim of study is clearly explained.
2	YES	(who): patients with multiple sclerosis - (where): Sheba MS Center – (when): between December 2020 and February 2021
3	N/R	
4	YES	The inclusion and exclusion criteria were applied uniformly among all patients.
5	NO	No prior-to-study estimates on minimum required sample size are reported.
6	YES	This study was performed prospectively; exposure was assessed prior to outcome measurement.
7	YES	A minimum of interval between the second dose and serum sample acquisition of 28 days was set, which is long enough to detect the outcome.
8	YES	DMTs (exposure) were categorized in terms of medication type. Also, time from the last treatment dose and the first vaccination was taken into account and analysis of effect on outcome measure.
9	YES	The exposure measures in this study are parts of the patients' medical history, which are usually objective and reliable if registered by health care providers.
10	N/A	The exposure measures in this research scenario do not require multiple assessments at different time points.
11	YES	The outcome measurement methods were clearly defined and implemented consistently across all study participants. [Serum samples were examined for anti-SARS-COV-2 IgG using ELISA kit based on the recombinant S1 protein from the SARS-COV-2 spike protein (Euroimmun, Lubeck, Germany). Index values (signal to cut-off ratios) >1.1 were considered positive (EUROIMMUN. Anti-SARS-COV-2 ELISA IgG, [Package Insert], Moutain Lakes, NJ: EUROIMMUN US, 2020). Absolute lymphocyte count (ALC, cells/mm3) in the peripheral blood were collected at the same date of IgG serology and determined by a DxI hematology analyzer (Beckman Coulter USA).]
12	N/R	
13	YES	No follow-up losses are reported.
14	YES	Possible covariates were taken into account in the analysis of treatment groups and healthy subjects.

Study: Ali et al. DOI: 10.1016/j.vaccine.2021.08.078

1	YES	The probable factors impacting the outcome aimed in the study are documented. The necessity of performing the study is explained. The aim is clearly stated.	YES
2	YES	(who): participants with MS and other demyelinating diseases (Those who completed 2 doses of SARS-CoV-2 mRNA vaccines)- (where): University of Michigan Multiple Sclerosis Center- (when): between December 21, 2020 and May 19, 2021.	YES
3	N/R		N/R
4	NO	Patients were selected from one single center (University of Michigan Multiple Sclerosis Center) but from different populations (people with multiple sclerosis and other demyelinating diseases). The included participants were patients who had received 2 doses of SARS-CoV-2 mRNA vaccines during the same time period; inclusion criteria were applied uniformly (December 21, 2020 and May 19, 2021)	NO
5	N/R		NO
6	YES	The exposure status of participant prior to the study is clear (disease modifying therapies); participants were recruited based on their exposure.	YES
7	YES	The second blood sample was obtained approximately 3 weeks after the second dose of vaccination. This time frame is sufficiently enough.	YES
8	YES	The DMTs were categorized into different groups (B-cell depleting and non-B-cell depleting); further, both “the time between vaccination and the last dose of DMT” and “the duration of treatment with DMTs”, as two possible measures of exposure were taken into account.	YES
9	NO	The exposure measures mentioned above were clearly defined, and are parts of the patients’ medical history, which are usually objective and reliable if registered by health care providers.	YES
10	N/A	The exposure measures in this research scenario, i.e., DMT type, DMT receiving duration, and interval between the last DMT dose and vaccination, do not require multiple assessments at different time points.	N/A
11	YES	[The Roche anti-SARS-CoV-2 nucleocapsid assay works is an electrochemiluminescent immunoassay (ECLIA) which utilizes recombinant biotinylated and ruthenium-labeled nucleocapsid protein. The Siemens SARS-CoV-2 Spike RBD total antibody assay works as a chemiluminescent immunoassay (CLIA) by utilizing recombinant S1 subunit receptor-binding domain as a biotinylated and acridinium ester-conjugated antigen.]	YES
12	N/R		N/R
13	N/R		N/R
14	YES	Age, BMI, and total treatment duration didn’t differ between negative and positive antibody groups.	YES

Study: Apostolidis et al. DOI: 10.1038/s41591-021-01507-2

1	YES	The research questions and objectives are clearly stated.	YES
2	NO	(who): MS patients treated with anti-CD20 monotherapy (n=20) + healthy controls (n=10). (when): December 2020 - April 2021; (where): not specified	NO
3	N/R		N/R
4	N/R	Although subjects in each group (i.e., MS and HC) seem to have fulfilled uniform inclusion criteria, which were specified before the study was begun, details of the inclusion criteria are not stated.	N/R
5	N/R		N/R
6	YES	The exposure status of participant prior to the study is clear (anti-CD20 monotherapy); participants were recruited based on their exposure.	YES
7	YES	The follow-up time was longer that one month which is fairly sufficient in this study.	YES
8	YES	The authors have taken into account the type of the anti-CD20 (i.e., ocrelizumab and rituximab), the number of previous cycles of anti-CD20, and the interval between the last cycle and vaccination; which enables fairly sufficient power for stratification of exposure risk.	YES
9	YES	The exposure measures mentioned above were clearly defined, and are parts of the patients' medical history, which are usually objective and reliable if registered by health care providers.	YES
10	N/A	The exposure measures in this research scenario do not require multiple assessments at different time points.	N/A
11	YES	[Plasma samples were tested for SARS-CoV-2-specific antibody by ELISA59. The estimated sensitivity of the test is 100% (95% confidence interval (CI), 89.1 to 100.0%) and specificity is 98.9% (95% CI, 98.0 to 99.5%). Plasmids encoding the recombinant full-length spike protein and the RBD were provided by F. Krammer and purified by nickel-nitrilotriacetic acid resin (QIAGEN). Monoclonal antibody CR3022 was included on each plate to convert optical density values into relative antibody concentrations.]	YES
12	YES	[All experiments were conducted in blinded fashion with designated members of the clinical team (who were not part of running the assays) having access to the sample key until data were collected, at which point all researchers were unblinded for the analysis.]	YES
13	N/R		N/R
14	N/R		N/R

