Risk of COVID-19 in people with multiple sclerosis who are seronegative following vaccination.

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Abstract

Background: People with MS (pwMS) treated with certain disease modifying therapies have attenuated IgG response following COVID-19 vaccination, however the clinical consequences remain unclear.

Objective: To report COVID-19 rates in pwMS according to vaccine serology.

Methods: PwMS with available (i) serology 2-12 weeks following COVID-19 vaccine 2 and/or vaccine 3 and (ii) clinical data on COVID-19 infection/hospitalisation were included. Logistic regression was performed to examine whether seroconversion following vaccination predicted risk of subsequent COVID-19 infection after adjusting for potential confounders. Rates of severe COVID-19 (requiring hospitalisation) were also calculated.

Results: 647 pwMS were included (mean age 48 years, 500 (77%) female, median EDSS 3.5 and 524 (81%) exposed to DMT at the time of vaccine 1). 472/588 (73%) were seropositive after vaccines 1&2, and 222/305 (73%) after vaccine 3. Seronegative status after vaccine 2 was associated with significantly higher odds of subsequent COVID-19 infection (odds ratio (OR) 2.35 [1.34-4.12], p=0.0029), whereas seronegative status after vaccine 3 was not (OR 1.05 [0.57-1.91]). Five people (0.8%) experienced severe COVID-19, all of whom were seronegative after most recent vaccination.

Conclusions: Attenuated humoral response to initial COVID-19 vaccination predicts increased risk of COVID-19 in pwMS but overall low rates of severe COVID-19 were seen.

Introduction

Reduced vaccine responses in people with multiple sclerosis (MS) taking disease modifying therapies (DMTs) have been subject to substantial study in the context of the COVID-19 pandemic. Attenuated vaccine responses have been described across multiple cohorts in people with MS (pwMS) exposed to anti-CD20 monoclonal antibodies and sphingosine-1-phosphate (S1P) modulators.[1-4] Studies of large MS cohorts during 2020, before the widespread vaccine rollout, highlighted an excess risk of COVID-19-related morbidity and mortality in pwMS treated with certain DMTs, including some of those associated with reduced vaccine response.[5,6]

Whilst the development of vaccines against COVID-19 has markedly reduced hospitalisations for the general public,[7] the risk of COVID-19 infection and severe outcomes in people with MS with attenuated vaccine responses is not yet fully understood. Some authors have demonstrated that COVID-19 vaccination reduces infection risk in all people with MS regardless of DMT exposure,[3,8,9] whilst others suggest that people taking anti-CD20 and S1P modulating drugs do not derive as much benefit from COVID vaccines at a population level.[10] However, these studies did not measure serology in all participants, instead focussing on clinical infection. Lack of seroconversion after initial vaccination, especially in the context of anti-CD20 and S1P treatments, has been reported to be associated with increased rates of COVID-19 by some,[11-14] but not all,[15] groups. The differential protective effect of vaccination against severe infections in those exposed to DMT associated with lower seroconversion is less clear, given the relative rarity of severe infection in the post-vaccination era.[16,17] Alongside this, there remains uncertainty about the additional value of booster vaccination on infection rates.[12,18]

Clinical correlates of attenuated vaccine responses have been uncertain to date, raising challenges in providing guidance for this group. Early studies have indicated an increased rate of breakthrough infection in those with suboptimal vaccine response, however seroconversion following third dose (booster) vaccination was not measured.[12] Those with attenuated humoral immune responses may

still have intact T-cell responses, and both humoral and T-cell vaccine responses appear to augment following booster vaccine administration.[19] The impact of vaccination programmes on both infection risk and infection severity in people taking immunomodulatory and immunosuppressive agents remains a source of concern to many, and in particular pwMS.

In order to address this area of need, we aimed to report COVID-19 infection rates and severity in a large cohort of pwMS for whom post-vaccine humoral response data was available.

