Predictors of an Immunogenic Response to the BNT162b2 mRNA COVID-19 Vaccination in Patients with Autoimmune Inflammatory Rheumatic Diseases Treated with Rituximab: A Multicenter Study

Victoria Furer (furer.rheum@gmail.com)
Tel Aviv Sourasky Medical Center, Tel Aviv University

Tali Eviatar
Tel Aviv Sourasky Medical Center, Tel Aviv University

Devy Zisman
Carmel Medical Center

Hagit Peleg
Hebrew University of Jerusalem

Yolanda Braun-Moscovici
Rambam Health Care Campus

Alexandra Balbir-Gurman
Rambam Health Care Campus

Daphna Paran
Tel Aviv Sourasky Medical Center, Tel Aviv University

David Levartovsky
Tel Aviv Sourasky Medical Center, Tel Aviv University

Michael Zisapel
Tel Aviv Sourasky Medical Center, Tel Aviv University

Ofir Elalouf
Tel Aviv Sourasky Medical Center, Tel Aviv University

Ilana Kaufman
Tel Aviv Sourasky Medical Center, Tel Aviv University

Adi Broyde
Tel Aviv Sourasky Medical Center, Tel Aviv University

Ari Polachek
Tel Aviv Sourasky Medical Center, Tel Aviv University

Katya Meridor
Tel Aviv Sourasky Medical Center, Tel Aviv University
Research Article

Keywords: COVID-19, vaccination, rituximab, immunogenicity, prediction model

Posted Date: December 28th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-1189980/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License
Abstract

Background

Treatment with rituximab (RTX) blunts SARS-CoV-2 vaccination-induced humoral response. We sought to identify predictors of a positive immunogenic response to the BNT162b2 mRNA vaccine in patients with autoimmune inflammatory rheumatic diseases (AIIRD) treated with RTX (AIIRD-RTX).

Methods

We analyzed 108 AIIRD-RTX patients and 122 immunocompetent controls immunized with BNT162b2 mRNA vaccine participating in a multicenter vaccination study. Immunogenicity was defined by positive anti-SARS-CoV-2 S1/S2 IgG measured at 2 to 6 weeks after the second vaccine dose. We used a stepwise backward multiple logistic regression to identify predicting factors for a positive immunogenic response to vaccination and develop a predicting calculator, further validated in an independent cohort of AIIRD-RTX patients (n=48) immunized with the BNT162b2 mRNA vaccine.

Results

AIIRD-RTX patients who mounted a seropositive immunogenic response significantly differed from non-responders by lower number of RTX courses (median (range) 3 (1-10) vs 5 (1-15), p=0.007; lower cumulative RTX dose 6943.11±5975.74 vs 9780.95±7240.12 mg, p=0.033; higher IgG level prior to last RTX course (mean ± SD), 1189.78±576.28 vs. 884.33±302.31 mg/dL, p=0.002, and extended interval between RTX treatment and vaccination, 469.82±570.39 vs 162.08±160.12 days, p=0.0009, respectively. Patients with ANCA-associated vasculitis and inflammatory myositis had a low likelihood of a seropositive immunogenic response compared to patients with rheumatoid arthritis, odds ratio (OR) 0.209, 95% confidence interval (CI) 0.046-0.96, p=0.044 and OR 0.189, 95% CI 0.036-0.987, p=0.048, respectively. Based on these findings, we constructed a calculator predicting the probability of a seropositive immunogenic response following BNT162b2 mRNA vaccination which performed with 90.5% sensitivity, 59.3% specificity, 63.3% positive and 88.9% negative predictive values.

Conclusions

The predicting calculator might guide clinicians for optimal timing of BNT162b2 mRNA vaccination in AIIRD-RTX patients.

Background

The SARS-CoV-2 provoked COVID-19 pandemic has urged the development and authorization of novel messenger RNA (mRNA) vaccines proved to be immunogenic and effective among the immunocompetent population.(1,2) Patients with autoimmune inflammatory rheumatic diseases (AIIRD), susceptible to infections in general and bearing worse outcomes of COVID-19 disease(3,4), have been prioritized for COVID-19 vaccination.(5) As immunosuppressed patients were excluded from the landmark
vaccines trials, uncertainty regarding the response to vaccination in AIIRD patients was raised by the medical community. Reassuringly, several prospective controlled studies proved that most patients with rheumatic diseases could mount an adequate immunogenic response to SARS-CoV-2 vaccination, acknowledging a lower post-vaccination level of anti-SARS-CoV-2 immunoglobulin G (IgG) antibodies in AIIRD patients compared to immunocompetent controls. (6–11)

