THERAPEUTIC ADVANCES in
Neurological Disorders

Original Research

Long-term immunological consequences of anti-CD20 therapies on humoral responses to COVID-19 vaccines in multiple sclerosis: an observational study

Ther Adv Neurol Disord

2022, Vol. 15: 1–9

DOI: 10.1177/17562864221092092

Tobias Moser, Ciara O'Sullivan, Ferdinand Otto, Wolfgang Hitzl, Georg Pilz, Kerstin Schwenker, Cornelia Mrazek, Elisabeth Haschke-Becher, Eugen Trinka, Peter Wipfler* and Andrea Harrer*

Correspondence to:
Tobias Moser
Department of Neurology, Christian Doppler University Hospital, Paracelsus Medical University and Center for Cognitive Neuroscience, European Reference Network EpiCARE, Ignaz-Harrer-Straße 79, 5020 Salzburg, Austria.
t.moser@salk.at

Ciara O'Sullivan
Ferdinand Otto
Georg Pilz
Kerstin Schwenker
Peter Wipfler
Department of Neurology, Christian Doppler University Hospital, Paracelsus Medical University and Center for Cognitive Neuroscience, European Reference Network EpiCARE, Salzburg, Austria

Wolfgang Hitzl
Research Management (RM): Biostatistics and Publication of Clinical Studies Team, Paracelsus Medical University, Salzburg, Austria

Department of Ophthalmology and Optometry, Paracelsus Medical University, Salzburg, Austria

Research Program Experimental Ophthalmology and Glaucoma Research, Paracelsus Medical University, Salzburg, Austria

Eugen Trinka
Department of Neurology, Christian Doppler University Hospital, Paracelsus Medical University and Center for Cognitive Neuroscience, European Reference Network EpiCARE, Salzburg, Austria

Neuroscience Institute, Christian Doppler University Hospital, Paracelsus Medical University and Center for Cognitive Neuroscience, Salzburg, Austria

Abstract

Background: Anti-CD20 therapies induce pronounced B-cell depletion and blunt humoral responses to vaccines. Recovery kinetics of anti-CD20 therapy-mediated cellular and humoral effects in people with multiple sclerosis (pwMS) are poorly defined.

Objective: To investigate the duration of the anti-CD20 treatment-induced effects on humoral responses to COVID-19 vaccines.

Methods: This retrospective observational study included pwMS who had discontinued anti-CD20 therapy for ⩾12 months and remained without immunomodulation. We retrieved demographics and laboratory parameters including B-cell counts and immunoglobulin (IgG, IgM, IgA) levels prior to anti-CD20 commencement (baseline) and longitudinally after anti-CD20 treatment discontinuation from electronic medical records. Humoral responses to SARS-CoV-2 vaccines were compared with a population of 11 pwMS with ongoing anti-CD20 medication (control cohort).

Results: A total of 24 pwMS had discontinued anti-CD20 therapy for a median of 34 months (range: 16–38 months). Antibody responses to COVID-19 vaccines were available in 17 (71%). Most individuals ($n = 15$, 88%) elicited a measurable antibody response [mean: 774 BAU/ml (±SD 1283 BAU/ml)] to SARS-CoV-2 immunization on average 22 months (range: 10–30 months) from the last anti-CD20 infusion, which was higher compared with the population with ongoing anti-CD20 therapy ($n = 11$, mean: 12.36 ± SD 11.94 BAU/ml; $p < 0.00001$). Significantly increased antibody levels compared with the control cohort were found among pwMS who were vaccinated >18 months after treatment discontinuation (19–24 months: $n = 2$, $p = 0.013$; 25–36 months: $n = 9$; $p < 0.001$). The interindividual kinetics for B-cell reconstitution were heterogeneous and mean B-cell counts approached normal ranges 18 months after treatment discontinuation. There was no correlation of B-cell repopulation and vaccine responses. Mean total IgG, IgM, and IgA levels remained within the reference range.

Conclusion: Anti-CD20-induced inhibition of humoral responses to COVID-19 vaccines is transient and antibody production was more pronounced >18 months after anti-CD20 treatment discontinuation. The immunological effect on B-cell counts appears to wane by the same time.

Keywords: antibody titers, B-cell depletion, B-cell therapy, immune reconstitution, immunoglobulins, long-term effects, SARS-CoV-2

Received: 7 February 2022; revised manuscript accepted: 15 March 2022.

Highlights

- Anti-CD20 medications induce long-lasting immunological consequences.
- The immunological scar of anti-CD20 medication includes cellular and humoral effects.
- B-cell recovery after anti-CD20 therapy is heterogeneous.
- Humoral responses to COVID-19 vaccines are resumed 18 months after discontinuation.

