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BRIEF REPORT

Impact of rituximab on humoral response to SARS-CoV-2 vaccination in previously vaccinated patients with autoimmune diseases

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Abstract

SARS-CoV-2 infection is more severe in patients undergoing rituximab (RTX) treatment. Humoral response to vaccination is severely impaired in patients already treated with RTX, but data on antibody persistence in patients initiating RTX are lacking. We evaluated the impact of RTX initiation on humoral response to SARS-CoV-2 vaccination in previously vaccinated patients with immune-mediated inflammatory diseases. We performed a retrospective, multicenter study evaluating the evolution of anti-spike antibodies and breakthrough infections after initiation of RTX in previously vaccinated patients with protective levels of anti-SARS-CoV-2 antibodies. Threshold for anti-S antibodies positivity and protection were 30 and 264 BAU/mL, respectively. We included 31 previously vaccinated patients initiating RTX (21 female, median age 57 years). At first RTX infusion, 12 (39%) patients had received 2 doses of vaccine, 15 (48%) had received 3 doses, and 4 (13%) had received 4 doses. The most frequent underlying diseases were ANCA-associated vasculitis (29%) and rheumatoid arthritis (23%). Median anti-S antibody titers at RTX initiation, 3 months, and 6 months were 1620 (589–2080), 1055 (467–2080), and 407 (186–659) BAU/mL, respectively. Overall, antibody titers waned by almost two-fold at 3 months and four-fold at 6 months. Median antibody titers were significantly higher in patients who received ≥3 doses compared to those who received only 2 doses. Three patients developed SARS-CoV-2 infection without any severe symptom. Anti-SARS-CoV-2 antibody titers in previously vaccinated patients decline after RTX initiation similarly to general population. Specific monitoring is useful to anticipate prophylactic strategies.

Key Points

- *Anti-SARS-CoV-2 antibody titers in previously vaccinated patients decline after rituximab initiation similarly to the general population.*
- *The number of dose of vaccine before rituximab initiation is associated with higher antibody titers at month 3.*
- *Monitoring antibody levels is mandatory to initiate prophylactic strategies in this population.*

Keywords Biological therapy · Rituximab · SARS-CoV-2 · Vaccines

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Introduction

Patients receiving immunosuppressive agents are at high risk of severe forms of SARS-CoV-2 infection [1, 2]. Rituximab (RTX) was identified as an independent risk factor for severe COVID-19 and death in observational cohorts of patients with immune-mediated inflammatory diseases [1–3]. Vaccination offers protection against severe forms of the disease, but can be less efficient in immunosuppressed patients with immune-mediated inflammatory diseases. Production of anti-spike protein (S) antibodies is currently the main correlate of protection used to evaluate the efficacy of anti-SARS-CoV-2 vaccines [4].

Impaired antibody response to SARS-CoV-2 vaccination is observed in patients treated with RTX. Profound B cell depletion induced by RTX is associated with the absence of anti-S antibodies production after 2 doses of vaccine and the loss of humoral response is maximum after 6 months following treatment [5, 6]. Detection of circulating B cells correlates with anti-S antibodies production and even patients with a low number of B cells (<1%) are able to mount a detectable antibody response [6]. Adjunction of supplemental doses of vaccine allows seroconversion in a small number of non-responders patients [7, 8]. Conversely, functional T cell response to SARS-CoV-2 vaccines is not altered compared to healthy controls and patients with autoimmune disease treated with other immunosuppressants [5, 9].

All studies evaluated de novo immune response to vaccination in patients already treated with RTX. No data are currently available on the impact of RTX initiation on preestablished humoral immunity in patients with immune-mediated inflammatory diseases. The goal of our study was to evaluate the impact of RTX initiation on humoral response to SARS-CoV-2 vaccination in a cohort of patients with immune-mediated inflammatory diseases and previously vaccinated with an efficient level of antibody protection.

Methods

Study design

We conducted a retrospective descriptive multicenter study including consecutive patients initiating RTX to treat systemic immune-mediated inflammatory diseases. Main inclusion criteria were as follows: (i) age over 18 at the time of RTX initiation, (ii) initiation or resumption of RTX between January 2021 and May 2022 to treat immune-mediated inflammatory diseases, (iii) patients should have received at least two doses of anti-SARS-CoV-2 vaccination, (iv) protective level of antibodies defined by anti-S antibodies >264 binding antibody unit (BAU)/mL [4] within the month preceding RTX initiation. Exclusion criteria were as follows: (i) diagnosis of hematological malignancy or solid organ transplant, (ii) previous RTX treatment within the last 12 months before inclusion, and (iii) anti-SARS-CoV-2 monoclonal antibody treatment prior to, or within the first 3 months of RTX treatment.