Among other factors, B-cell depleting therapy significantly contributes to a reduced immunogenic response to SARS-CoV-2 vaccination in patients with AIIRD. (8–10,12–18) Indeed, B-cell depleting therapy has been consistently associated with reduced immunogenicity induced by influenza and pneumococcal vaccines in patients with AIIRD.(19) Rituximab (RTX), a widely used B-cell depleting therapy for rheumatoid arthritis (RA), anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV), and other rheumatic diseases, was identified as a risk factor for COVID-19 complications and a severe disease course(20–24), emphasizing the importance of SARS-CoV-2 vaccination in vulnerable RTX-treated patients. On the other hand, RTX was linked to a reduced immunogenic response to SARS-CoV-2 vaccination, corresponding to a low seroconversion rate ranging between 24% and 49%.(8,10,11,14–18,25) Furthermore, B-cell depleting therapy was reported to impair not only humoral but also a cell-mediated immune response to SARS-CoV-2 mRNA vaccination(11), although two recent studies detected T-cell-mediated immune response in the majority of RTX-treated patients irrespective of the humoral response.(12,16) However, the extent of anti-COVID-19 protection conferred by T-cell-mediated immune response in RTX treated AIIRD (AIIRD-RTX) patients remains unknown.

In the setting of the COVID-19 pandemic, clinicians commonly face a challenge concerning the optimal timing of vaccination in relation to RTX treatment, despite a general recommendation to delay the B-cell depleting therapy in relation to SARS-CoV-2 vaccination.(5) Identifying routine available predictors of a seropositive immunogenic response to SARS-CoV-2 vaccination in AIIRD-RTX patients may assist in a patient-tailored vaccination approach. Indeed, several studies found a direct correlation between detectable CD19 peripheral B cell counts and an immunogenic response to mRNA SARS-CoV-2 vaccination.(11,15,16) However, routine measurement of CD19 B cells prior to RTX treatment has not been recommended for most AIIRD and, therefore, might be unavailable in daily practice. In a prospective study conducted in a large cohort of AIIRD patients and immunocompetent controls reported by our group, we found that time interval between RTX administration and vaccination had a critical role in predicting an immunogenic response to the BNT162b2 mRNA vaccine .(8) Therefore, we sought to investigate additional predictors associated with an immunogenic response to mRNA BNT162b2 vaccination in AIIRD-RTX patients participating in our ongoing prospective vaccination study. We further developed a calculator based on clinical and laboratory data available in daily clinical practice to predict a seropositive immunogenic response conferred by SARS-CoV-2 vaccination in AIIRD-RTX patients.

**Methods**

This study is part of the ongoing prospective observational multicenter study investigating immunogenicity and safety of the BNT162b2 mRNA COVID-19 vaccine in adult AIIRD patients, focusing
on RTX-treated patients compared to immunocompetent controls. The study was conducted at the Rheumatology Departments of Tel Aviv Sourasky, Carmel, and Hadassah Medical Centers, Israel, between December 2020 and June 2021. The study protocol was described in detail elsewhere.(8) For the prediction model validation, data from an independent cohort of RTX-treated BNT162b2 mRNA vaccinated AIIRD patients from the Rambam Medical Health Care Campus were used.

The study was performed in accordance with the principles of the Declaration of Helsinki and approved by the research ethics committees of the four medical centers: TLV-1055-20, CMC-0238-20, HMO-0025-21, RMB-417-20, respectively. All study participants gave written informed consent on recruitment into the study.

Study aims

The primary end point was to identify independent predictors associated with seropositive immunogenic response to the BNT162b2 (Pfizer-BioNTech) vaccine in adult AIIRD-RTX patients.

Secondary end points included

1. Immunogenicity of the BNT162b2 mRNA vaccine in adult AIIRD-RTX patients compared with immunocompetent controls.

2. Development of a calculator to predict the probability of a seropositive immunogenic response to the BNT162b2 mRNA vaccination in AIIRD-RTX patients and its validation in an independent cohort of vaccinated AIIRD-RTX patients.

3. Safety of vaccination.

4. Effect of vaccination on disease activity in AIIRD-RTX patients stratified by positive and negative immunogenic response to vaccination.