Study: Bigaut et al. DOI: 10.1016/j.neurol.2021.05.001

1	YES	The aim of study was clearly defined which analyzing humoral response after COVID-19 vaccination and COVID-19 contracting in MS patients on DMTs.
2	YES	(who): MS patients with serological results after COVID-19 vaccination and 61 MS patients with serological results after COVID-19_ (when): between January and April 2021_ (where): MS center of Strasbourg, France.
3	N/R	
4	NO	The time period for all patients was the same but the inclusion criteria differs, one group is people with multiple sclerosis receiving COVID-19 vaccination and the other is people with multiple sclerosis who contracted COVID-19.
5	NO	Given the different types of medications, receivers of which were included in the study; and also given the inclusion of two types of immune triggers (i.e., vaccine and virus), the sample size may have been insufficient for subgroup analysis with regards to different medication types. This is stated as a limitation of the study by the very authors as well.
6	YES	The last dose of DMTs consumed by the patients is documented from their medical history and participant were exposed to the DMTs before the aimed outcome.
7	YES	The serology was assessed more than one month after COVID-19 and COVID-19 vaccination.
8	YES	The DMTs were categorized to Glatiramer acetate, Interferon b-1a, Teriflunomide, Dimethyl fumarate, Natalizumab, Mycophenolate mofetil, S1PR modulator, Anti-CD20 monoclonal antibody; each dose of DMT received by patients during the study, was documented.
9	YES	The exposure measures mentioned above were clearly defined, and are parts of the patients' medical history, which are usually objective and reliable if registered by health care providers.
10	N/A	The exposure measures in this research scenario do not require multiple assessments at different time points.
11	NO	Two different anti-SARS-CoV-IgG assays were used; no further information is given in this regard, e.g., how many serum samples were assessed by each tests.
12	N/R	
13	N/R	
14	YES	The univariate analysis incorporated a linear regression model adjusted for possible confounder, such as age and sex.

Study: Brill et al. DOI: 10.1001/jamaneurol.2021.3599

1	YES	The exposure, the aimed outcome and the participants relevant to the aim, are clearly defined.
2	YES	(who): people with MS (pwMS)- (where): Hadassah Medical Center in Jerusalem, Israel – (when): between December 2020 and April 2021
3	N/R	The number of screened patients and the final eligible participants is not reported.
4	NO	The inclusion and exclusion criteria were not clearly reported.
5	NO	No pre-enrollment estimates on the minimum sample size to detect a difference are provided; the study is extrapolating in nature and this is not a fatal flaw in this case.
6	YES	Participants were recruited considering their exposure of interest (Ocrelizumab) and were followed up prospectively to assess the aimed outcome for a specific timeframe after vaccination.
7	YES	Serum samples were taken 2-4 weeks after the second vaccine dose; the time frame is fairly sufficient.
8	YES	The duration of treatment is documented, but it is not clear if the whole treatment duration included only ocrelizumab therapy, and if not, for how long has ocrelizumab been given. However, it is interpretable from the results, and not from the methods section, that the time from the last ocrelizumab infusion and vaccination were investigated.
9	YES	The exposure measures mentioned above were clearly defined, and are parts of the patients' medical history, which are usually objective and reliable if registered by health care providers.
10	N/A	The exposure measures in this research scenario do not require multiple assessments at different time points.
11	YES	Outcome measures (dependent variables) were clearly defined, valid, reliable and used uniformly within all the participants. [Serology response was measured using Liaison SARS-CoV-2 S1/S2 IgG (DiaSorin) and spike receptor-binding domain (RBD) Architect SARS-CoV-2 IgG II Quant assay (Abbott Diagnostics) with a positive response defined by IgG titer of 19 or more or 50 or more arbitrary units (AU) per mL, respectively. T-cell immune response to SARS-CoV-2 was assessed by detecting interferon γ using T-SPOT Discovery SARS-CoV-2 (Oxford Immunotec), a modified enzyme-linked immunospot technology, IVD CE–marked assay, using freshly isolated peripheral blood mononuclear cells.]
12	N/R	
13	N/R	
14	YES	It is interpretable from the results and the supplemental data that possible confounders were considered in the association/correlation analyses; e.g., the confounder "age" did show an impact on the outcome measure "antibody levels".

Study: Capuano et al. DOI: 10.1007/s10072-021-05397-7

1	YES	the exposure (Natalizumab), the aimed outcome (humoral response) and the participants (people with MS (pwMS) and healthy controls receiving BNT162b2 mRNA Covid-19 vaccine) are clearly defined.
2	NO	The time frame of patient enrollment is not clear; (who): people with MS (pwMS) treated with Natalizumab and healthy controls. (where): the healthy controls were enrolled in the Clinic of University of Campania 'Luigi Vanvitelli' Naples, Italy.
3	YES	31 people with MS (pwMS) were screened and 26 were eligible for the study (83.87%). 31 healthy controls were recruited from a large dataset of healthy controls enrolled in a surveillance program at the Clinic of University of Campania 'Luigi Vanvitelli' Naples, Italy.
4	YES	The exclusion and inclusion criteria were applied uniformly across both groups.
5	N/R	
6	YES	The exposure in this study was being treated with Natalizumab; assessment of which was done before the study was initiated.
7	YES	Although 7 days after the second dose seems shorter than other similar studies, it maintains a more than a month interval from the first dose. Given the mechanism of action of Natalizumab, our interpretation is that a 5-week period after the first dose of vaccination is a reasonable interval for a non-B-cell depleted immune system to mount humoral immune responses against SARS-CoV-2.
8	YES	The time from the last Natalizumab infusion and vaccination was documented and taken into account.
9	YES	The exposure measures mentioned above were defined and investigated; these measures are parts of the patients' medical history, which are usually objective and reliable if registered by health care providers.
10	N/A	The exposure measures in this research scenario do not require multiple assessments at different time points.
11	YES	Outcome measures (serum anti-SARS-CoV-IgG presence) were clearly defined, valid, reliable and used uniformly within all the participants. [Sera were tested at virology laboratory of our University Hospital, using the LIAISON® SARS-CoV-2 TrimericSIgG assay (DiaSorin-S.p.A.), for the detection of IgG antibodies to SARS-CoV-2 spike protein including neutralizing antibodies.]
12	N/R	
13	YES	Follow-up data from all of the 26 patients with MS included were presented; thus, no follow-up losses are expected.
14	YES	Age and sex were matched between the people with multiple sclerosis (pwMS) and the healthy controls; possible confounding factors were taken into account through their analyses.