Cornelia Mrazek
Elisabeth Haschke-Becher
Department of Laboratory Medicine, Paracelsus Medical University, Salzburg, Austria

Andrea Harrer
Department of Neurology, Christian Doppler University Hospital, Paracelsus Medical University and Center for Cognitive Neuroscience, European Reference Network EpiCARE, Salzburg, Austria

Department of Dermatology and Allergology, Paracelsus Medical University, Salzburg, Austria

* Equally contributing senior authors.

- Humoral responses to COVID-19 vaccine are not correlated to B-cell recovery.

Introduction

Multiple sclerosis (MS) is an autoimmune disorder of the central nervous system (CNS) presumably mediated by auto-aggressive lymphocytes with encephalitogenic potential.[1–4] In fact, therapies that target B cells have demonstrated high efficacy in reducing cerebral inflammation in people with MS (pwMS).[5,6] Anti-CD20 therapeutics selectively target B cells and induce a profound, continuous depletion of peripheral B cells based on a 6-monthly dosing interval. Despite an overall favorable safety profile, selective B-cell medications allegedly increase the risk of infections and pwMS on anti-CD20 medication may be more prone to a severe course of COVID-19.[7,8] Moreover, long-term anti-CD20 administration induces a decrease in immunoglobulin levels, and anti-CD20-therapy-associated hypogammaglobulinemia may increase susceptibility to pathogens in patients with MS and with other autoimmune conditions.[9,10] Finally, anti-CD20 therapy inhibits seroconversion to COVID-19 immunization in a majority of individuals,[11–13] which may, in turn, explain COVID-19 susceptibility and needs to be considered in the care of patients.

How long anti-CD20 therapy-induced immunological consequences last once therapy has been suspended is, however, poorly defined. We therefore aimed to determine the pattern of cellular and humoral immune reconstitution following the discontinuation of selective B-cell therapy. B-cell recovery kinetics, the course of immunoglobulin levels, and humoral responses to SARS-CoV-2 vaccines were assessed in pwMS who had discontinued anti-CD20 therapy for ⩾1 year and not received any successive MS medication.

Methods

Recruitment and data extraction

We conducted a retrospective observational analysis including pwMS who had stopped anti-CD20 medication for at least 12 months without receiving any further immunomodulatory agent. Demographics, clinical, and laboratory data were retrieved from electronic medical records. We also reviewed for infectious adverse events leading to hospitalization after treatment discontinuation.

We collected B-cell and immunoglobulin values before start of anti-CD20 therapy (baseline) and at five post-treatment periods (<6; 7–12; 13–18; 19–24; and 25–36 months). Immunological recovery was assessed by comparison to baseline values, normal range, and lower limit of normal (LLN). Humoral response to COVID-19 vaccine was evaluated in terms of anti-spike IgG antibody levels and compared with a cohort of 11 pwMS with ongoing anti-CD20 therapy (control cohort), who had received three doses of COVID-19 vaccines prior to investigation. This control cohort has been published previously.[14]

Laboratory parameters

Peripheral venous blood samples were collected into 3 ml Vacuette® K3EDTA tubes (Greiner Bio-One, Kremsmünster, Austria) and 10 ml BD Vacutainer® CAT (Clot Activator Tube) tubes (Becton Dickinson, Franklin Lakes, NJ, USA). B cells, total immunoglobulin levels (IgG, IgM and IgA), and humoral responses to SARS-CoV-2 vaccination were assessed using standard laboratory methods at the Department of Laboratory Medicine at the University Hospital of Salzburg, certified according to the ISO-9001 standard and working according to ISO-15189 standards. SARS-CoV-2-specific IgG antibody levels to the spike receptor-binding domain (RBD) were evaluated by the SARS-CoV-2 IgG II Quant assay (Abbott Laboratories) measured on the Architect i 2000 SR in accordance with the manufacturer's instructions. The detection limit of the antibody assay is 7 BAU/ml. Values below the detection limit were set as 7 BAU/ml for statistical reasons.

The B-cell count is part of the routinely performed lymphocyte subset analysis, which is assessed using a dual-platform method. The white blood cell (WBC) count and the differential are measured on the Sysmex XN-9000 hematology analyzer (Sysmex Corporation, Kobe, Japan). Since microscopic examination of blood cells is performed on all specimens sent for lymphocyte subset analysis, the lymphocyte count of the manual WBC differential is used for further calculation of the B-cell count in the laboratory information system (GLIMS®, MIPS). For flow cytometric analysis, 50 µl of the EDTA sample is incubated with 15 µl of BD Multitest™ six-color TBNK reagent (Becton, Dickinson and Company, BD Biosciences, San Jose, USA, #644611, consisting of CD3 FITC/CD16 PE + CD56 PE/CD45 PerCP-Cy™5.5/