National council for vaccination strategy in France recommended serological monitoring at least every 3 months in immunocompromised patients [10]. Serologies for this study were thus performed as part of routine care for included patients and written informed consents were not required.

This study was conducted in compliance with the Good Clinical Practice protocol and the Declaration of Helsinki principles, and was approved by local Institutional Review Board (2022-07-03, Ethical Review Committee for publications of the Cochin University Hospital (CLEP) N°AAA-2022-08041).

Data collection and outcome

All medical records were retrospectively reviewed by the first author (EO). Demographic and clinical data (gender, age, immune-mediated inflammatory disease, date of diagnosis, other immunosuppressive treatments received) were collected as well as biological data (SARS-CoV-2 anti-S serology, $CD19^+$ B cells, and gammaglobulin dosage) and breakthrough infections.

Primary endpoint was the level of anti-S antibodies at 3 and 6 months after RTX initiation.

Anti-S antibodies were detected using ELISA commercial kits 3 and 6 months after RTX initiation and expressed in BAU/mL. Threshold for anti-S antibodies positivity and protection were 30 BAU/mL and 264 BAU/mL, respectively. Patients that received prophylactic monoclonal antibodies following a negative serology at 3 months were considered also negative at 6 months.

Statistical analysis

Descriptive analyses were performed using median and interquartile range (IQR) for continuous variables and number (percentage) for categorical variables. We used two-sided chi-square to compare categorical variables and Student *t*-test to compare continuous variables. Mann-Whitney test was used to compare anti-S antibody levels between subgroups of patients. Paired Student *t*-test was used to compare paired samples at 0, 3, and 6 months. p value <0.05 was considered significant. All statistical analyses were performed using R version 3.5.3 (R foundation for Statistics, Vienna).

Results

Between January 2021 and May 2022, among 285 patients with immune-mediated inflammatory diseases in whom treatment with RTX was initiated or resumed, 31 vaccinated cases with pre-RTX serology above 264 BAU/mL were included in the study (Supplementary Figure S1). Baseline patients' characteristics are shown in Table 1. Median age was 57 years (40–71) and 68% were female. The most frequent underlying diseases were ANCA-associated vasculitis (29%) and rheumatoid arthritis (23%). Oral glucocorticoids (GCs) were associated to RTX in 68% of cases with a median prednisone dose of 20 mg/day (5–40). Six (19%) patients were also treated with methotrexate or azathioprine. Three (10%) patients had a previous history of SARS-CoV-2 infection. Twenty-six (84%) patients had received BNT161B2 (Pfizer-BioNTech) vaccine and 5 received the mRNA-1273 (Moderna). Before first RTX infusion, 12 (39%) patients had received 2 doses of vaccine, 15 (48%) had received 3 doses, and 4 (13%) had received 4 doses. Median time between last dose of vaccine and first RTX infusion was 44 (17–84) days. Nine (29%) patients received an additional dose of vaccine between the first RTX infusion and the 6-month analysis, within a median interval of 81 (63–139) days between the first RTX infusion and the vaccine dose (Supplementary Figure S1).

Anti-S antibodies were available for 19 patients at 3 months, for 25 at 6 months, and for 14 patients at both time points. Median time between last dose of vaccine and

Table 1 Patients' characteristics at rituximab initiation

Characteristics	Number of patients $N = 31$
Demography	
Median age (IQR[a])	57 (40–71)
Female, n[a] (%)	21 (68)
Disease, n (%)	
ANCA-associated vasculitis	9 (29)
Rheumatoid arthritis	7 (23)
Membranous glomerulonephritis	4 (13)
Mixed connective tissue disease	2 (7)
Systemic sclerosis	2 (7)
Other[b]	7 (23)
Disease duration (years), median (IQR)	9.8 (3–13)
Previous rituximab treatment > 1 year	8 (26)
Rituximab regimen, n (%)	
1000 mg day 1 and day 15	22 (71)
375 mg/m^2 once weekly × 4 doses	8 (26)
500 mg once	1 (3)
Associated treatments, n (%)	
Pulses of methylprednisolone	2 (7)
Oral prednisone	21 (68)
Median prednisone dose (mg/day) (IQR)	20 (5–40)
Methotrexate	5 (16)
Azathioprine	1 (3)
SARS-CoV2 history, n (%)	
Previous SARS-CoV-2 infection	3 (10)
Number of vaccine doses before the first RTX[a] infusion	
2 doses	12 (39)
3 doses	15 (48)
4 doses	4 (13)
Time between vaccination and RTX (days), median (IQR)	44 (17–84)