Study: Disanto et al. DOI: 10.1001/jamaneurol.2021.3609

1	YES	The aim of the study is clearly defined as if to confirm the previous studies and investigate a new exposure- outcome assessing. The previous studies and the exposures and outcomes of interest are clearly cited.
2	YES	(who): people with MS (pwMS) older than 18 years old. - (when): between February 25, 2021,and May 11, 2021 – (where): Neurocenter of the southern Switzerland
3	N/R	
4	YES	The participants were recruited from the same population. The same inclusion and exclusion criteria were applied uniformly among all participants.
5	NO	No pre-enrollment estimates on the minimum sample size to detect a difference are provided; the study is extrapolating in nature and this is not a fatal flaw in this case.
6	YES	Since this study is a prospective observational cohort study, thus the exposure to DMTs in people with MS (pwMS) was assessed in the beginning of the study, and the participants were followed up and assessed for the aimed outcome.
7	YES	The time frame between the second vaccine dose and serological test (21-35 days) seems fairly sufficient for the desired outcome (seroconversion) to surface.
8	YES	The DMTs were subdivided into 9 main categories; time from last anti-CD20 infusion was also documented and taken into account.
9	YES	The exposure measures in this study are parts of the patients' medical history, which are usually objective and reliable if registered by health care providers.
10	N/A	The exposure measures in this research scenario do not require multiple assessments at different time points.
11	YES	Outcome measures (dependent variables) were clearly defined, valid, reliable and used uniformly within all the participants. [Quantification of IgG against SARS-CoV-2 spike receptor binding domain was performed using a chemiluminescence microparticle immunoassay (Abbott; quantification limits, 21- 40000AU/mL; cutoff for seropositivity = 50AU/mL).4 CD19+ B cells were measured in the first serum sample obtaining using fluorescence-activated cell sorting.]
12	N/R	
13	YES	Only 4 patients after testing positive within 2 weeks prior to first vaccine dose were excluded. (4/120 – 3.34%)
14	YES	Patients receiving medical treatments influencing response to vaccines other than MS DMTs were excluded. Sex and age were adjusted.

Study: Etemadifar et al. DOI: 10.1016/j.msard.2021.103417

1	YES	The aim of the study is clearly defined. The exposure and desired outcome are noted. Exposure: disease-modifying therapies (DMTs) – outcome: humoral response to COVID-19 inactivated virus vaccination – population: people with MS
2	YES	(who): exposed group: people with multiple sclerosis (pwMS) on DMTs, unexposed group: people having no history of immunosuppression and absence of any special condition. – (where): Isfahan-Iran. – (when): from August until October 2021.
3	N/R	
4	YES	The participants were recruited from the same population. The inclusion and exclusion criteria for all the participants were applied uniformly.
5	YES	The minimum sample size justification and power description are provided.
6	YES	This study is a retrospective study, and the participants were included considering their status of exposure (disease-modifying therapies (DMTs)). Patients were on DMTs before they receive vaccination.
7	YES	It is interpreted from the results (table 1) that nearly all second dose-to-phlebotomy values from the included participants were more than 14 days, which seems fairly sufficient for mounting a humoral immune response.
8	YES	The DMTs as the exposures, were categorized into 6 groups. Time from the last anti-CD20 infusion-to-first vaccination is also documented and taken into account.
9	YES	The exposure measures in this study are parts of the patients' medical history, which are usually objective and reliable if registered by health care providers; cases included in this study were patients from three private neurology clinics, thus, a reliably registered record is expected.
10	N/A	The exposure measures in this research scenario do not require multiple assessments at different time points.
11	YES	Quantification of the post-vaccination humoral responses among the participants was performed using an anti-Spike IgG enzyme-linked immunosorbent assay (ELISA) kit (Quanti SARS-CoV-2 Anti-Spike IgG, Pishtazteb Diagnostics, Iran). [The mentioned kit has a reported sensitivity and specificity of 98.16% and 99.01%, respectively, and an approved accuracy (Pishtazteb, 2021). The testing was carried out per manufacturer's instructions (Pishtazteb, 2021) three times for each specimen, and the mean results were reported quantitatively in relative units (RU)/ml with a cut-off index (COI) value equal and above eight considered positive.]
12	N/R	
13	N/R	
14	YES	Identifying, controlling, and accounting for the possible confounders in the analysis were performed and the possible confounders were considered. Confounding variable controlling was performed using Multivariable logistic regression model.

Study: Gadani et al. DOI: 10.1016/j.ebiom.2021.103636

1	YES	The aim of the study is clearly defined. The exposure (anti-CD20 therapy) and desired outcome (humoral and T cell immune responses to SARS-CoV-2) are noted. Population: people with multiple sclerosis.
2	NO	(who): people with multiple sclerosis (pwMS) who had recently received the COVID-19 vaccine and were part of the COVID-RIMS study. – (where): Johns Hopkins MS center – (when): not specified.
3	N/R	101 patients volunteered for this study; the participation rate remains unclear.
4	YES	All participants were recruited from the same population. Similar inclusion and exclusion criteria were applied uniformly to all participants.
5	N/R	
6	YES	Patients were on DMTs before they receive vaccination. Also, the participants were recruited when they had been recently vaccinated.
7	YES	The phlebotomy was performed variably 4 to 8 (average 6.8) weeks after the terminal COVID-19 vaccination dose. This timeframe is relatively sufficient.
8	YES	The exposures were subdivided into 2 categories. Injectable therapy and Oral therapy. Time from last infusion of anti-CD20 therapies was also taken into consideration.
9	YES	The exposure measures in this study are parts of the patients' medical history, which are usually objective and reliable if registered by health care providers
10	N/A	The exposure measures in this research scenario do not require multiple assessments at different time points.
11	YES	Same humoral and T-cell response assays were consistently utilized for all participants. The outcome measures (dependent variables) were clearly defined, valid, reliable.
12	N/R	
13	YES	Data from all of the 101 patients with MS included were presented; thus, no follow-up losses are expected.
14	YES	Age, sex, and time from first dose of COVID-19 vaccination blood collection were adjusted using a logistic regression model.