Methods

PwMS who had previously taken part in a large seroprevalence study,[1] were considered for inclusion. In brief, as part of the seroprevalence study, people with MS provided one or more dried blood spots for measurement of antibodies to SARS-CoV-2 (S1 subunit of the spike protein) during 2020-2022, and provided consent for review of their medical records. For this study, we selected those fulfilling the following criteria:

(i) available data on humoral response to COVID-19 spike protein from a sample taken 2-12 weeks after COVID-19 vaccine 2 and/or vaccine 3 and/or vaccine 4,

(ii) available medical records providing longitudinal information on dates of vaccination, NHS-recorded COVID-19 infection (confirmed by either PCR or point of care antigen testing [lateral flow]), and hospitalisation.

A sample window of 2-12 weeks was chosen based on data showing the pattern of waning humoral response following COVID-19 vaccination, showing that the interval taken to wane from seropositive to seronegative responses almost always exceeds 84 days, even in people on immunosuppression.[20] Dried blood spots were collected prospectively, and IgG against the SARS-CoV2 spike protein was determined using either the FDA-approved EuroImmun (PerkinElmer) enzyme-linked immunosorbent assay (ELISA), or Kantaro (EKF Diagnostics, UK) assay according to the manufacturer's instructions, as previously described.[19] Validation of the EuroImmun assay has

previously demonstrated that plasma/serum and DBS specimens produce equivalent results,[21] and agreement between assays for positive versus negative is high.[19]

We combined data from electronic medical records and questionnaires completed by patients at the time of each blood sampling in order to identify any symptomatic episodes. We cross-referenced patient-reported infections with lateral flow (self-reported and/or electronic health record) and PCR (electronic health record) data. Medical records were also used to confirm the severity and outcomes of any COVID-19 illness. Participants were considered to have a mild COVID-19 infection if they had symptoms of infection, confirmed by either lateral flow or PCR testing, which did not require supplemental oxygen and hospitalisation. Severe COVID-19 infection was defined as laboratory (PCR)-confirmed COVID-19 requiring hospitalisation with oxygen administration and/or death from COVID-19.

We used medical records to obtain information on dates of exposure to DMTs, and most recent disability status according to Expanded Disability Status Scale (EDSS). Participants were categorised according to DMT exposure status at the time of first COVID-19 vaccination. People were considered exposed to DMT if they had received fingolimod, dimethyl fumarate, teriflunomide, glatiramer acetate or beta-interferon within 4 weeks of their vaccine. For infrequently dosed DMTs people were considered exposed if they had received alemtuzumab or cladribine within 4 years,[22,23] ocrelizumab within 12 months,[24] and natalizumab within 8 weeks[25] of their vaccine. These intervals were selected by the authors based on the different schedules of delivery and expected duration of action of the DMTs being studied. People who switched to an alternative DMT from ocrelizumab or fingolimod during the course of the study were excluded due to the known differing impact of these DMT on vaccine response. All other participants were categorised as unexposed to DMTs.

Ethical approval

This study has Research Ethics Committee approval (REC 20/SW/0104 [South East Wales REC – covering samples processed in Cardiff] and 20/NE/0176 [Newcastle North Tyneside REC – covering QMUL samples]).

Statistical analysis

Categorical variables were reported in terms of counts (%) and continuous variables were reported in terms of mean (SD) or median (IQR) if skewed. Univariate and multivariate logistic regression were used to identify whether seroconversion following a 2nd or 3rd COVID-19 vaccine was associated with differential risk of COVID-19 infection, adjusting for other potential factors. Data on serostatus after 4th COVID-19 vaccine was not incorporated into the analysis due to low numbers (n=50) and limited follow up duration.

For participants receiving ocrelizumab, univariate and multivariate logistic regression were used to investigate (1) whether time since most recent ocrelizumab infusion at vaccination predicted vaccine response (time since ocrelizumab was dichotomised to 0-3 months and >3 months prior to vaccine 1, or 0-5 months and >5 months prior to vaccine 2), and (2) whether COVID-19 infection at any time prior to vaccine 2, was associated with seroconversion following vaccine 2. Other potentially mediating factors included in the analysis were age at vaccine 1, sex, disability (EDSS <6,>=6 or unknown), inter-vaccine interval (days between vaccine 1 to vaccine 2), and COVID-19 infection prior to vaccine 2 (for analysis (1). Duration of treatment prior to vaccination and switches other than from ocrelizumab/fingolimod were not included in the model due to power considerations, the risk of introducing type 2 statistical error, and previously demonstrated equivalence between many DMT in terms of vaccine response with no impact of duration of treatment.[1,4] Descriptive statistics were used to report rates of severe COVID-19; further analysis was not performed due to low numbers.