[a]*IQR*, interquartile range; *n*, number; *RTX*, rituximab

[b]Retroperitoneal fibrosis ($n = 1$), systemic lupus erythematosus ($n = 1$), anti-MAG neuropathy ($n = 1$), cryoglobulinemia ($n = 1$), rapidly progressive glomerulonephritis ($n = 1$), IgG4-related disease ($n = 1$), IgA vasculitis ($n = 1$)

pre-rituximab serology was 35 (17–82) days. One patient received prophylactic monoclonal antibody therapy due to negative serology at 3 months. Median $CD19^+$ B cells prior to RTX initiation was 169/mm^3 (45–242) and dropped down to 1/mm^3 (0–10) at 3 months and 6/mm^3 (2–28) at 6 months. Median gammaglobulin levels at RTX initiation were 11 g/L (10–15), 10 g/L (9–10) at 3 months, and 12 g/L (8–16) at 6 months.

Figure 1A shows the evolution of anti-S antibody titers 3 and 6 months after RTX initiation. Median anti-S antibody titers at RTX initiation, 3 months, and 6 months were 1620 (589–2080), 1055 (467–2080), and 407 (186–659) BAU/mL, respectively. Anti-S antibody titers decreased in 25 patients and remained stable in 6. Overall, median antibody titers waned by almost two-fold at 3 months and by four-fold at 6 months after rituximab initiation. At 3 months, 18/19 (95%) patients still had detectable anti-S antibodies and 16 (84%) had protective antibody titers (i.e., >264 BAU/mL).

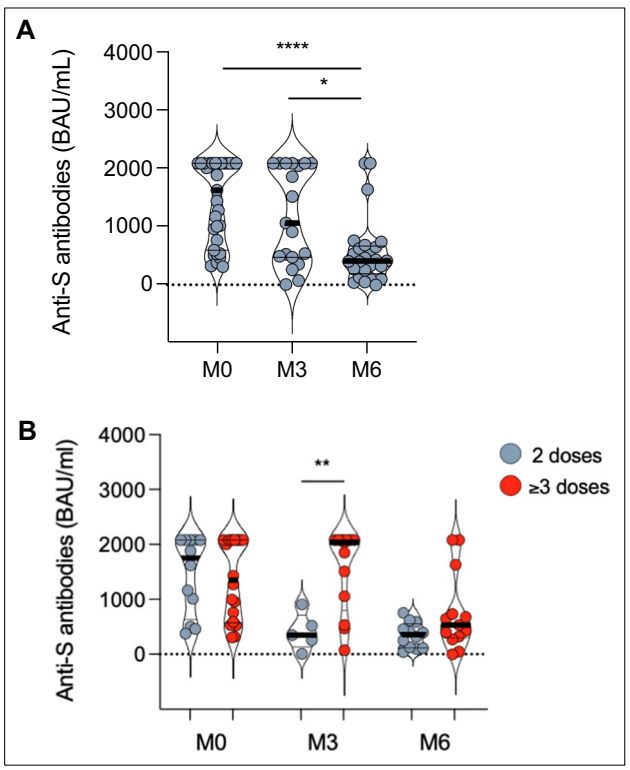

Fig. 1 Humoral response to SARS-CoV-2 vaccination before first rituximab infusion, 3-months and 6-months after, as determined by ELISA. Levels of anti-S IgG antibodies are indicated in BAU/mL. Each point is an individual. **A** Levels of anti-S antibodies in the whole population. The thick black line indicates median level of antibody. **B** Comparison of antibody titers at baseline, 3 months, and 6 months after first rituximab infusion between patients who received 2 doses of vaccine before rituximab initiation ($N = 12$) and patients who received ≥3 doses ($N = 10$). **A**–**B** Two-tailed p values were determined in a Mann-Whitney test. $^{****}p < 0.0001$, $^{**}p < 0.01$, $^{*}p < 0.05$

At 6 months, 24/25 (96%) patients still had detectable anti-S antibodies including 19 (61%) with protective level. Nine patients received a supplementary dose of vaccine between first RTX infusion and 6-month analysis (Fig. 1) without significative impact on anti-S antibody levels.

Despite a low number of patients, we tried to identify factors associated with protective levels of antibodies at 6 months. The absence of seroprotection at 6 months was only significantly associated with shorter median time between last dose of vaccine and first RTX infusion ($p = 0.049$) (Supplementary Table S1). Underlying disease, associated treatments, supplementary doses of vaccine between RTX and serology, and median gammaglobulin levels at 6 months had no significant impact on seroprotection. We did not have enough data to analyze correlation between CD19 count and seroprotection. We also compared anti-S antibody titers between patients who received 2 or ≥3 doses of vaccine before first RTX initiation (Fig. 1B). Median antibody titers were significantly higher in patients who received ≥3 doses compared to those who received only 2 doses at 3 months (2040 versus 346 BAU/mL, $p = 0.009$) but not at 6 months (531 versus 355 BAU/mL, $p = 0.15$).