Study: Gallo et al. DOI: 10.1007/s10072-021-05397-7

1	YES	The aim of the study is clearly defined. The exposure (Ocrelizumab) and desired outcome (humoral response to SARS-CoV-2 mRNA vaccine) are noted. Population: patients with multiple sclerosis and healthy controls
2	YES	(who): people with multiple sclerosis (pwMS) on Ocrelizumab and healthy subjects. – (where): Neurology clinic, University of Campania “Luigi Vanvitelli”, Naples, Italy. – (when): Since January 5th, 2021,
3	N/R	
4	YES	The inclusion and exclusion criteria were applied uniformly to all participants. Apparently, all participants were included in the same time period.
5	NO	No pre-enrollment estimates on the minimum sample size to detect a difference are provided. Only 4 patients are included, but, the study is extrapolating in nature and this is not a fatal flaw in this case.
6	YES	The exposure in this study is being treated with Ocrelizumab in people with multiple sclerosis. These participants were enrolled based on their exposure to Ocrelizumab. All subjects were seronegative at the baseline; the exposure had taken place prior to the outcome.
7	NO	Only 7 days after the second dose of vaccination, the serum samples were obtained. Given that subjects on ocrelizumab may need more doses of the vaccine to increase their chance of seroconversion, the incorporated interval seems less sufficient, even though it still is a month and a week later than the first dose.
8	YES	The exposure (ocrelizumab) is investigated at different levels, i.e., type, duration, previous DMT, and time from the last infusion to the first vaccine dose.
9	YES	The exposure measures in this study are parts of the patients’ medical history, which are usually objective and reliable if registered by health care providers
10	N/A	The exposure measures in this research scenario do not require multiple assessments at different time points.
11	YES	The outcome measures (dependent variables) were clearly defined, valid, reliable. [Sera were stored at − 20 °C and tested using the LIAISON ®SARS-CoV-2 TrimericS IgG assay (DiaSorin S.p.A,.Saluggia, Italy), an indirect chemiluminescence immunoassay (CLIA) technology for the detection of serum IgG antibodies to SARS-CoV-2 trimeric spike protein (anti-TSP IgG), including neutralizing antibodies . Performance and interpretation of results were done in accordance with the manufacturer’s instructions, and the IgG titers were expressed in Binding Antibody Units (BAU), an international standard unit, with 33.8 BAU/mL as cut-off value.]
12	N/R	
13	YES	All 4 patients had completed the follow-up.
14	N/R	

Study: Katz et al. DOI: 10.1016/j.msard.2021.103382

1	YES	The aim of the study is clearly defined. The exposure (Ocrelizumab) and desired outcome (Humoral and T-Cell Responses to SARS-CoV-2 Vaccination) are noted. Population: people with multiple sclerosis.
2	YES	(who): people with multiple sclerosis. – (where): Elliot Lewis Center for Multiple Sclerosis Care (MA, USA). – (who): between March 2, 2021, and July 1, 2021.
3	N/R	
4	YES	All participants were enrolled from the same population. Inclusion and exclusion criteria for being in the study were pre-specified and applied uniformly to all participants.
5	NO	No pre-enrollment estimates on the minimum sample size to detect a difference are provided.
6	YES	The exposure was assessed prior to the outcome. The investigators screened participants for the presence of SARS-CoV-2 antibodies prior to vaccination, to ensure the antibodies (outcome) were result of vaccination and not the preexisting antibodies. This study is a prospective observational cohort study.
7	YES	Serology tests were done 3 to 4 weeks after final dose of vaccination. This timeframe is relatively sufficient.
8	YES	The exposure (DMT) was categorized and inspected regarding the type (i.e., Ocrelizumab or Natalizumab), number of previous cycles, time between the last infusion and first vaccination.
9	YES	The exposure measures in this study are parts of the patients' medical history, which are usually objective and reliable if registered by health care providers.
10	N/A	The exposure measures in this research scenario do not require multiple assessments at different time points.
11	YES	The outcome measures (dependent variables) were clearly defined, valid, reliable. [The Roche Elecsys Anti-SARS-CoV-2 S immunoassay was performed per the manufacturer's regulations using the cobas e602 analyzer to identify individuals with an antibody response to SARS-CoV-2. This was done prior to the first COVID-19 vaccination dose and 3-4 weeks following the final vaccination dose. The Adaptive Biotechnologies T-Detect COVID Test was performed per the manufacturer's regulations using the Kingfisher Flex 711 system and the MagMax DNA Multi-sample Ultra 2.0 reagent kits to identify individuals with an adaptive T-cell immune response to SARS-CoV-2.]
12	N/R	
13	YES	Given the inclusion of follow-up data from all 48 participants in the final analysis, no losses in follow-up are expected.
14	YES	Demographic and clinical factors including weight, BMI, age, race, baseline level of disability, MS disease subtype, duration of disease, duration of treatment, time from prior relapse, and smoking status were considered and assessed as potential confounders and predictors of vaccine response (aimed outcome). Patients with multiple comorbidities were excluded to avoid immuno-senescence and other health conditions as confounders.