CD4 PE-Cy™7/CD19 APC/CD8 APC-Cy™7), 5 µl of BD Anti-HLA-DR (L243) V450 (Becton, Dickinson and Company, BD Biosciences, #655874), and 5 µl of BD Horizon™ BV510 Mouse Anti-Human CD14 (MϕP9) (Becton, Dickinson and Company, BD Biosciences, #563079) for 15 min in the dark at room temperature. After adding 500 µl of BD FACS™ Lysing Solution (Becton, Dickinson and Company, BD Biosciences), the sample is again incubated in the dark for 15 min at room temperature. Acquisition is performed on the BD FACSLyric™ flow cytometer with BD FACSuite™ software (Becton, Dickinson and Company, BD Biosciences). The percentage of CD19-positive B cells, which is determined using the gating strategy displayed in the supplementary Figure S1, is entered into the laboratory information system where the B-cell count is calculated by multiplication with the absolute lymphocyte count of the manual differential.

IgG, IgA, and IgM are measured using the Siemens BN II nephelometer (Siemens Healthcare Diagnostics Products GmbH, Germany) with reagents of the manufacturer (Siemens N Antiserum against human IgG, IgA, and IgM, respectively). During the whole study period, there were no changes in analyzer platforms or reagents.

Statistics and ethics

Data were checked for consistency. Dependent and independent *t*-tests and, in the case of non-normality, randomization tests with and without the assumption of variance homogeneity were used. Normality was tested using Kolmogrov–Smirnov test (K-S test) and skewness tests, and variance homogeneity was tested using modified Levene's test. Spearman's correlations were computed and tested. The correlation between humoral responses to COVID-19 vaccines and B-cell counts was computed with and without adjustment for age. The former calculation was computed using a partial correlation coefficient with age as covariate. In some cases, bootstrap BCa confidence intervals were used. All reported tests were two-sided, and p-values <0.05 were considered statistically significant. To adjust for multiple comparisons, the method of Holmberg–Bonferroni was used. All statistical analyses in this report were performed by the use of NCSS (NCSS 10; NCSS, LLC. Kaysville, UT, USA) and STATISTICA 13 (Hill, T. & Lewicki, P. Statistics: Methods and Applications. StatSoft, Tulsa, OK, USA).

For this retrospective study, we included pwMS participating in the ongoing study 'Immunological processes in multiple sclerosis and clinically isolated syndromes' with the votum of the local Ethics Committee (415-E/161 2111-2018). The observational study was conducted in accordance with Good Clinical Practice (GCP) as defined by the International Conference on Harmonization (ICH), WHO and any local directives. All patients gave written informed consent.

Results

Patient characteristics

We included a total of 24 pwMS who had received a mean of five anti-CD20 infusions (±SD 2 infusions). Anti-CD20 therapy had been discontinued for a median of 34 months [interquartile range (IQR): 25–36 months, range: 16–38 months], resulting in a total follow-up of 724 patient-months. The mean age was 56 years (±SD 9 years), 67% were woman, and the median Expanded Disability Status Scale (EDSS) was 6.0 (IQR: 5–7). Demographic features and patient characteristics are summarized in Table 1, and patient variables of the control cohort with ongoing anti-CD20 therapy are presented in the supplementary Table 1. Rituximab was given in doses of 500 mg very 6 months. Ocrelizumab was administered according to the manufacturer's recommendations. Due to the retrospective character of the study, data availability was incomplete. The number of samples included at each investigation period is illustrated within each figure. A flowchart gives an overview of patient selection and data assessment (Figure 1).

Vaccine responses

Data on humoral responses to SARS-CoV-2 immunization were available in 17 individuals. The majority (16/17) had received mRNA-based SARS-CoV-2 vaccines.

The doses of the basic immunization were administered according to the manufacturer's recommendation. The booster vaccine was given in mean 24 weeks (±SD 6.5 weeks, range 18–46 weeks) after the basic immunization. Median times from last infusion to first immunization dose were 26 months (IQR: 18–28 months, range: 10–30 months). Most pwMS (15/17) elicited measurable antibodies to the COVID-19 vaccine.

Table 1. Patient characteristics ($n = 24$).

Age (y), mean (±SD)	56 (9)
Female, *n* (%)	16 (67)
MS duration (y), mean (±SD)	16 (10)
Type of MS, *n* (%)	
• SPMS	19 (79)
• PPMS	5 (21)
EDSS, median (range)	
• Start of study	6 (3–8)
• End of study	6 (4–8)
CD20-depleting treatment	
• Rituximab, *n* (%)	21 (87.5)
• Ocrelizumab, *n* (%)	3 (12.5)
No. infusions, median (range)	5 (1–8)
Duration anti-CD20 Tx (y), median (range)	2.5 (1–4)
Anti-COVID-19 antibodies available, *n* (%)	17 (71)
Type of vaccine administered, *n* (%)	
• Pfizer (mRNA)	13 (76)
• Moderna (mRNA)	3 (18)
• Pfizer (mRNA)/ Moderna (mRNA)	1 (6)
• Janssen (Vector)/Pfizer (mRNA)	1 (6)

EDSS, Expanded Disability Status Scale; MS, multiple sclerosis; no., number; No. infusions, number of anti-CD20 infusions received; PPMS, primary progressive MS; SD, standard deviation; SPMS, secondary progressive MS; Tx, treatment; y, years.