Study population

This study included consecutive AIIRD patients treated with RTX up to 8 years prior to the BNT162b2 vaccination. The AIIRD-RTX group included 86 patients who participated in the vaccination study reported by our group (8) and 22 patients added to this analysis. Adult AIIRD patients ≥18 years of age, fulfilling the ACR/EULAR criteria for RA (26), CASPAR criteria for psoriatic arthritis (PsA)(27), Systemic Lupus International Collaborating Clinics classification criteria for systemic lupus erythematosus (SLE)(28), Chapel Hill classification criteria for systemic vasculitis (AAV, giant cell arteritis, other systemic vasculitides)(29), and the EULAR/ACR classification criteria for idiopathic inflammatory myopathy (IIM) (30) patients were recruited.

The control group included a sample of the immunocompetent population, with one additional participant added to the original study control group (n=122).
An independent prospective cohort of BNT162b2 mRNA vaccinated AIIRD-RTX patients (n=48) from the Rambam Medical Health Care Campus was used for the validation of the prediction model.

Exclusion criteria for all study groups included pregnancy, history of past vaccination allergy, and previous COVID-19 infection and for controls - history of AIIRD, immunosuppressive treatment, and previous COVID-19 infection.

Data collection

Demographic and clinical characteristics, including AIIRD diagnosis and anti-rheumatic medications, were reported by the participants and confirmed by reviewing the electronic medical records (EMR) by the study investigators (VF, TE, DZ, HP). The dates of the seasonal 2020 influenza vaccination and BNT162b2 mRNA vaccination were recorded. Medications included conventional synthetic disease modifying antirheumatic drugs (csDMARDs), glucocorticoids (GC), other immunosuppressive medications (mycophenolate mofetil), and intravenous immunoglobulin (IVIg). Doses of methotrexate (MTX) and prednisone were recorded. Specific details regarding RTX treatment were collected from the EMR, including immunoglobulin G levels (IgG, mg/dL) up to 3 months prior to last RTX course; total number of RTX courses, regardless of indication; the dose of each RTX course and the cumulative RTX dose (mg); the date of last RTX course. Hypogammaglobulinemia was defined as a total IgG level (prior to last RTX course) of less than 500 mg/dL. The time interval between last RTX course and BNT162b2 vaccine was calculated in days. Data on CD19-positive B cell counts at the time of RTX administration prior the vaccination were unavailable.

Vaccination procedure

All participants were administered the two-dose regimen of the BNT162b2 Pfizer BioNTech mRNA vaccine according to national guidelines. Each 30-µg dose was given as an intramuscular injection in the deltoid muscle, the second dose given 3 weeks after the first dose.

Vaccine Immunogenicity

All study participants had a serological test performed 2 to 6 weeks after the second vaccine dose. SARS-CoV-2 S1/S2 IgG antibodies were measured by the food and drug administration (FDA) authorized LIAISON (Diasorin) quantitative assay, with 98% sensitivity and specificity.(31) A cut-off of 15 binding antibody units (BAU) was considered as a positive immunogenic response, according to the manufacturer's instructions. In the validation group, anti-spike- receptor-binding- domain (RBD) antibodies were measured by the SARS-Cov-2 IgG II Quant (Abbott) assay (a chemiluminescent microparticle immunoassay) on the ARCHITECT ci8200system from Abbott. This test was considered positive when titers were above 50 AU/ml, according to the manufacturer's instructions. Whereas an inter-assay validation test between the two assays was out of this study scope, published data suggest a good diagnostic performance and strong correlations with neutralizing antibodies for both.(32,33)

Vaccine safety
Study participants were questioned (by phone or in-person) regarding adverse events 2 weeks after the 1st vaccine dose and 2-6 weeks after the 2nd vaccine dose.

Assessment of AIIRD activity

Pre-vaccination disease activity was retrieved from the medical records within 3 months before vaccination. Post-vaccination disease activity was clinically assessed 2-6 weeks after the 2nd dose. Disease activity indices used were Clinical Disease Activity Index (CDAI), Simplified DAI (SDAI), and DAS-28-CRP for RA, Systemic Lupus Disease Activity Index (SLEDAI) for SLE, and patient and physician global assessment (PGA and PhGA, respectively), using a visual analogue scale (VAS) of 0–10 mm, for vasculitis and IIM.