Study: König et al. DOI: 10.1136/jnnp-2021-327612

1	YES	The aim of the study is clearly defined. The exposure (Humoral immunity to SARS-CoV-2 mRNA vaccination) and aimed outcome (DMTs) are noted. Population: people with multiple sclerosis.
2	YES	(who): people with multiple sclerosis (pwMS). – (when): all patients who donated a blood sample were reported by 30 June 2021. – (where): Norway (invitations were sent on a national level)
3	N/R	
4	YES	The inclusion and exclusion criteria were applied uniformly to all participants. Apparently, all participants were included in the same time period.
5	NO	No pre-enrollment estimates on the minimum sample size to detect a difference are provided.
6	YES	The exposure was assessed prior to the study initiation and outcome measurement.
7	YES	Participants were assessed for the aimed outcome 3 to 12 weeks after full vaccination which is fairly sufficient.
8	YES	The exposure (DMT) is categorized and investigated at different levels, i.e., type, time from the last infusion to the first vaccine dose, etc.
9	YES	The information acquisition about the exposure history (i.e., DMTs) was done through digital questionnaire and national MS registry, which seems relatively reliable and valid.
10	N/A	The exposure measures in this research scenario do not require multiple assessments at different time points.
11	YES	The outcome measures (dependent variables) were clearly defined, valid, reliable. [Antibodies to full length Spike (HexaPro) from SARS-CoV-2 and the receptor-binding domain (RBD) were measured using a bead-based Flow cytometric assay13 in all included patients 3–12 weeks after full vaccination. Post-immunisation IgG titres were used as a correlate of protection,14 and reduced immunity was assumed in cases of IgG <70 arbitrary units (AU) corresponding to a level which was lower than found in 99% of all healthy vaccinated subjects.].
12	N/R	
13	YES	No follow-up losses are reported; also, none is expected, given the inclusion of data from all 1155 participants in the final analysis.
14	N/R	

Study: Madelon et al. DOI: 10.1101/2021.07.21.21260928

1	YES	The objective of the study is clearly defined. It is clearly mentioned that the study includes exposed and control participants. Exposure : anti-CD20 therapy – outcome: T cell responses 2 to mRNA-based COVID-19 vaccines
2	NO	(who): people with multiple sclerosis, people with rheumatic diseases, healthy controls
3	N/R	
4	YES	For enrolling the participants (3 groups), general and group-specific inclusion and exclusion criteria were applied uniformly among all participants. No specific information on study enrollment/conduction period is given, however, it is negligible and a short variance of participants' vaccination and enrollment is expected
5	NO	No pre-enrollment estimates on the minimum sample size to detect a difference are provided.
6	YES	The study is done prospectively. The Exposure (DMTs) were measured prior to outcome (T cell responses).
7	YES	The immune responses were assessed 30 day after the second dose of vaccination which is sufficient.
8	YES	The exposure in the participants (being treated with DMTs) were subdivided into 5 categories. Glucocorticoid, Methotrexate, Leflunomide, Rituximab, Ocrelizumab. The timeframe between the last dose of medications (exposure) receiving and the vaccination, the medications (exposure) doses receiving by the participants (pwMS and people with rheumatic diseases) were documented.
9	YES	The exposure measures in this study are parts of the patients' medical history, which are usually objective and reliable if registered by health care providers.
10	N/A	The exposure measures in this research scenario do not require multiple assessments at different time points.
11	YES	All antibody measurements were performed utilizing the Elecsys platform (Roche Diagnostics) and seroconversion was defined as > 0.8 IU/ml.
12	N/R	
13	YES	No follow-up losses are reported; also, none is expected, given the inclusion of data from all 59 participants in the final analysis.
14	YES	Individuals in the control group were matched for age.

Study: Pitzalis et al. DOI: 10.1101/2021.09.26.21264067

1	YES	The objective of the study is clearly defined. The exposure (disease-modifying therapies), participants (people with multiple sclerosis) , and the aimed outcome (humoral response to BNT162b2 vaccine) is clearly described.
2	YES	(who): people with multiple sclerosis (pwMS) and healthy controls. – (where): MS clinical centers in Cagliari and Sassari in Sardinia (Italy). – (when): between April and June 2021.
3	N/R	
4	NO	Although patients were recruited from a relatively homogenous population as controls; the inclusion and exclusion criteria were not clearly defined.
5	NO	No pre-enrollment estimates on the minimum sample size to detect a difference are provided.
6	YES	The measurement of exposure had preceded the outcome.
7	YES	The serum samples were obtained 30 days after the second dose of vaccination which is fairly sufficient.
8	YES	The type of the DMTs received by each patient is documented along with treatment duration and time between the last dose of treatment and inoculation.
9	YES	The exposure measures in this study are parts of the patients' medical history, which are usually objective and reliable if registered by health care providers.
10	N/A	The exposure measures in this research scenario do not require multiple assessments at different time points.
11	YES	[Detection of anti-SARS-CoV-2-S and anti-SARS-CoV-2-N antibodies in serum samples was performed using the electrochemiluminescence immunoassays Elecsys® Anti-SARS-CoV-S and Elecsys® Anti-SARS-CoV-N (Roche) on the automated Cobas e-411 analyzer, according to the manufacturer's instructions.]
12	N/R	
13	YES	No follow-up losses are reported; also, none is expected, given the inclusion of data from all 912 patients in the final analysis.
14	YES	The potential confounders were considered, including sex, age, EDDS, and smoking status.

Study: Sabatino et al. DOI: 10.1101/2021.09.10.21262933

1	YES	The objective of the study is clearly defined. The exposure (disease-modifying therapies), participants (people with multiple sclerosis), and the aimed outcome (SARS-CoV-2 vaccineinduced antibody and T cell immunity) is clearly described.
2	NO	(who): people with multiple sclerosis and healthy controls. (where): five UK MS centres (Cardiff, Newport, Nottingham, Royal London Hospital (Barts Health NHS Trust) and Swansea) – (when): not specified
3	N/R	
4	YES	Inclusion and exclusion criteria were uniformly applied.
5	NO	No pre-enrollment estimates on the minimum sample size to detect a difference are provided.
6	YES	The measurement of exposures preceded the outcome.
7	YES	Blood samples were obtained two weeks (for Comirnaty/BNT162b2 and mRNA-1273) or four weeks (for Ad26.COV2) after final dose of vaccination. Follow-up time is fairly sufficient.
8	YES	The exposure in the participants (being treated with DMTs) were subdivided into 6 categories. glatiramer acetate (GA), dimethyl fumarate (DMF), natalizumab (NTZ), sphingosine- 1-phosphate receptor modulator (S1P), rituximab (RTX), ocrelizumab (OCR); time from last infusion of anti-CD20 mAb was also documented.
9	YES	The exposure measures in this study are parts of the patients' medical history, which are usually objective and reliable if registered by health care providers.
10	N/A	The exposure measures in this research scenario do not require multiple assessments at different time points.
11	YES	The outcome measures (dependent variables) were clearly defined, valid, reliable. [Spectrally distinct Luminex beads were conjugated with trimeric spike protein (residues 1-1213), spike RBD (residues 328-533) (generously provided by Dr. John Pak, Chan Zuckerberg Biohub), or bovine-specific albumin fraction V (BSA) (Sigma-Aldrich #10735094001) at a concentration of 5 µg of protein per 1 million beads. Conjugation was done via an EDC/sulfo-NHS coupling strategy to terminal amines using antibody coupling kit following manufacturer's instructions (Luminex #40-50016) as performed previously. Antibody analysis is performed by coronaphage VirScan.]
12	N/R	
13	YES	No follow-up losses are reported; also, none is expected, given the inclusion of data from all 80 participants in the final analysis.
14	N/R	