The mean antibody level to SARS-CoV-2 vaccine among this cohort was 774 BAU/ml (±SD 1283 BAU/ml). The two individuals who did not elicit a humoral response after immunization were vaccinated 10 and 13 months after treatment discontinuation. When comparing the antibody levels to a cohort of 11 individuals with ongoing anti-CD20 therapy, we found significantly higher antibody response among the population after treatment discontinuation (774 ± SD 1283 BAU/ml *versus* mean 12.36 ± SD 11.94 BAU/ml; $p < 0.001$, Figure 2(a)).

The one patient mentioned above with incomplete B-cell recovery at 38 months from anti-CD20 cessation elicited a robust antibody response (1698 BAU/ml) to the COVID-19 vaccination 29 months after the last infusion.

We performed a subgroup analysis to investigate the time at which humoral responses to vaccinations recovered. The population was divided according to time from last anti-CD20 infusion to first SARS-CoV-2 vaccine (Figure 2(b)). Significantly increased antibody levels were elicited among pwMS who were vaccinated >18 months after treatment discontinuation. We found no correlations between antibody responses to SARS-CoV-2 vaccines and age ($r = 0.04$, $p = 0.89$, after adjustment for age: $r = -0.46$, $p = 0.074$), number of previous anti-CD20 infusions ($r = -0.02$, $p = 0.93$), B-cell counts at the time of antibody evaluation ($r = -0.29$, $p = 0.26$), and total IgG levels ($r = 0.03$, $p = 0.92$).

Cellular effects

B-cell recovery started 7–12 months after the last anti-CD20 dose and B-cell counts approached the lower limit of normal (LLN) 13–18 months from anti-CD20 treatment discontinuation. Compared with baseline values, B-cell counts were reduced throughout the 0–18 months post-treatment phase (<6 months $p = 0.000009$; 6–12 months $p = 0.00007$; 12–18 months $p = 0.049$). B-cell recovery kinetics were, however, heterogeneous and 4 of 10 had not reached LLN in year 3 after stop of anti-CD20 therapy. In one patient, B cells did not repopulate to LLN within the 38-month follow-up (59 cells/µl, normal range: 80–616 cells/µl). Despite general B-cell recovery, baseline values were not reached during the whole follow-up (Figure 3).

Impact on immunoglobulins

Mean IgG, IgM, and IgA values did not fall below the LLN throughout the post-treatment follow-up, while several single data points were outside the normal range and a trend toward decreased levels was observable. Pretreatment values were not reached by any immunoglobulin type throughout the follow-up. The long-term effects of anti-CD20 therapy on immunoglobulins G, M, and A are shown in Figure 3.

Disease activity

The median EDSS of our cohort remained unchanged throughout the follow-up [start: EDSS 6 (IQR 5–7) *versus* termination EDSS 6

(IQR 6–7)]. We recorded three relapses among two patients.

Discussion

Anti-CD20 monoclonal antibodies induce prominent effects on cellular and humoral components of the immune system, which makes them powerful treatment options for several autoimmune disorders and B-cell malignancies. However, the ongoing pandemic has raised safety concerns as to whether anti-CD20 therapies increase COVID-19 severity.[7,15] It has become clear that anti-CD20 substances blunt humoral responses to pathogen-specific vaccines.[11–13] Given evidence of pathogen-specific neutralizing antibodies inhibiting severe SARS-CoV-2 infections and humoral immunity to be more relevant than T-cell responses for successful COVID-19 control,[16–18] we investigated the duration of the anti-CD20 treatment-induced negative effect on humoral responses to COVID-19 vaccines. At a median of 26 months after last anti-CD20 infusion, COVID-19 vaccines elicited significantly higher antibody levels compared with patients with ongoing anti-CD20 therapy, indicating that the inhibiting effect on humoral responses to vaccines is transient. Our results are in accordance