Statistical Analysis

Differences between categorical variables were tested with the Fisher's exact test. Differences between numeric variables were tested with t-test. A stepwise backward multiple logistic regression for predicting a seropositive response to vaccination was applied to AIIRD patients with all data available (n=104). The AIIRD diagnosis was a dummy variable, meaning that each participant could have only one diagnosis. The model included all individual variables that showed p<0.2 significance level between seropositive and seronegative result. The rule for leaving the variable in the model was p < 0.2. Multicollinearity between significant variables was assessed by Pearson correlations. The prediction calculator was tested on an independent cohort of 48 BNT162b2 vaccinated AIIRD-RTX patients (validation group), including 21 patients with a positive immunogenic response (responders) to vaccination and 27 patients without a detectable immunogenic response (non-responders) to vaccination. The calculated fixed values for the tested population were plotted on a receiver operator characteristic (ROC) curve to select the optimal discriminative cut-off to predict a positive response to vaccination. Sensitivity, specificity, positive predictive, and negative predictive values (PPV and NPV, respectively) were calculated based on this optimal cut-off of the ROC curve.

Results

Study participants

The study included 108 AIIRD-RTX patients and 122 controls, all vaccinated with the two-dose regimen of the BNT162b2 mRNA vaccine. Demographics and clinical data of the AIIRD-RTX population are presented in Table 1. AIIRD-RTX patients were significantly older than controls, mean ± standard deviation (SD) 61.45±14.96 vs 50.83±14.64 years, p<0.0001. In both patient and control groups, the majority were females, 76.85% (n=83) and 64.75% (n=79), p=0.06, with a high uptake of the seasonal 2020 influenza vaccination prior to the BNT162b2 mRNA vaccination, 84.11% (n=90) and 81.65% (n=89), p=0.72, respectively. The most common AIIRD diagnosis was RA in 45.37% (n=49), followed by AAV (21.3%, n=23), IIM (16.67%, n=18), SLE (10.19%, n=11), and other vasculitides (5.66%, n=6).
Table 1. Demographic and clinical characteristics of AIIRD patients treated with rituximab vaccinated with the BNT162b2 mRNA COVID-19 vaccine (n=108).
| Age, years, median (range) | 65.5 (23-88) |
|----------------------------|-------------|
| Female gender, n (%)       | 83 (76.85)  |
| Seasonal influenza vaccine uptake, n/total (%) | 90/107 (84.11) |
| **AIIRD diagnosis, n (%)** |            |
| RA                        | 49 (45.37)  |
| SLE                       | 11 (10.19)  |
| ANCA-associated vasculitis | 23 (21.3)   |
| Other systemic vasculitis | 6 (5.56)    |
| IIM                       | 18 (16.67)  |
| Concomitant lymphoma, n/total (%) | 5/88 (5.68) |
| **AIIRD duration, years, median (range)** | 10.5 (0.75-45) |
| **Rituximab-relevant details, (mg), median (range)** |  |
| Serum IgG level prior last RTX course (mg/dL) (n=105) | 911 (357-3405) |
| Hypogammaglobulinemia<500mg/dL (prior to last RTX course), n/total (%) | 6/106 (5.66) |
| RTX cumulative dose       | 6000 (1000-30,000) |
| RTX dose of last course prior to vaccination | 2000 (500-3210) |
| Total number of RTX courses | 4 (1-15) |
| Interval between last RTX course and BNT162b2 vaccination, days | 162.5 (2-2794) |
| **Concomitant immunosuppressive medications, n (%)** |  |
| csDMARDs                  | 34 (31.48)  |
| Methotrexate              | 16 (14.81)  |
| Methotrexate dose, mg/week, mean±SD | 13.1±5.32 |
| Prednisone                | 54 (50)     |
| Prednisone dose, mg/d, mean±SD | 5.9±3.44 |
| **Other immunosuppressants, n (%)** |  |
| Leflunomide               | 2 (1.85)    |
| Mycophenolate mofetil     | 5 (4.63)    |
| IVIG                      | 9 (8.33)    |
Legend: AIIRD, autoimmune inflammatory rheumatic diseases; ANCA, antineutrophil cytoplasmic antibody; csDMARDs, conventional synthetic disease modifying antirheumatic drugs; IIM, idiopathic inflammatory myopathy; IgG, immune globulin G; IVIG, intravenous immune globulin; n, number; RA, rheumatoid arthritis; RTX, rituximab; SD, standard deviation, SLE, systemic lupus erythematosus.

