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*Brief Report*

# Determining the best window for BNT162b2 mRNA vaccination for SARS-CoV-2 in patients with multiple sclerosis receiving anti-CD20 therapy

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***Abstract***

We studied the serologic response to the BNT162b2 mRNA vaccine at four weeks after the second dose in patients with RRMS treated with rituximab with extended-interval dosing ($n = 26$). At four weeks, 73% of patients were seropositive. No patient without B cells at the first dose ($n = 4$) was seropositive. Four of seven (57%) patients with B-cell proportion >0% and ≤5% were seropositive. All patients with B-cell proportion >5% ($n = 15$) were seropositive. In all patients, quantitative ELISA measures after vaccination were correlated with B-cell counts measured before vaccination. In patients receiving rituximab, seropositivity after BNT162b2 mRNA vaccination emerged only after B-cell repopulation.

***Keywords:*** disease-modifying therapies, multiple sclerosis

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## Introduction

In people with multiple sclerosis (PwMS), anti-CD20 therapies are associated with increased risk of severe COVID-19,[1] blunted antibody response to SARS-CoV-2 infection[2] and reduced immune response to COVID-19 vaccines.[3] In these patients, reducing the B-cell count could be the main factor altering the immunogenicity of the vaccines.

We report here the serologic response to BNT162b2 mRNA vaccination for SARS-CoV-2 in patients with relapsing-remitting MS (RRMS) treated with rituximab (RTX) with extended-interval dosing.[4] This therapeutic scheme gives a unique opportunity to assess the potential impact of B-cell repopulation on the immunogenicity of the vaccine for determining the best biological window for BNT162b2 mRNA vaccination for SARS-CoV-2 in PwMS under anti-CD20 therapy.

## Methods

### *Protocol and participants*

In 2018, our department initiated a change concerning the dosing interval used for off-label RTX therapy in patients with RRMS. We decided to extend the interval between two infusions beyond six months and up to 24 months, maintaining visits every six months and MRI monitoring at least annually. Extending dosing was used for only patients showing no disease activity since the last RTX infusion six months previous. This decision was based on the absence of a standardized administration scheme for RTX in RRMS as demonstrated by the heterogeneity of dosing intervals reported in the literature.[5–7] Particularly, the 24-month interval was chosen according to a recent study finding a potential slight waning of the RTX effect at 24 months after the last infusion.[8] Considering the potential risk of reduced immunologic response to vaccination in the context of anti-CD20 therapy, we performed a prospective observational study. We checked B-cell count at the time of the first dose and the serologic response at four weeks after the second dose in patients vaccinated in our department between February and March 2021.

This study was approved by the institutional review board of the University Hospital of Marseille (RGPD/Ap-Hm 2021-19).

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*Biological analysis*

B-cell count was measured by flow cytometry immunophenotyping. Sera samples were tested for anti-SARS-CoV-2 IgG antibodies directed against the S1 domain of the spike protein of the virus by using a commercial ELISA kit (Quantivac IgG, Euroimmun, Lübeck, Germany). Quantitative results are expressed in standardized units (binding antibody units per mL) as recommended by the manufacturer. For all samples with a semi-quantitative ratio result ≥0.7, neutralizing antibodies against SARS-CoV-2 were detected by using a virus neutralization test (VNT100) as described.[9] Serial dilutions of 1/10 to 1/160 serum were tested, and specimens with VNT100 titers ≥40 were considered seropositive, titers = 20 undetermined, and titers <20, seronegative.

*Statistical analysis.* Potential correlations between quantitative ELISA measures obtained four weeks after the second dose and B-cell counts at the time of the first dose or time since the last infusion were tested with Spearman's rank correlation analysis. Comparisons between the characteristics of patients with and without IgG antibodies directed against the S1 protein four weeks after the second dose were tested with Fisher's exact test and Wilcoxon signed-rank test $p < 0.05$ was considered statistically significant.

## Results

We included 26 patients (Table 1). The median time since the last RTX infusion was 20 months (range 5–24). At the first dose of the BNT162b2 mRNA vaccine, the mean (SD) proportion of B cells to total lymphocytes was 7% (7) (Table 1).