All p-values were evaluated at 95% significance. Missing data were either placed into an "Unknown" category or were not imputed. All statistical analysis of the data was conducted using R software version 4.1.1 (R Core Team, 2020).

Results

647 participants fulfilled the inclusion criteria. 588 participants had provided samples post vaccine 2, 305 participants post vaccine 3, and 50 participants post vaccine 4 (Figure 1). Demographic and clinical characteristics of the study population are summarised in Table 1. Mean age at initial COVID-19 vaccine was 48 years, 500 (77%) were female, 522 (80%) had relapsing MS, and 524 (81%) were exposed to DMT at the time of initial vaccination. Overall, 427 out of 588 (73%) were seropositive after vaccines 1&2, and 222 out of 305 (73%) after vaccine 3. The observed similarity in seropositive status after vaccines 2 and 3 can be explained by responder bias leading to cohort enrichment with people who were seronegative after vaccine 2. For those 49 people with MS who provided blood samples after each of COVID-19 vaccines 2, 3 and 4, there was a sequential increase in seropositive rates with each booster (8%, 27%, 33%). Median time between vaccine 2 and 3 was 197 days (IQR 183-218) and median follow-up time after vaccine 3 was 265 days (IQR 226-291 days). The time between vaccine doses did not differ significantly between participants on different DMT (median vaccine 1-2 interval 77 days for people receiving ocrelizumab (IQR 54-77 days), natalizumab (IQR 60-82.5 days), dimethyl fumarate (IQR 69-78 days) and 74 days for those on alemtuzumab (IQR 47-77 days). Similarly, there was no significant difference in the vaccine 2-3 interval, although a trend towards a shorter interval was seen in those on ocrelizumab (median interval: ocrelizumab 182 days [IQR 158-208], natalizumab 196 days [IQR 180-221], alemtuzumab 203 days [IQR 184-219], dimethyl fumarate 195 days [IQR 183-208].

173/647 (27%) participants experienced at least one documented COVID-19 infection. 63 people reported COVID-19 infection between vaccines 2 and 3, of whom 56 had available serology following vaccine 2. 118 people reported COVID-19 following vaccine 3, of whom 68 had serology available following vaccine 3. Three people reported COVID-19 following each vaccine, meaning that 124 infections in 121 people were included overall. The proportions of people who seroconverted and

proportion who developed symptomatic COVID by DMT class are shown in Figure 2, which includes only those people with available serology at the relevant timepoint.

Between vaccines 2 and 3, 25/161 (15.5%) people who were seronegative following vaccine 2 developed COVID-19 infections, of which 4 were severe. 31/427 (7.3%; no severe infections) of those who were seropositive at this time developed COVID-19. Following vaccine 3, 19/83 (22.9%) people who were seronegative following vaccine 3 developed COVID-19 infections, of which 1 was severe, compared to 49/222 (22.1%; no severe infections) of those who were seropositive. It is important to note that the background incidence of COVID-19 was not stable during these two periods, and the follow-up time was different following each vaccination time point; thus the proportion of people developing COVID-19 cannot be directly compared between post-vaccine 2 and post-vaccine 3 time periods.

Seronegative status after vaccine 2 was associated with significantly higher odds of subsequent COVID-19 infection (odds ratio (OR) 2.35 [1.34-4.12], p=0.0029) (Table 2), whereas seronegative status after vaccine 3 was not (OR 1.05 [0.57-1.91], p=0.88). These results did not change substantially after adjustment for age, sex, EDSS and COVID-19 infection prior to vaccination in multivariate analysis (infections after vaccine 2: OR 2.29, 95% CI 1.29-4.06, p=0.0047; infections after vaccine 3: OR 1.08, 95% CI 0.58-2.03, p=0.80). Overall, 119 out of 124 (96%) COVID-19 infections were mild, while 5 (4%) were severe.