Immunogenicity of the BNT162b2 vaccine

The BNT162b2 vaccine-induced positive immunogenic response rate and serum S1/S2 IgG titers were significantly lower in the AIIRD-RTX group compared to controls, 41.67% (n=45) vs 100%, n=122 (p<0.0001) and 51.01±79.17 vs 218.39±81.76 BAU, p<0.0001, respectively. The lowest S1/S2 IgG titer (BAU, mean±SD) was detected in AAV and IIM patients, 36.25±73 and 25.19±45.07, respectively, followed by patients with other non-AAV systemic vasculitides (48.8±74.29), whereas the highest titers were detected in patients with SLE and RA, 99.84±110.55 and 55.19±81.55, respectively. Both vaccine responders and non-responders were similar with regard to age, gender, and concomitant immunosuppressive medications use. The rate of seropositive and seronegative vaccine response was similarly distributed across all rheumatic diseases, except for SLE patients who had a high prevalence of a seropositive immunogenic response, 81.82% (n=9) vs 18.18% (n=2), p=0.007, respectively. Collectively, SLE patients had the lowest cumulative RTX dose, the highest mean IgG levels prior to last RTX course, and a longer interval between last RTX course and BNT162b2 vaccine. (table 2 and supplementary table S1).

Table 2. Comparison of AIIRD patients treated with rituximab who did or did not mount a positive immunogenic response following vaccination with the BNT162b2 mRNA COVID-19 vaccine.
|                                | Vaccine responders, n=45 | Vaccine non-responders, n=63 | p value |
|--------------------------------|--------------------------|-----------------------------|---------|
| Age, years, median (range)     | 64 (29-88)               | 67 (23-87)                  | 0.075   |
| Gender, female, n (%)          | 37 (82.22)               | 46 (73.02)                  | 0.356   |
| Seasonal influenza vaccine uptake, n/total (%) | 35/44 (79.55) | 55/63 (87.3) | 0.296   |
| Disease duration, years, median (range) | 13 (0.75-45) | 9 (1-42) | 0.146   |

**AllIRD diagnosis, n (%)**

- RA: 23 (51.11) vs 26 (41.27), p = 0.333
- SLE: 9 (20) vs 2 (3.17), p = 0.007
- ANCA-associated vasculitis: 6 (13.33) vs 17 (26.98), p = 0.101
- Other systemic vasculitis: 2 (4.44) vs 4 (6.35), p = 1
- IIM, n (%): 4 (8.89) vs 14 (22.22), p = 0.074
- History of lymphoma, n/total (%): 4/39 (10.26) vs 1/49 (2.04), p = 0.166

**Rituximab-relevant details, (mg), median (range)**

- Serum IgG level prior last RTX (mg/dL)**: 1189.78±576.28 vs 884.33±302.31, p = 0.002
- Hypogammaglobulinemia<500mg/dL (prior to last RTX course), n/total (%): 1/44 (2.27) vs 5/62 (8.06), p = 0.397
- RTX cumulative dose: 4000 (2000-20,000) vs 8000 (1000-30,000), p = 0.033
- RTX dose of last course prior to vaccination: 2000 (500-3210) vs 2000 (500-2000), p = 0.168
- Total number of RTX courses: 3 (1-10) vs 5 (1-15), p = 0.007
- Time interval between last RTX course and BNT162b2 vaccination, days: 255 (6-2794) vs 130 (2-1163), p = 0.0009
  - Up to 180 days, n (%): 14 (31.11) vs 49 (77.78), p <0.0001
  - 181-365 days, n (%): 13 (28.89) vs 11 (17.46)
  - Over 365 days, n (%): 18 (40) vs 3 (4.76)

**Concomitant immunosuppressive medication, n (%)**

- csDMARDs: 18 (40) vs 16 (25.4), p = 0.142
- Methotrexate: 6 (13.33) vs 10 (15.87), p = 0.789
| Methotrexate dose, mg/week, mean±SD | 10.63±4.27 | 14.17±5.59 | 0.287 |
|-----------------------------------|------------|------------|--------|
| Prednisone                        | 18 (40)    | 36 (57.14) | 0.118  |
| Prednisone dose, mg/d, mean±SD ***| 5.47±3.23  | 6.16±3.56  | 0.505  |
| Other immunosuppressants, n (%)   |            |            |        |
| Leflunomide                       | 1 (2.22)   | 1 (1.59)   | 1      |
| Mycophenolate mofetil             | 1 (2.22)   | 4 (6.35)   | 0.399  |
| IVIG                              | 3 (6.67)   | 6 (9.52)   | 0.732  |

* Fisher exact test p-value for the comparison between the relevant diagnosis (RA, SLE, etc.) and all other diagnoses.