At the first vaccine dose, no patient had anti-SARS-Cov-2 IgG antibodies directed against the S1 protein of SARS-CoV-2. One month after the second dose, 19/26 patients were seropositive (73%) (Table 1). All seropositive patients were positive for neutralizing antibodies against SARS-Cov-2 (VNT100 titers ≥40). No patient without B cells at the time of the first dose ($n = 4$) became seropositive. Four of seven (57%) patients with B-cell proportion >0% and ≤5% became seropositive. All patients with B-cell proportion >5% ($n = 15$) became seropositive. In all patients, quantitative ELISA measures obtained 1 month after the second dose were correlated with B-cell counts at the time of the first dose (Spearman's rank correlation, $\rho = 0.75$, $p < 0.0001$) (Figure 1) but not with time since the last infusion ($\rho = 0.07$, $p = 0.73$).

One patient experienced symptomatic SARS-CoV-2 infection 20 days after the second vaccine dose. Infection was characterized by fever, cough and major fatigue for 10 days. This patient had received an RTX infusion eight months previous, and the B-cell proportion was 0.2% at the first vaccine dose. One month after the second vaccine dose, the patient was not seropositive.

## Discussion

The present study reveals that an effective humoral immune response to the BNT162b2 mRNA vaccine for SARS-CoV-2 in PwMS receiving RTX is strongly associated with the level of B-cell repopulation. Patients without B cells never showed seropositivity, but the seropositivity percentage increased to 57% in patients with B-cell proportion >0 and ≤5% and 100% with proportion >5%.

Patients receiving anti-CD20 therapies have shown lack of humoral immune response to the BNT162b2 mRNA vaccine. In most PwMS receiving ocrelizumab with a six-month dosing interval, the anti-SARS-CoV-2 S antibody response was negative 28 days after the second dose of BNT162b2 mRNA vaccine.[3] In patients with chronic lymphocytic leukemia treated with anti-CD20 therapy, better responses were observed in those who completed anti-CD20 therapy at least 12 months before vaccination, which suggests that the humoral response increases with B-cell repopulation.[10] This finding has been also suggested in patients with inflammatory rheumatic diseases. In five patients receiving RTX and the BNT162b2 mRNA vaccine, humoral response to vaccination was found in only two patients with detectable B cells.[11] Recently, Sormani and colleagues demonstrated reduced humoral response to SARS-CoV-2 mRNA vaccination in patients under anti-CD20 therapy.[12] Moreover, this study demonstrated an association between time since last infusion and antibody levels. We did not find a similar association probably because all patients included in the present study received the first vaccination at least five months after the last infusion. However, we found that B-cell count at the time of first vaccination could be more relevant to predict the response to vaccination, as found recently by Ali and colleagues.[13]

For SARS-CoV-2 infection, the humoral response seems particularly important because B-cell–depleting therapies have been associated with increased risk of severe COVID-19.[1,14] However, a recent study demonstrated that virus-specific T-cell responses also contribute to survival in patients with COVID-19,[15] which suggests that the CD8-T-cell response to vaccination could also participate in protecting against SARS-CoV-2 infection. The T-cell response to BNT162b2 mRNA vaccine for

**Table 1.** Characteristics of patients with relapsing-remitting multiple sclerosis under anti-CD20 therapy and vaccinated for SARS-CoV-2.

| | | Response to vaccination[§] | | |
|---|---|---|---|---|
| | All patients ($n = 26$) | Patients with IgG antibodies against the S1 protein ($n = 19$) | Patients without IgG antibodies against the S1 protein ($n = 7$) | $p$ value[#] |
| Sex (F/M) | 19/7 | 15/4 | 4/3 | $p = 0.34$ |
| Age (years), mean (SD) | 40 (11) | 41 (11) | 38 (11) | $p = 0.38$ |
| Disease duration (years), mean (SD) | 12 (7) | 13 (7) | 10 (6) | $p = 0.38$ |
| EDSS score, median (range) | 2.5 (0-6) | 2 (0–6) | 4 (0–4) | $p = 0.83$ |
| Time since the last RTX infusion (months), median (range) | 20 (5–24) | 21 (6–24) | 7 (5–24) | $p = 0.28$ |
| Number of RTX infusions before the first vaccine dose, median (range)* | 4 (1–8) | 5 (1–8) | 4 (1–6) | $p = 0.41$ |
| % of positive CD19 B-cells at the time of the first vaccine dose, median (range) | 6 (0.3–26) | 9 (0.3–26) | 0 (0–4.6) | $p = 0.0005$ |

RTX, rituximab; EDSS, Expanded Disability Status Scale.
* BNT162b2 mRNA vaccination.
[#]Comparison between patients with and without IgG antibodies directed against the S1 protein.
[§]Serum samples were tested for anti-SARS-CoV-2 IgG antibodies directed against the S1 domain of the spike protein four weeks after the second vaccine dose.