The 5 severe infections all occurred in pwMS who were seronegative after their most recent COVID vaccine. Four out of 5 had been receiving ocrelizumab since 2019/2020, of whom two (EDSS 1 and EDSS 2.5) received ward-based care for 9-10 days, including oxygen +/- Ronapreve (casirivimab and imdevimab) +/- tocilizumab, and subsequently recovered. One pwMS in their 40s with EDSS 5.5, treated with ocrelizumab, who smoked but had no other co-morbidity, required ITU care for COVID-19 occurring after vaccine 2. They received dexamethasone, sarilumab, remdesivir, Ronapreve, Meropenem and non-invasive ventilation, but were ultimately discharged home on oxygen. The

other pwMS who experienced severe COVID-19 after ocrelizumab was in their late 60s, and had EDSS 6.5, but no comorbidity. They were treated for 10 days with oxygen and ward-based care but sadly died. The other pwMS who experienced severe COVID-19 was in their late 60s, with EDSS 1.0, and had previously received alemtuzumab in 2016/2017. They received ward-based care for approximately 6 weeks including dexamethasone and antibiotics, and subsequently recovered.

Time from most recent ocrelizumab dose to first vaccine dose (vaccine 1) appeared to influence odds of seroconversion following vaccine 2, with those having their initial vaccination within 3 months of ocrelizumab having a 5-fold increase in risk of being seronegative (7/68 (10.3%) versus 21/61 (34.4%) of those dosed more than 3 months prior; univariate model odds ratio, OR of seronegative status 4.57, 95% CI 1.78-11.76, p=0.0016) (Figure 3). This relationship persisted in a multivariate model accounting for age, sex, COVID-19 infection prior to vaccine 2, and inter-vaccine interval (OR 4.52, 95% CI 1.66-12.29, p=0.0031). Where time between ocrelizumab dosing and vaccine 2 was studied, capturing infusions occurring between vaccines 1 and 2, a similar relationship was seen albeit with lower point estimates (12/88 (13.6%) dosed within 3 months seroconverted vs 16/51 (31.4%) of those dosed more than 3 months prior; OR for seronegative status: univariate analysis OR 2.51, 95% CI 1.07-5.90, p=0.034; multivariate analysis OR 2.55, 95% CI 1.04-6.24, p=0.041).

In pwMS treated with ocrelizumab, COVID-19 infection prior to vaccination was associated with increased odds of seroconverting following vaccine 2. Infection prior to vaccine 2 was associated with an OR of 4.02 (95% CI 1.56-10.35, p=0.0039; multivariate model with time between ocrelizumab and vaccine 1, age, sex and inter-vaccine interval OR 3.32, 95% CI 1.2-9.00. p=0.0184). This relationship persisted when time between ocrelizumab dosing and vaccine 2 was used as a mediator in the multivariate analysis, capturing those dosed between vaccinations (multivariate OR 4.02, 95% CI 1.52-10.61, p=0.0050).

Discussion

A key observation from this study was that in a vaccinated cohort of people with MS, severe COVID-19 was uncommon, and the vast majority (96%) of reported infections were mild. However, we identified a greater than 2-fold excess risk of COVID-19 in pwMS who remained seronegative after their initial course of COVID-19 vaccination. The second key finding in our cohort was that this excess risk was no longer evident after the third (booster) COVID-19 vaccination, possibly due to the incremental effect of repeated vaccination on T-cell immune responses[19], although other factors need to be considered. It must be noted that this cohort was enriched for people who were seronegative following vaccination, and so rates of infection are likely to be an overestimate for the total vaccinated MS population.