** Data were missing for 3 patients.

*** Data were missing for 1 patient.

Legend: AIIRD, autoimmune inflammatory rheumatic diseases; ANCA, antineutrophil cytoplasmic antibody; csDMARDS, conventional synthetic disease modifying antirheumatic drugs; IIM, idiopathic inflammatory myopathy; IgG, immune globulin G; IVIG, intravenous immune globulin; n, number; RA, rheumatoid arthritis; RTX, rituximab; SD, standard deviation; SLE, systemic lupus erythematosus.

Predictors associated with seropositive immunogenic response to the BNT162b2 vaccine in AIIRD patients treated with RTX

Vaccine responders significantly differed from non-responders by the following parameters (table 2): 1) higher total IgG level prior to last RTX course (mean ± SD), 1189.78±576.28 vs. 884.33±302.31 mg/dL, p=0.002, respectively; 2) lower cumulative RTX dose, 6943.11±5975.74 vs 9780.95±7240.12 mg, p=0.033, respectively; 3) lower total number of RTX courses (median (range) 3 (1-10) vs 5 (1-15), respectively, p=0.007. The time interval between RTX treatment and vaccination was more than twice longer in responders, 469.82±570.39 vs 162.08±160.12 days, p=0.0009, respectively.

In the stepwise backward logistic regression model predicting seropositive immunogenic response to vaccination (table 3), AAV and IIM diagnoses significantly decreased the likelihood of the response, odds ratio (OR) 0.209, 95% confidence interval (CI) 0.046-0.96, p=0.044, and OR 0.189 (95% CI 0.036-0.987), p=0.048, respectively, while higher serum total IgG levels prior to last RTX course and longer time interval between RTX treatment and BNT162b2 vaccine increased the likelihood of the response, OR 1.1 (95% CI 1.019-1.196) for each IgG level increment by 50 mg/dL, p=0.016 and OR 1.048 (95%CI 1.018-1.079) for each passing week after last RTX course, p=0.002, respectively (figure 1). There was a moderate
correlation between the IgG levels and a total number of RTX courses, but not strong enough to assume multicollinearity, permitting to consider both variables as independent predictors.

Table 3. Stepwise backward logistic regression predicting seropositive immunogenic response following BNT162b2 mRNA vaccination (n=104)

| Predictors                                                                 | OR     | 95% CI          | p value |
|---------------------------------------------------------------------------|--------|-----------------|---------|
| RA Ref                                                                    | Ref    | Ref             | Ref     |
| **AllIRD diagnosis**                                                      |        |                 |         |
| SLE                                                                       | 4.225  | 0.543-32.89     | 0.169   |
| ANCA-associated vasculitis                                                | 0.209  | 0.046-0.96      | 0.044   |
| Other systemic vasculitis                                                 | 0.478  | 0.044-5.244     | 0.546   |
| IIM                                                                       | 0.189  | 0.036-0.987     | 0.048   |
| **Rituximab-relevant details**                                            |        |                 |         |
| Serum IgG level (50 mg/dL increments, prior to last RTX course)           | 1.104  | 1.019-1.196     | 0.016   |
| Total number of RTX courses                                               | 0.874  | 0.75-1.018      | 0.084   |
| Time interval between last RTX course and BNT162b2 vaccine (weeks)        | 1.048  | 1.018-1.079     | 0.002   |

The model included the following variables: AllIRD diagnosis, disease duration, serum IgG levels, cumulative rituximab dose, rituximab last course dose, total rituximab courses, and time interval between last rituximab course and BNT162b2 vaccine. The rule for leaving the variable in the model was p < 0.2.

Legend: AllIRD, autoimmune inflammatory rheumatic diseases; ANCA, antineutrophil cytoplasmic antibody; IIM, idiopathic inflammatory myopathy; IgG, immune globulin G; RA, rheumatoid arthritis; RTX, rituximab; SLE, systemic lupus erythematosus.