Study: Sormani et al. DOI: 10.1016/j.ebiom.2021.103581

1	YES	The aim of this study is clearly defined. Exposure (disease modifying therapies), outcome (side effects and immunogenicity of SARS-CoV-2 mRNA vaccination), and the population (people with multiple sclerosis) are mentioned.
2	YES	(who): people with multiple sclerosis (pwMS). – (where): 35 MS Italian MS centers. - (when): between March 4, 2021 and July 9, 2021.
3	YES	85% of enrolled patients accepted to participate.
4	YES	Participants were recruited from a relatively homogenous population (MS centers across Italy) Inclusion and exclusion criteria were applied uniformly across all patients.
5	YES	The results in this article are interim results of an ongoing, large study; the planned number of participants is given, i.e., 2000.
6	YES	This study is performed prospectively and the exposures were measured prior to the outcome.
7	YES	The follow-up time of this study is relatively sufficient.
8	YES	The DMTs were categorized into 11 groups. Time since last infusion of an anti-CD20 agent (rituximab or ocrelizumab) is reported
9	YES	Data on patients' exposure were presented by their neurologists, as part of their medical history, registered by the respective clinics; thus, the data acquisition method appears reliable.
10	N/A	The exposure measures in this research scenario do not require multiple assessments at different time points.
11	YES	High-affinity pan-Ig antibodies to SARS-CoV-2 were measured by a centralized laboratory with a double-antigen sandwich-based electrochemiluminescence immunoassay (ECLIA), using commercial kits (Elecsys, Roche Diagnostics Ltd, Switzerland).
12	N/R	
13	NO	24 % of the eligible included patients didn't have the blood sample assessed. 780/1022 (76%)
14	YES	Age, sex, BMI, EDSS level, disease duration, presence of comorbidities, antibody levels in the pre-vaccination samples and vaccine type were adjusted among participants.

Study: Tallantyre et al. DOI: 10.1101/2021.07.31.21261326

1	YES	The aim of this study is clearly defined. Exposure (disease modifying therapies), outcome (serological response to COVID-19 vaccination), and the population (large multi-center cohort) are mentioned.
2	NO	(who): people with multiple sclerosis. – (where): five UK MS centers (Cardiff, Newport, Nottingham, Royal London Hospital (Barts Health NHS Trust) and Swansea) – (when): not reported.
3	N/R	
4	YES	The inclusion and exclusion criteria were applied uniformly among all participants.
5	N/R	
6	YES	The participants were exposed to the DMTs before the study.
7	YES	The follow-up time is around 4 weeks which is fairly adequate.
8	YES	Exposures (DMTs) were categorized to different groups. Details of DMT start dates, doses, total time on DMT, were documented from participants medical report.
9	YES	Details of DMT, e.g., start date, dose, total duration, were extracted from the patients' official medical records, as researchers were given permission to access by patients. Thus, the data acquisition method appears sound.
10	N/A	The exposure measures in this research scenario do not require multiple assessments at different time points.
11	NO	Dried blood spots were analyzed in two different laboratories with two different techniques. [In UHW (376), samples were analysed with the COVID-SeroKlir two-step enzyme-linked immunosorbent assay (ELISA) (Kantaro Biosciences, USA - supplied by EKF Diagnostics, UK). At QMUL(37) , samples were analysed using the Globody technique.]
12	N/R	
13	YES	Only one of the participants was excluded after initiation of the study.
14	YES	Logistic and linear regression models were used to account for the impact of possible confounders.

Study: Giossi et al. DOI: 10.1016/j.msard.2021.103415

1	YES	The aim of this study is clearly defined. Exposure (disease modifying therapies or immunosuppressant), outcome (serological response to BNT162b2 COVID-19 vaccination), and the population (people with multiple sclerosis) are mentioned.
2	YES	(who): consecutive healthcare workers with multiple sclerosis and controls. – (when): From February 2nd to April 2nd, 2021. – (where): three participating centers in Italy (Fondazione I.R.C.C.S. Istituto Neurologico Carlo Besta, Milano; I.R.C.C.S. Fondazione Mondino, Pavia; Azienda Ospedaliera Policlinico Umberto I, Roma)
3	N/R	
4	YES	The study population was relatively homogenous; the inclusion and exclusion were applied uniformly for each group of participants.
5	NO	No pre-defined sample size estimates were provided.
6	YES	This study is performed prospectively. Participants were exposed to medications prior to receiving vaccination.
7	YES	The follow-up duration is nine weeks from the first vaccine dose which is fairly sufficient.
8	YES	The exposures were subdivided to 9 groups; further data on starting date, last administration date, etc. were also investigated.
9	YES	The exposure measures in this study are parts of the patients' medical history, which are usually objective and reliable if registered by health care providers.
10	N/A	The exposure measures in this research scenario do not require multiple assessments at different time points.
11	YES	The outcome measures (dependent variables) were clearly defined, valid, reliable. [Samples were analyzed with SARS-CoV-2 IgG II Quant (Abbott) on the Architect instrument (Abbott) to quantitatively and qualitatively detect IgG antibodies against the Spike protein S1 receptor-binding domain (RBD).]
12	N/R	
13	Y	No follow-up losses are reported; also, none is expected, given the inclusion of data from all 39 MS patients in the final analysis.
14	N/R	