Whilst all episodes of severe COVID-19 in this study occurred in pwMS who were seronegative following two or more vaccinations, the majority of COVID-19 cases were mild and did not result in hospital admission. This may highlight the role of T-cell immunity, and we and others have shown that anti-SARS-CoV2 T-cell responses are measurable in many people who remain seronegative after COVID-19 vaccine 3.[19,26] People with MS treated with anti-CD20 DMTs have consistently been shown to have low rates of seroconversion but relatively normal T-cell responses to SARS-CoV2 after COVID-19 vaccination.[27-37] In contrast, in people treated with S1P modulators, initial COVID-19 vaccination is associated with attenuated humoral response but that responds somewhat to re-vaccination, while persistently low or absent T-cell responses to SARS-CoV2 are seen in this group.[31,33,35,36,38]

It is also likely that changes in the treatment of COVID-19, emerging concurrently with the provision of booster vaccines, may have improved outcomes for people with MS on treatments associated with reduced vaccine responses who developed COVID-19 after vaccine 3. Additionally, the potential ability to generate antibodies in response to infection even when not present on routine testing may have played a role. Whilst it could be argued that differences in vaccination schedule offered to people on DMT may have played a role in differential infection risk, we did not see statistically

significant evidence of this at group level, although it must be noted that this can obscure marked differences in individual behaviours.

The fact that all 5 severe COVID-19 cases occurred in those who remained seronegative following vaccination (four after vaccine-2 and one after vaccine-3), highlights several important issues. It raises a question about whether pwMS on certain DMTs should have their vaccine responses measured routinely, especially as response durability has not been assessed in those on DMTs. Identification of people with suspected or proven secondary immune deficiency may allow personalisation of their care e.g. they should be prioritised for booster vaccinations, should be able to promptly access antivirals and antibody therapies in the event of COVID-19, and certain individuals may even benefit from prolonged courses of antivirals.

Our finding that time from ocrelizumab dosing appears to impact the odds of seroconversion is not unexpected. Whilst we analysed our cohort according to a binary definition of time between dosing and vaccination, the relationship between the timing of infusion/vaccination and odds of seroconversion is complex and likely incorporates multiple individual-level factors including time on therapy, time to B-cell reconstitution, and age. Our data indicate that where it is possible to wait 3-5 months from ocrelizumab dosing to vaccination, this has a positive impact, increasing the proportion of serological responders from 10-15% to over 30%. However, vaccinating remains preferable to not vaccinating in periods of high background prevalence, since some of those vaccinated early after ocrelizumab do still develop a serological response. It may emerge that an additional, appropriately timed booster vaccination will benefit pwMS who receive vaccination(s) during the immediate interval following ocrelizumab infusion. Where other anti-CD20 therapies are used with differing dosing schedules (for example extended interval dosing of anti-CD20s),[39] optimal timing of vaccination in those people with MS established on therapy remains unclear. Similarly, how to manage dosing of sphingosine-1-phosphate inhibitors, of which fingolimod has been associated with

reduced vaccine efficacy, requires further study. In general, evaluation of serostatus and, where warranted, vaccination prior to therapy initiation is recommended.

This work is subject to several limitations. By focusing on infection severity rather than population PCR screening for infection, it is likely that we have missed asymptomatic infection. However, as SARS-CoV-2 has become an endemic infection with seasonal transmission, infection severity and hospitalisation (the focus of our study) have become major concerns. It is also possible that people with mild COVID-19 (not requiring hospitalisation) may have experienced long-term symptoms and/or worsening of their neurological status, since this was not measured. It is also the case that the prevalence of COVID-19 and thresholds/methods for testing both evolved over the duration of this study. Our cohort is enriched for people with RRMS, as patients on DMT were more likely to be engaged with the project for various reasons, so may not fully reflect the wider MS population. We did not systematically collect data on other risk factors for COVID-19. It is possible that survivor bias may have played some part in our results, if those pwMS most vulnerable to COVID-19 experienced it early, and gained some additional protection as a result, making them more resilient to infection during the later follow-up period.

This study predated the widespread use of ofatumumab, ponesimod or siponimod in the UK, which were therefore not studied. By using assays directed against the spike protein, it is conceivable that some participants who were documented to have seroconverted following vaccination had in fact seroconverted due to infection prior to sampling. Despite this, the ability to generate antibody responses to either infection or vaccination remains an important marker of immunocompetence. The use of slightly different assays in this cohort, albeit with high agreement on positive versus negative status, limited our ability to explore the relationship between antibody titre and infection rates. The addition of T-cell measures of immunity, particularly in those who do not generate humoral immune responses to vaccination remains important. However, measuring T cell responses

requires in-person attendance for blood sampling, and given concerns around hospital attendance in people with MS, such investigations were not feasible to carry out at scale in this cohort.