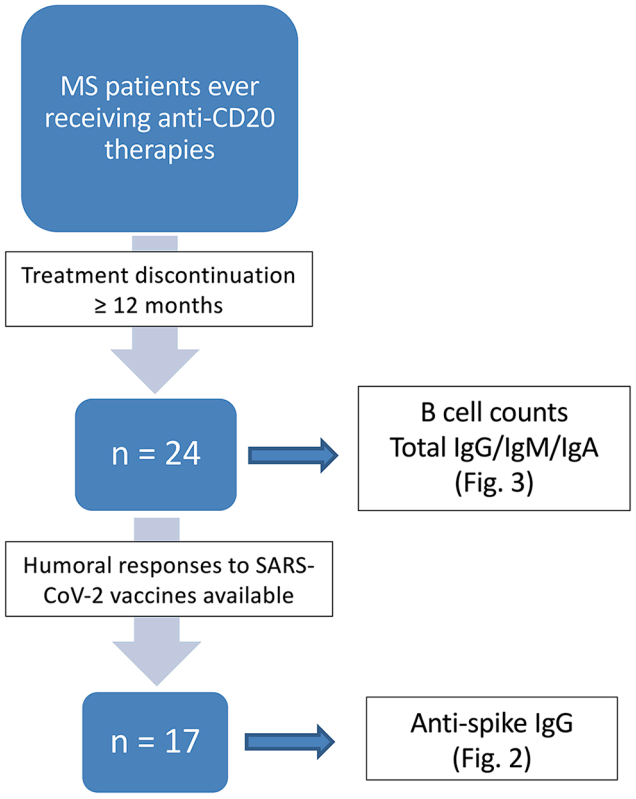

Figure 1. Flowchart illustrating the strategy of patient selection and reference to the respective figures.

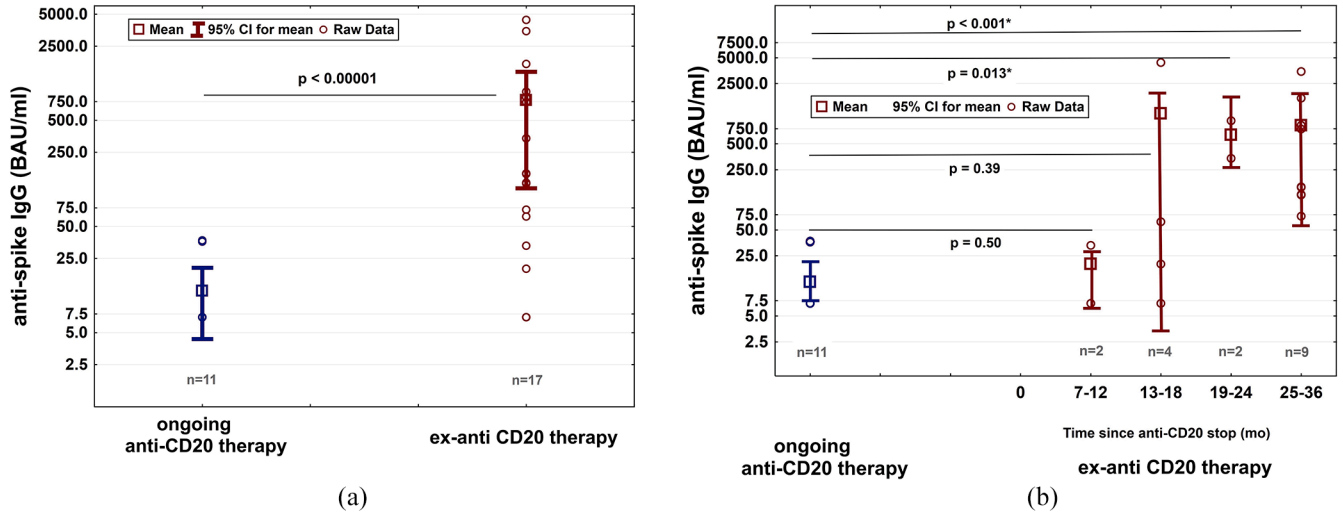

Figure 2. Antibody responses to SARS-CoV-2 immunization under and post anti-CD20 therapy. (a) Vaccine-induced antibody levels are significantly higher among individuals who have discontinued anti-CD20 therapy ($n = 17$, red) compared with patients with ongoing anti-CD20 therapy ($n = 11$, blue). (b) The treatment-associated inhibiting effect on the SARS-CoV-2-specific antibody response to vaccinations appears to wane 19 months after the last anti-CD20 infusion. Humoral responses represented by time elapsed between anti-CD20 discontinuation and first COVID-19 vaccination. Mo, months.