After a median follow-up of 227 (176–272) days after first RTX infusion in the whole study population, 3 (10%) patients developed mild symptomatic breakthrough infection but did not require hospitalization. The median interval between the last rituximab infusion and SARS-CoV-2 infection was 209 (132–211) days whereas median interval between the last dose of vaccine and SARS-CoV-2 infection was 193 (131–219) days. Among these 3 patients, 2 had protective levels of anti-S antibodies at the time of breakthrough infection.

Discussion

RTX was demonstrated to be associated with severe form of COVID-19 and complete abolition of antibody response after vaccination within the 6 months following first infusion [9], but data on the persistence of preestablished humoral immunity after SARS-CoV2 vaccination in patients initiating new RTX therapy are lacking. In the present study, we show that RTX initiation in previously vaccinated patients with immune-mediated inflammatory disease was associated with anti-S antibody level decline by two-fold at 3 months and four-fold at 6 months. The number of doses of vaccine before RTX initiation was associated with higher anti-S antibody titers at month 3. Also, the absence of seroprotection at 6 months was significantly associated with shorter time between last dose of vaccine and first RTX infusion.

Recent studies focused on the evolution of anti-S antibodies after vaccination. In the general population, anti-S antibody levels after 2 doses of BNT161B2 peaked around

21 days after the second dose and declined slowly later [11]. In patients without prior infection, mean half-live of anti-S antibodies after a second BNT162b2 dose was 52 days but 100% of subjects remained positive (i.e., >23 BAU/mL) for anti-S antibodies at 6 months post-immunization [11, 12]. Also, after a second dose of BNT162b2 vaccine or mRNA-1273 vaccine, anti-S antibody levels decreased by 6-fold from peak to 6 months [12]. Thus, anti-S antibody decline in our study population seems comparable to the general population of these studies.

Defining a protective threshold is more challenging with new SARS-CoV-2 variants. Indeed, the level of 264 BAU/mL was defined for alpha and delta variants [4], but is probably less suitable with recent variants, even if some papers demonstrated that this threshold could be considered protective in immunocompromised populations even during the omicron wave [13].

French guidelines suggest to evaluate individually the risk of SARS-CoV-2 infection in immunosuppressed patients, considering the type of immunosuppression and comorbidities rather than using only this threshold. Thus, identifying factors associated with faster decline in antibody titers is essential to prevent at-risk situations. We observed higher antibody titers at 3 months in patients who had received three or four doses of vaccine than in those who received only two doses before RTX initiation. These data are consistent with modeling studies showing that duration of protection (i.e., anti-S levels > 264 BAU/mL) seems longer in patients who received 3 doses (9 months) than those who received 2 doses (5.8 months) [14]. Decline of antibody titers remains of concern in this immunosuppressed population at high risk of severe forms of COVID-19 [1–3]. Under RTX, as shown in this study, revaccination often fails to maintain protective levels of anti-S antibodies [8]. Therefore, in case of RTX continuation and antibody titer decline, prophylactic interventions should be initiated such as revaccination after postponing RTX on clinically stable patients or prophylactic treatment with monoclonal antibodies.

This study has several limitations. We could not have a proper control population in order to compare our results to healthy individuals but we used strong data from other studies conducted in the general population. The small size of the population is a limit of our study and some serologies were lacking during the follow-up. Indeed, many patients had to be excluded because SARS-CoV-2 serology before RXT was lacking and because systematic monitoring of serology was gradually implemented in our centers. When possible, RTX initiation was postponed because of the pandemic, thus limiting inclusions. The main strength of our study is its novelty. To our knowledge, this is the first study reporting this type of results, including after influenza or pneumococcal vaccination. Even if serologies were performed in different laboratories, antibody titers were standardized according to OMS recommendations and expressed in BAU/mL. Heterogeneity of vaccine schedules allowed us to compare subgroups of patients who received different doses before RTX initiation.

In conclusion, RTX initiation in patients with preestablished immunity following SARS-CoV-2 vaccination was associated with a decline in antibody titers at 3 and 6 months, which seemed comparable to the general population. A majority of patients still showed protective anti-S antibodies after 6 months and very few developed symptomatic infection, but patients receiving RTX did not increase their antibody titers following vaccine booster after RTX treatment. Even if T cell response to SARS-CoV-2 vaccines is preserved under RTX, this particular population might still be at risk of severe forms of COVID-19 and monitoring antibody levels could help initiating prophylactic strategies.

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Author contribution All authors whose names appear on the submission (1) made substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data; or the creation of new software used in the work; (2) drafted the work or revised it critically for important intellectual content; (3) approved the version to be published; and (4) agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Compliance with ethical standards

Disclosures None.

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