Study: Pompsch et al. DOI: 10.1186/s42466-021-00158-5

1	YES	The aim of this study is clearly defined. Exposure (Ocrellizuamb), outcome (T-cell response to COVID-19 vaccination), and the population (people with multiple sclerosis) are mentioned.
2	NO	(who): people with multiple sclerosis. – (where): Outpatient clinic for MS of the Alfried Krupp Hospital in Essen, Germany. – (when): not reported
3	N/R	
4	YES	The inclusion and exclusion criteria were applied uniformly to all participants.
5	N/R	
6	YES	Participants were exposed to Ocrelizumab(exposure) prior to vaccination and outcome measurement (T-cell response)
7	YES	The assays were performed about 28 days after the second dose of vaccination which is fairly sufficient.
8	YES	The level of exposure to Ocrelizumab is investigated as time from last infusion to first inoculation.
9	YES	The exposure measures in this study are parts of the patients' medical history, which are usually objective and reliable if registered by health care providers.
10	N/A	The exposure measures in this research scenario do not require multiple assessments at different time points.
11	YES	The outcome measures (dependent variables) were clearly defined, valid, reliable. [To assess SARS-CoV-2-specific cellular immunity, ELISpot assays were performed, using peptide pools of the Spike (S) 1, the S1/S2, the membrane (M) and the nucleocapsid (NC) protein (Miltenyi Biotec, Bergisch Gladbach, Germany) and an S1 protein of SARS-CoV-2 (S Sino, Sino Biological, Wayne, PA, USA). Antibodies were determined by a CE marked Anti-SARS-CoV-2 IgG semi-quantitative ELISA (Euroimmun, Lübeck, Germany).]
12	N/R	
13	N/R	
14	YES	Healthy controls were matched for confounders, such as age and sex.

Study: Tortorella et al. DOI: 10.1212/wnl.0000000000013108

1	YES	The aim of this study is clearly defined. Exposure (disease modifying therapies), outcome (anti Region-Binding-Domain (RBD) neutralizing antibodies and Spike (S)-specific T-cell response to full COVID-19 vaccination), and the population (people with multiple sclerosis) are mentioned.
2	NO	(who): health care workers and patients with multiple sclerosis. – (where): MS Centre of the Department of Neurosciences of San Camillo Forlanini Hospital (Rome, Italy). – INMI Lazzaro Spallanzani. – (when): not reported
3	N/R	
4	YES	Inclusion and exclusion criteria were applied uniformly among each group.
5	NO	No pre-estimated minimum of sample size with respect to the statistical power needed to detect a potential difference was reported.
6	YES	This study was performed prospectively.
7	YES	Blood samples were collected 23 days after the second dose of vaccination. This time frame is more than 3 weeks which is fairly sufficient.
8	YES	The DMTs (exposure) were subdivided into different categories; other treatment characteristics, e.g., duration, were assessed.
9	YES	The exposure measures in this study are parts of the patients' medical history, which are usually objective and reliable if registered by health care providers.
10	N/A	The exposure measures in this research scenario do not require multiple assessments at different time points.
11	YES	The outcome measures (dependent variables) were clearly defined, valid, reliable. [IFN-γ levels were quantified in the plasma samples using an automatic ELISA (ELLA, Protein Simple). Humoral response to vaccination was assessed by quantifying the anti-Nucleoprotein IgG and the anti-RBD IgG (Architect® i2000sr Abbott Diagnostics, Chicago, IL, USA).]
12	N/R	
13	YES	No follow-up losses are reported; also, none is expected, given the inclusion of data from all 108 MS patients in the final analysis.
14	YES	Multivariable adjustment for confounding factors was done.

Study: van kempen et al. DOI: 10.1016/j.msard.2021.103416

1	YES	The exposure, and the aimed outcome is clearly defined.
2	NO	(who): people with multiple sclerosis. – (where): Amsterdam MS Center. – (when): not reported.
3	YES	Only 2 out of the initial 89 enrolled patients were excluded from the study.
4	YES	All patients with multiple sclerosis were recruited from same population. The inclusion and exclusion criteria were uniformly applied to each group.
5	NO	No pre-estimated minimum of sample size with respect to the statistical power needed to detect a potential difference was reported.
6	YES	This study is performed prospectively. The group of exposed patients were receiving Ocrelizumab prior to the outcome.
7	YES	The time of follow up is around 28 days after the second vaccine dose which is sufficient.
8	YES	The exposure (ocrelizumab) was further assessed with respect to time from last infusion to vaccination, number of previous infusions, etc.
9	YES	Clinical data were retrieved from patients' medical files, which can be considered reliable and valid.
10	N/A	The exposure measures in this research scenario do not require multiple assessments at different time points.
11	YES	The outcome measures (dependent variables) were clearly defined, valid, reliable. [SARS-CoV-2 antibodies against RBD were measured at Sanquin using an IgG specific ELISA (Steenhuis et al., 2021) Fresh whole blood was drawn at baseline (prior to vaccination) for measuring CD19+ B-cells with a highly sensitive assay (Koutsakos et al., 2021). a qualitative anti-RBD bridging assay was also used as this assay has better sensitivity to detect low levels of antibodies (Vogelzang et al., 2020).].
12	N/R	
13	YES	No follow-up losses are reported; also, none is expected, given the inclusion of data from all 87 MS patients in the final analysis.
14	N/R	

Study: Capone et al. DOI: 10.1007/s13311-021-01165-9

1	YES	The aim of study is clearly explained.
2	YES	(who): people with multiple sclerosis. – (where): three MS centers of Rome, Italy (Università Campus Bio-Medico, Fondazione Policlinico Universitario "A. Gemelli" IRCCS, and San Filippo Neri Hospital). – (when): between 26 April 2021 and 4 June 2021.
3	YES	Only four out of 140 patients, initially enrolled, were excluded (previous COVID-19 infection).
4	YES	The inclusion and exclusion criteria were applied uniformly among all patients.
5	NO	No prior-to-study estimates on minimum required sample size were given.
6	YES	This study was performed prospectively.
7	YES	The blood test was performed 30 days after the second dose of vaccination. This time frame is sufficient.
8	YES	The DMTs (exposure) were subdivided into different categories.
9	YES	Clinical data were retrieved from patients' electronic health records in MS centers, which can be considered reliable and valid.
10	N/A	The exposure measures in this research scenario do not require multiple assessments at different time points.
11	YES	The outcome measures were clearly defined, valid, reliable, and implemented consistently across all study participants. [The detection of SARS-CoV-2 IgG antibodies in blood samples was performed using a chemiluminescent microparticle immunoassay for quantitative and qualitative detection of IgG against SARS-CoV-2 nucleoprotein (Spike-RBD S1) (SARSCoV- 2 IgG II for use with ARCHITECT; Abbott Laboratories, Abbott Park, IL, USA; ref: 6S60-22).]
12	N/R	
13	YES	No follow-up losses are reported; also, none is expected, given that the whole study follow-up period was 1 month after second inoculation, and authors have reported that no case of SARS-CoV-2 infection has been detected among the participants during this period.
14	YES	Demographic and clinical factors of potential confounding effect were assessed and were not different between participants with different outcomes.