Development and validation of the predicting calculator for a seropositive immunogenic response to the BNT162b2 mRNA vaccine in RTX-treated patients

We used stepwise backward multiple logistic regression to identify the following independent predictors for a seropositive immunogenic vaccine response: AllIRD diagnosis, total number of RTX courses prior to vaccination, serum IgG level prior to last RTX course administered before vaccination, and time interval between last RTX course and vaccination date. Notably, MTX and CS variables were not significant according to the model. We further developed a prediction calculator by the equation presented in figure 2.
Next, we validated the model using data from an independent AIIRD-RTX cohort (n=48) vaccinated with the BNT162b2 mRNA vaccine, including 21 responders and 27 non-responders. The characteristics of the validation and main study groups were similar regarding age, gender, and the rate of seropositive response to vaccination. In contrast to the main study group, there were only three cases of AAV in the validation group. (supplementary table S2) We further applied a ROC curve to select the optimal discriminative cut-off value of 0.41, with a sensitivity of 90.5%, specificity of 59.3%, PPV 63.4%, and NPV 89.9.1% (figure 3). As an example of the calculator use, an AAV patient with a treatment history of 4 RTX courses, IgG level of 600 mg/dl, and 100 days interval to a planned vaccination would have a very low probability (5.3%) of seropositive response, thus suggesting to test a serologic response following vaccination and consider a vaccination booster later on. In opposite, an RA patient with a treatment history of 2 RTX courses, IgG level of 1100 mg/dl, and 100 days interval to a planned vaccination would have a higher chance for a seropositive response estimated as 42%.

BNT162b2 vaccine safety in seropositive and seronegative RTX-AIIRD patients

Vaccine responders and non-responders had a similar profile and rate of vaccine-related adverse events. (supplementary table S3) Among non-responders, one patient died due to fulminant haemorrhagic cutaneous vasculitis with subsequent fatal sepsis. She had AAV in clinical remission following RTX treatment in October 2017 and was treated only with low-dose prednisone (5 mg/day) at the time of vaccination.(8)

BNT162b2 vaccine impact on disease activity in RTX-AIIRD patients

Following vaccination, disease activity indices of RA patients worsened in 26.5%-32.6% of patients, were stable in 52.9%-60% of patients, and improved in 12.5-20.6% of patients, depending on the score used. (supplementary table S4 and figure S1) The pattern of disease activity changes was similar in RA patients who mounted a seropositive immunogenic response to vaccination and those who did not. SLE disease activity measured by SLEDAI remained stable for 8 of 9 patients who had available pre- and post-vaccination SLEDAI. PGA and PhGA-VAS scores before and after vaccination were overall stable in AAV, IIM, and patients with other vasculitides.

**Discussion**

The optimal timing of COVID-19 vaccination in AIIRD patients treated with RTX remains debatable. Herein, we report the analysis of a large group of RTX treated AIIRD patients (n=108) vaccinated with the two-dose BNT162b2 mRNA vaccine regimen, representing a subset of the ongoing multicenter controlled vaccination trial conducted within a nationwide vaccination campaign.

An immunogenic serologic response against the BNT162b2 mRNA vaccine was observed in all immunocompetent controls, yet in only 41.7% of the RTX-treated patients, with significantly lower post-vaccination anti-spike S1/S2 IgG antibody levels in the latter group. This finding aligns with the previous studies confirming the negative impact of RTX on vaccine-induced immunogenicity. (8–18) The safety
profile of vaccination was similar among patients with positive and negative immunogenic responses to vaccination and consistent with the report of the main study. We further identified predictors for a positive immunogenic response to vaccination defined in a binary mode by the positive versus negative anti-spike S1/S2 antibodies, without considering the antibodies’ titer. The predictors included the diagnosis of rheumatoid arthritis as opposed to AAV and IIM, a low number of total RTX courses prior to vaccination, high serum total IgG levels prior to last RTX course, and a longer interval between RTX treatment and vaccination.

To date, a number of studies addressed this topic, reaching a consensus that CD19 reconstitution at the time of vaccination plays a critical role in mounting the immunogenic response to vaccination. Mrak et al reported that among 74 RTX-treated AIIRD patients immunized with mRNA vaccines, only patients with measurable peripheral B cells developed an immunogenic response to vaccination, measured by antibodies to the receptor-binding-domain (RBD) of the spike protein and the number of peripheral B cells correlated with levels of anti-RBD antibodies. Interestingly, T-cell-mediated immune response was observed independently of both peripheral B cell presence and antibody response. Similar results were reported by Prendecki et al based on 44 RTX-treated AIIRD patients. As data on CD19 peripheral B cells were not available in our study and are not available in routine practice, we chose to use commonly collected variables to develop the predicting calculator. It is plausible to assume that the extent of exposure to RTX reflected by the total number of RTX courses and the time interval between RTX treatment and vaccination indicate the extent of B cell reconstitution at the time of vaccination. Indeed, several studies also confirmed the impact of the time interval between RTX treatment and vaccination on predicting the serological response.