For many pathogens, epidemiological studies and consensus on assays have evolved over years to provide quantifiable laboratory correlates (typically IgG concentrations) that infer protection against infection and/ or severe outcomes of infection [13]. Due to its recency, the clinical correlates of measurable antibody and T-cell responses to COVID-19 are not yet established. Further standardisation of assays, as well as investigation of the role of humoral versus T-cell immunity to SARS-CoV2, are both needed to establish the laboratory metrics most predictive of protection against (severe) COVID-19.

Conclusion

Whilst failure to mount a humoral response to initial COVID-19 vaccination appears to expose pwMS to a higher risk of COVID-19 infection, the overall rates of severe infection remain low. Our data suggest that booster vaccines provide incremental benefits for humoral response, but could also mitigate infection risk even in those who remain seronegative. All pwMS should be encouraged to follow vaccination schedules to obtain maximum possible protection. T-cell and antibody testing of pwMS on certain DMTs may allow more individualised counselling on infection risk. Uncertainties remain over whether DMTs should be interrupted to augment the immune response to vaccination. The rapidly evolving MS therapeutic landscape provides a challenge; analyses of vaccine response will be essential for new agents, particularly those with new modes of action.

Author contributions

ECT, RD, SJ, NPR, NV, GG, KS, DB, MW and SM contributed to the conception and design of the study; SAZ, MU, CW, PT, NV, AR, VA, RC, NE, KG, AG, KEH, AH, GI, MJ, AK, SJM, SJ and SL contributed to the acquisition and analysis of data; SHZ, ECT, PT and RD contributed to drafting the text or preparing the figures.

Declaration of Conflicting Interests

Biogen, Merck, Novartis, Roche, Sanofi/Genzyme, Teva all manufacture multiple sclerosis disease modifying therapies that were used in this study, or which could be affected by the study. The following authors have received speaker fees, consultancy fees and/ or travel expenses to attend educational meetings from one or more of these companies: ECT, DB, RD, GI, KEH, NE, GG, AK, NPR, MW, KS. The following authors declare no conflicts of interest: SAZ, MU, CW, AR, PT, NV, AR, VA, RC, KG, AG, AH, ASK, SL, SJM, SJ.

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Figure Legends

Figure 1: Flow diagram illustrating patients, samples and outcomes in the current study

Figure 2: Proportion of people who seroconverted, and who developed symptomatic COVID-19 infection, following each vaccination according to DMT class. DMT classes were collapsed in order to provide sufficient numbers. (a) between vaccines 2 and 3; (b) following vaccine 3.

Figure 3: Distribution of time from most recent ocrelizumab dose to COVID vaccine 1 and 2 (v1 and v2) for those who are seropositive and seronegative. (A) Boxplot illustrating distribution of time from ocrelizumab to v1 (days; displayed on the y axis) stratified by serostatus following vaccine 2. (B) Boxplot illustrating distribution of time from ocrelizumab to v2 (days; displayed on the y axis) stratified by serostatus following vaccine 2. Boxplots display median (central line), interquartile range (box margins) and total range (whiskers). (C) Density plot showing distribution of time from ocrelizumab dose to v1, versus seroconversion, in the whole cohort. The time in days between ocrelizumab infusion and v1 is displayed on the x axis, and the proportion of the total number of participants on the y axis. Seronegative participants are shaded turquoise and seropositive participants red. The cut off used in the binary analysis (3 months) is indicated by the solid red line. (D) Density plot showing distribution of time from ocrelizumab dose to v2 and seroconversion in the whole cohort and split by site. The time in days between ocrelizumab infusion and vaccine 2 is displayed on the x axis, and the proportion of the total number of participants on the y axis. Seronegative participants are shaded turquoise and seropositive participants red. The cut off used in the binary analysis (5 months) is indicated by the solid red line.