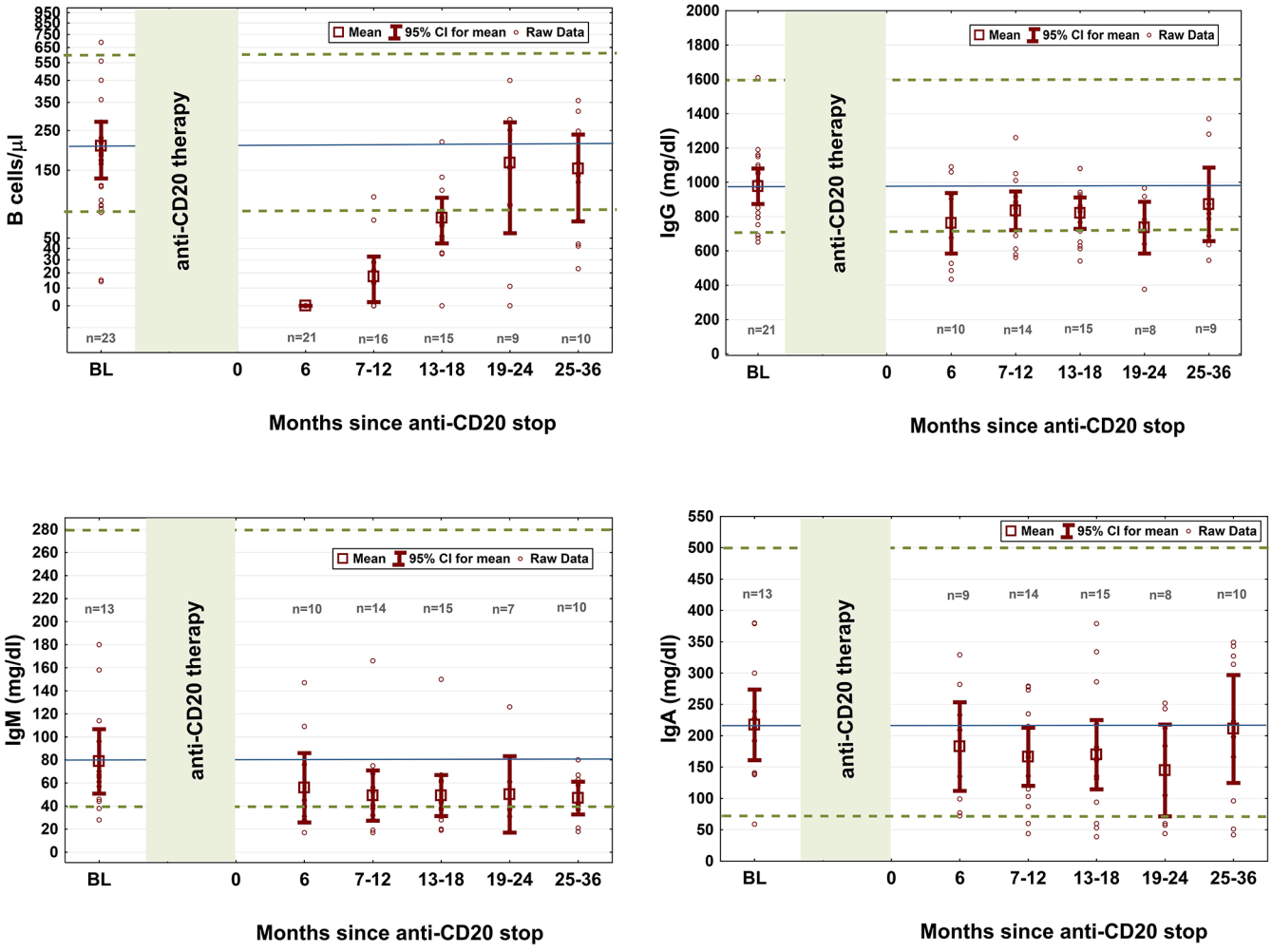

Figure 3. Dynamics of B-cell count reconstitution and immunoglobulin levels after anti-CD20 therapy. B cells recover to normal range within the second post-treatment year but do not reach pretreatment baseline levels until up to 36-month follow-up. Mean immunoglobulins levels (IgG, IgM, and IgA) were within the normal range at all time points. Solid line represents baseline values, dashed lines represent normal range. BL, baseline.

with recent studies that have suggested an association between time elapsed since last anti-CD20 infusion and humoral vaccine efficacy in COVID-19 immunization.[11,19] Regarding the best timing of post-anti-CD20 therapy vaccine administration to optimize antibody production, we found vaccine responses to be more pronounced ⩾18 months after treatment discontinuation. The ability to produce SARS-CoV-2 specific antibodies was not significantly increased in individuals vaccinated within 18 months from anti-CD20 stop compared with patients with ongoing B-cell therapy. Interestingly, we found no correlation between restoration of humoral vaccine responses and B-cell repopulation. In line with previous reports, we found a complete depletion of B cells at 6 months from anti-CD20 infusions and a stepwise approach toward the LLN within 18 months from last dosing.[20] Our real-life data corroborate findings from ocrelizumab phase II trials, showing a repopulation to the LLN by 72 weeks.[21] We observed a heterogeneity regarding B-cell recovery rates, ranging from repopulation to LLN within 1 year to incomplete recovery 36 months from the last anti-CD20 administration. The cause of different recovery paces between individuals is largely unknown and requires further attention. An association between higher body mass index and accelerated B-cell repopulation has been reported,[22] suggesting that personalized dosing regimens would improve efficacy and safety profiles.