Study: Maniscalco et al. DOI: 10.1007/s13311-021-01165-9

1	YES	The aim of study is clearly explained.
2	YES	(who): people with multiple sclerosis. – (where): MS center of the Cardelli hospital, Naples, Italy. – (when): from March 2021 to June 2021.
3	YES	85.1% (n=149) of eligible individuals (mentioned above) met the inclusion criteria.
4	YES	The inclusion and exclusion criteria were applied uniformly among all patients. All participants were Caucasian.
5	NO	No prior-to-study estimates on minimum required sample size are reported.
6	YES	This study was performed prospectively; exposure was assessed prior to outcome measurement.
7	YES	SARS-CoV-2 IgG was tested for 21 days after the second vaccination, which is relatively enough.
8	YES	The DMTs (exposure) were subdivided into different categories; in the case of depletant medications, interval between last dose and vaccination was also assessed.
9	YES	The exposure measures in this study are parts of the patients' medical history, which are usually objective and reliable if registered by health care providers.
10	N/A	The exposure measures in this research scenario do not require multiple assessments at different time points.
11	YES	The outcome measurement methods were clearly defined, valid, reliable, and implemented consistently across all study participants. [Quantitative determination of antibodies to the SARS-CoV-2 spike protein was carried out by Roche Elecsys® Anti-SARS-CoV-2 S assay (Roche Diagnostics International Ltd, Rotkreuz, Switzerland). The assay was performed using a recombinant protein representing the RBD of the S antigen leading to a double-antigen sandwich assay complex which favors detection of high affinity antibodies against SARS-CoV-2 (range between 0.4 to 250 U/mL), resulting in a sensitivity of 98.8% (95% CI: 98.1 – 99.3%).
12	N/R	
13	YES	No follow-up losses are reported; also, none is expected, given the inclusion of data from all 125 MS patients in the final analysis.
14	YES	Healthy controls were matched for age, and sex; possible confounding factors were adjusted for in the multilinear regression model.

Study: Türkoglu et al. DOI:10.1016/j.msard.2022.103524

1	YES	The aim of study is clearly explained.
2	NO	(who): consecutive patients with multiple sclerosis - (where): Amsterdam MS Center. – (when): not reported.
3	N/R	
4	YES	The inclusion and exclusion criteria were applied uniformly among all patients.
5	NO	No prior-to-study estimates on minimum required sample size are reported.
6	YES	This study was performed prospectively; exposure was assessed prior to outcome measurement.
7	YES	SARS-CoV-2 IgG was tested for 28 days after each vaccination dose, which is relatively enough.
8	YES	The duration of therapy with DMTs (exposure) were measured and taken into account.
9	YES	The exposure measures in this study are parts of the patients' medical history, which are usually objective and reliable if registered by health care providers.
10	N/A	The exposure measures in this research scenario do not require multiple assessments at different time points.
11	YES	The outcome measurement methods were clearly defined, valid, reliable, and implemented consistently across all study participants. [Sera were collected 28 days after both first (day 28) and second (day 58) vaccinations and kept at 80 ◦C freezer until analysis. Immunoassay for the detection of SARS-CoV-2 IgG antibodies in sera was performed using Euroimmun (Luebeck, Germany) quantitative ELISA kit, designed for detection of antibodies to spike protein of the SARS-CoV-2 virus. The assay was performed following the manufacturer's instructions and an index value higher than 1.1 was considered positive.]
12	N/R	
13	YES	No follow-up losses are reported.
14	YES	Healthy controls were matched for age, and sex; no significant correlation was found between the outcome and possible confounding factors in the regression model.

Study: Ozakbas et al. DOI: 10.1016/j.msard.2022.103486

1	YES	The aim of study is clearly explained.
2	YES	(who): patients with multiple sclerosis - (where): Dokuz Eylul University Izmir, Turkey – (when): May 2021 and September 2021
3	N/R	
4	YES	The inclusion and exclusion criteria were applied uniformly among all patients.
5	NO	No prior-to-study estimates on minimum required sample size are reported.
6	YES	This study was performed prospectively; exposure was assessed prior to outcome measurement.
7	YES	A minimum of interval between the second dose and serum sampling of two-weeks was set, which appears relatively enough.
8	YES	DMTs (exposure) were categorized in terms of medication type. However, treatment duration, or time from last dose of treatment to vaccination/serum sampling was not studied in depth for a better quantification of exposure into a continuous measure.
9	YES	The exposure measures in this study are parts of the patients' medical history, which are usually objective and reliable if registered by health care providers.
10	N/A	The exposure measures in this research scenario do not require multiple assessments at different time points.
11	YES	The outcome measurement methods were clearly defined and implemented consistently across all study participants. [The primary outcome was the quantification of antibody response after two doses of the SARS-CoV-2 vaccine. Antibody levels were transformed on a Log10 scale to normalize their distribution, and the 'AU/mL Log' name was used after that. For antibody titer less than the detectable limit of 21AU/mL, in order to prevent missing data during Log10 transformation, a titer of 0.01Au/mL was used. Antibody titer of patients with MS who did not use DMT (pwMSw/oT) was compared as a separate group with pwMS with treatment and HC.]
12	N/R	
13	YES	Only three (out of 593) patients were excluded from the study; one due to unsuccessful serum sample acquisition and the other due to incident hemolysis in the sample, and the other due to their medication type (RTX).
14	YES	Healthy controls were matched for age and sex.