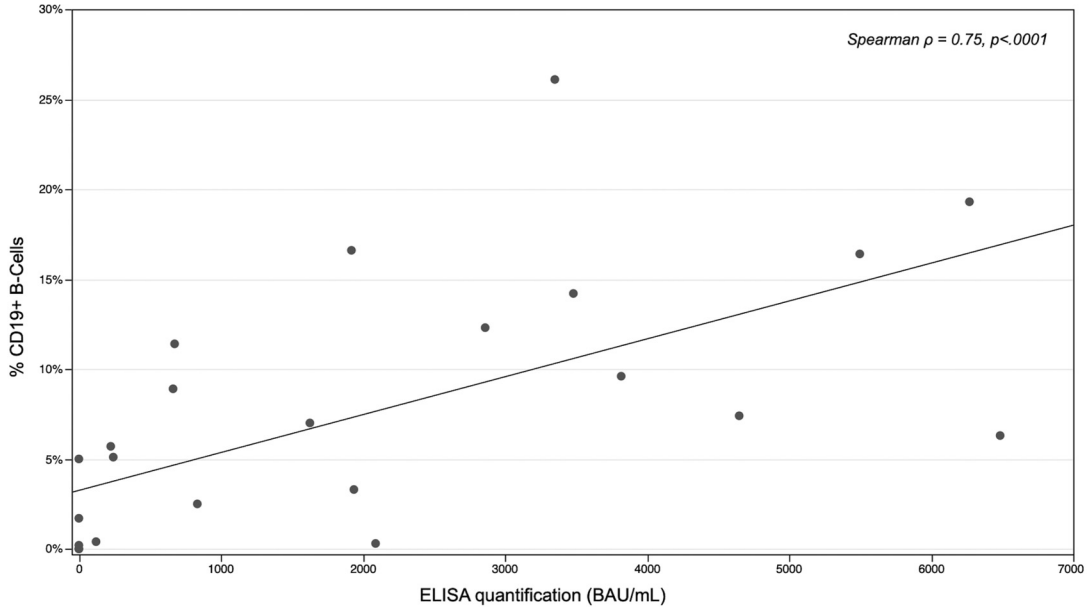


**Figure 1.** Correlation between quantitative ELISA measures of the serologic response to BNT162b2 mRNA vaccination for SARS-CoV-2 at four weeks after the second vaccine dose and proportion of B cells to total lymphocytes at the time of the first vaccine dose. BAU, binding antibody unit.

SARS-CoV-2 is maintained in MS patients under anti-CD20 therapy, but the ability of this isolated response to prevent COVID-19 in treated patients must be demonstrated.[15,16] In the present study, the occurrence of symptomatic SARS-CoV-2 infection in one patient at three weeks after the second vaccine dose suggests that the T-cell response seems not sufficient to prevent symptomatic infection.

The present study is not without limitations. First, the sample size was small, which limits the potential generalization of the findings. Second, the T-cell response to vaccination was not assessed. Third, the potential efficacy of a third vaccine dose and/or delayed seroconversion was not studied.

The present study reveals that in patients with RRMS treated with RTX, an effective immunologic response to the BNT162b2 mRNA vaccine emerged only after B-cell repopulation and was consistently observed with B-cell proportion >5%. Because the mean time to surpass 1% B-cell proportion after anti-CD20 infusion is 250 days (eight months),[17] this finding strongly argues for the use of extended-interval dosing with B-cell-depleting therapies to obtain the best "window for vaccination" against SARS-CoV-2 infection. In MS, this strategy could be easy because extended dosing with anti-CD20 agents is associated with low risk of relapse or MRI activity, as suggested by recent studies.[4,18]

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