In contrast to an evident inhibition of humoral vaccine responses, effects of anti-CD20 medications on total immunoglobulin levels was less pronounced. This is likely due to the fact that the main antibody source, plasma cells, does not carry CD20 and is therefore spared from depletion. Their precursors, however, which contain the pool of B cells able to respond to new antigenic stimuli, are major targets of B-cell depletion. This not only explains the poor seroconversion rates to neoantigens under concomitant anti-CD20 therapy, but also supports a recent finding that responses to recall COVID-19 antigens are preserved in pwMS under anti-CD20 therapy, once a basic immunity, likely in the form of antigen-specific, CD20-negative plasma cells, has been established.[14] A retained humoral vaccine efficacy despite complete peripheral B-cell depletion underpins the concept that generation of pathogen-specific antibodies occurs within the lymphoid tissues.[20] Together, these considerations argue against the hypothesis that vaccine efficacy strongly relates to the degree of B-cell repopulation.[20] Peripheral B-cell counts therefore appear not to be an appropriate biomarker to predict seroconversion rates in pwMS treated with anti-CD20 therapies.

This study carries the well-known limitations of retrospective analyses. Characterizing immunological consequences of immunotherapies in MS has some advantages in comparison with other indications, especially to hematological malignancies. First, MS patients usually receive monotherapies, rather than a combination of immunoactive substances. Second, the immune function required to combat pathogens is considered unimpaired in MS, offering an ideal setting to assess the long-term effect of anti-CD20 therapy. We have included a cohort of individuals primarily with secondary progressive MS, and anti-CD20 therapy was utilized in an off-label setting with no successive therapy being indicated. The conclusions therefore primarily regard progressive MS forms and we cannot exclude an impact of immunosenescence on cellular and humoral recovery, as the population included mainly elderly MS patients. Another major issue is the assessment of anti-SARS-CoV2 antibody levels, which occurred any time after vaccination and not at standard intervals after immunization.

To conclude, the immunological 'scar' of anti-CD20 therapy regarding B-cell counts and vaccine responses in pwMS appears to wane 18 months after anti-CD20 cessation.

Author contribution(s)

Tobias Moser: Conceptualization; Data curation; Investigation; Methodology; Project administration; Writing – original draft.

Ciara O'Sullivan: Investigation; Writing – original draft; Writing – review & editing.

Ferdinand Otto: Data curation; Methodology; Writing – review & editing.

Wolfgang Hitzl: Data curation; Formal analysis; Investigation; Validation; Visualization; Writing – review & editing.

Georg Pilz: Investigation; Writing – review & editing.

Kerstin Schwenker: Data curation; Investigation; Writing – review & editing.

Cornelia Mrazek: Data curation; Writing – review & editing.

Elisabeth Haschke-Becher: Data curation; Writing – review & editing.

Eugen Trinka: Conceptualization; Project administration; Writing – review & editing.

Peter Wipfler: Conceptualization; Data curation; Project administration; Supervision; Writing – review & editing.

Andrea Harrer: Conceptualization; Investigation; Methodology; Supervision; Visualization; Writing – original draft; Writing – review & editing.

ORCID iD

Tobias Moser https://orcid.org/0000-0002-5397-5595

Funding

The authors received no financial support for the research, authorship, and/or publication of this article.

Conflict of interest statement

The authors declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: TM received travel support and honoraria for presentations or participation on advisory boards from Biogen Idec, Celgene, Novartis, Roche, Sanofi, Merck and Teva. FO received travel support and honoraria for presentations or participation on

advisory boards from Biogen Idec, Celgene, Novartis, Roche, Sanofi, Merck and Teva. ET has received consultation fees and/or speakers' honoraria from Arvelle, Argenx, Angelini, Bial, Bio-gen-Idec, Boehringer Ingelheim, Eisai, Epilog, GL Pharma, GW Pharmaceuticals, Ever Pharma, Hikma, LivaNova, Marinus, Medtronics, Newbridge, Novartis, Sanofi, Genzyme, and UCB Pharma. PW received consultation fees and/or speakers' honoraria from Bayer, Biogen Idec, Bristol-Myers Squibb, Merck, Novartis, Roche, Sanofi Genzyme, Teva Pharmaceutical Industries Ltd. He received research grants from Biogen Idec and Merck. The remaining authors have no competing interests.

Data availability statement

The data that support the findings of this study are available on reasonable request from the corresponding author.

Supplemental material

Supplemental material for this article is available online.

References

1. Moser T, Akgun K, Proschmann U, *et al.* The role of TH17 cells in multiple sclerosis: therapeutic implications. *Autoimmun Rev* 2020; 19: 102647.
2. Margoni M, Preziosa P, Filippi M, *et al.* Anti-CD20 therapies for multiple sclerosis: current status and future perspectives. *J Neurol* 2022; 269: 1316–1334.
3. Cencioni MT, Mattoscio M, Magliozzi R, *et al.* B cells in multiple sclerosis – from targeted depletion to immune reconstitution therapies. *Nat Rev Neurol* 2021; 17: 399–414.
4. Dobson R and Giovannoni G. Multiple sclerosis – a review. *Eur J Neurol* 2019; 26: 27–40.
5. Hauser SL, Bar-Or A, Comi G, *et al.* Ocrelizumab versus interferon beta-1a in relapsing multiple sclerosis. *N Engl J Med* 2017; 376: 221–234.
6. Montalban X, Hauser SL, Kappos L, *et al.* Ocrelizumab versus placebo in primary progressive multiple sclerosis. *N Engl J Med* 2017; 376: 209–220.
7. Sormani MP, De Rossi N, Schiavetti I, *et al.* Disease-modifying therapies and coronavirus disease 2019 severity in multiple sclerosis. *Ann Neurol* 2021; 89: 780–789.
8. Luna G, Alping P, Burman J, *et al.* Infection risks among patients with multiple sclerosis treated with fingolimod, natalizumab, rituximab, and injectable therapies. *JAMA Neurol* 2020; 77: 184–191.
9. Barmettler S, Ong MS, Farmer JR, *et al.* Association of immunoglobulin levels, infectious risk, and mortality with rituximab and hypogammaglobulinemia. *JAMA Netw Open* 2018; 1: e184169.
10. Perriguey M, Maarouf A, Stellmann JP, *et al.* Hypogammaglobulinemia and infections in patients with multiple sclerosis treated with rituximab. *Neurol Neuroimmunol Neuroinflamm* 2022; 9: e1115.
11. Apostolidis SA, Kakara M, Painter MM, *et al.* Cellular and humoral immune responses following SARS-CoV-2 mRNA vaccination in patients with multiple sclerosis on anti-CD20 therapy. *Nat Med* 2021; 27: 1990–2001.
12. Novak F, Nilsson AC, Nielsen C, *et al.* Humoral immune response following SARS-CoV-2 mRNA vaccination concomitant to anti-CD20 therapy in multiple sclerosis. *Mult Scler Relat Disord* 2021; 56: 103251.
13. Tallantyre EC, Vickaryous N, Anderson V, *et al.* COVID-19 vaccine response in people with multiple sclerosis. *Ann Neurol* 2021; 91: 89–100.
14. Moser T, Otto F, O'Sullivan C, *et al.* Recall response to COVID-19 antigen is preserved in people with multiple sclerosis on anti-CD20 medications – a pilot study. *Mult Scler Relat Disord* 2022; 59: 103560.
15. Spelman T, Forsberg L, McKay K, *et al.* Increased rate of hospitalisation for COVID-19 among rituximab-treated multiple sclerosis patients: a study of the Swedish multiple sclerosis registry. *Mult Scler*. Epub ahead of print 2 July 2021. DOI: 10.1177/13524585211026272.
16. Corti D, Purcell LA, Snell G, *et al.* Tackling COVID-19 with neutralizing monoclonal antibodies. *Cell* 2021; 184: 4593–4595.
17. Khoury DS, Cromer D, Reynaldi A, *et al.* Neutralizing antibody levels are highly predictive of immune protection from symptomatic SARS-CoV-2 infection. *Nat Med* 2021; 27: 1205–1211.
18. Molodtsov IA, Kegeles E, Mitin AN, *et al.* SARS-CoV-2 specific T cells and antibodies in COVID-19 protection: a prospective study, 2021, https://www.medrxiv.org/content/10.1101/2021.08.19.21262278v2

19. Shree T, Shankar V, Lohmeyer JJ, *et al.* CD20-targeted therapy ablates de novo antibody response to vaccination but spares pre-established immunity. *Blood Cancer Discov* 2022; 3: 95–102.

20. Baker D, MacDougall A, Kang AS, *et al.* Seroconversion following COVID-19 vaccination: can we optimize protective response in CD20-treated individuals? *Clin Exp Immunol* 2021; 2021: uxab015.

21. Kappos L, Li D, Calabresi PA, *et al.* Ocrelizumab in relapsing-remitting multiple sclerosis: a phase 2, randomised, placebo-controlled, multicentre trial. *Lancet* 2011; 378: 1779–1787.

22. Signoriello E, Bonavita S, Di Pietro A, *et al.* BMI influences CD20 kinetics in multiple sclerosis patients treated with ocrelizumab. *Mult Scler Relat Disord* 2020; 43: 102186.