Remarkably, AAV and IIM conferred the risk for a negative or poor immunogenic response to vaccination as opposed to patients with RA and SLE, despite the highest cumulative dose of RTX being observed in patients with RA. A potential explanation may relate to the long-lasting RTX-induced depletion of B cells reported in AAV in contrast to patients with RA and connective tissue diseases. After a single course of RTX for remission induction, AAV patients might have a very long-lasting B cell depletion up to more than 60 months, particularly in patients with microscopic polyangiitis and advanced renal failure, indicating a profound dysregulation of the B-cell compartment in these patients. In addition, patients with AAV and IIM are commonly treated with several immunosuppressants for an extended period of time, contributing to an impaired immunogenic response to vaccination. The history of the previous use of immunosuppressants, such as cyclophosphamide or mycophenolate mofetil, was not accounted in the present model.

The high clinical relevance of our study relies on the development of an algorithm available as a simple for use calculator for predicting the probability of a seropositive immunogenic response to vaccination in RTX-treated AIIRD patients, validated in a separate independent cohort of RTX-treated AIIRD patients (n=48). The calculator is suitable for AIIRD patients with RA, AAV, and other systemic vasculitides, SLE, and IIM. RTX-related details required for the calculator use include the total number of RTX courses, serum IgG level prior to last RTX course, and the interval between last RTX course and vaccination.
Therefore, the calculator can be helpful in daily practice in the absence of peripheral B cell count. Yet, the result obtained by the calculator should be interpreted with a certain caution in view of a moderate specificity of 59.3% and moderate PPV of 63.3%, accounting for potential false positive results. On the other hand, relatively high sensitivity of 90.5% and NPV of 88.9% account for a low rate of false negative results.

Our study has several limitations. First, it should be underlined that seropositive immunogenic response by itself, defined as the main outcome of the study, does not necessarily imply protection from COVID-19 infection, especially in the case of low antibodies titers.(37) As there is no consensus on the protective antibody levels in vaccinated patients with rheumatic diseases, the use of a seropositive versus seronegative immunogenic response may be used in clinical practice. Second, there are a number of limitations pertaining to the model of the predicting calculator. We observed the difference in immunogenic response between RA and AAV/IIM patients treated by RTX not reported by other studies(10,11,17) potentially due to differences in cumulative immunosuppression not captured in our model. The diagnostic power of the model appeared to be moderate, at least partially explained by a small size of the validation cohort characterized by a different representation of AIIRD compared to the main cohort. The immunogenic response was evaluated by different laboratory assays in both cohorts, yet this difference should not significantly affect the results as both assays correlated well with anti-SARS-CoV-2 neutralizing antibodies levels.(31,32) Furthermore, no data on CD19 peripheral cells were available. Yet, clinical variables used in the predicting model indirectly reflect this parameter and are more accessible in daily practice. While assessment of T-cell induced immune response to vaccination may be important in patients with absent immunogenic response, this analysis was beyond the scope of this study. Finally, the study was performed with one vaccine type, with questionable applicability for other vaccines.

**Conclusion**

This is the first multicenter study based on a diverse AIIRD population treated with RTX to identify clinical predictors for a seropositive immunogenic vaccination response to BNT162b2 vaccination. A diagnosis of rheumatoid arthritis (as opposed to AAV and IIM), low number of total RTX courses prior to vaccination, high serum total IgG level prior to last RTX course, and extended interval between RTX treatment and vaccination confer a high probability to achieve a seropositive immunogenic response to vaccination. The study provides the clinicians with a practical prediction calculator for assessing the probability of seropositive response to mRNA BNT162b2 vaccination easily applied in daily practice. Taken together, the use of the predicting calculator may optimize the scheduling of COVID-19 vaccination in the setting of RTX treatment in AIIRD patients. Further investigation of predictors of vaccine-induced response and vaccine efficacy as well as validation of the proposed prediction algorithm in prospective cohorts are warranted.

**Abbreviations**
AAV - ANCA associated vasculitis
AIIRD - autoimmune inflammatory rheumatic diseases
AUC - area under curve
BAU - binding antibody units
cDMARD - conventional synthetic disease modifying antirheumatic drug
CDAI - clinical disease activity index
CI - confidence interval
DAS-28-CRP - disease activity score with c-reactive protein
GC - glucocorticoids
IgG - immunoglobulin G
IIM - idiopathic inflammatory myositis
IVIG - intravenous immunoglobulin
MTX - methotrexate
mRNA - messenger RNA
n - number
OR - odds ratio
PDA - patient global assessment
PhGA - physician global assessment
RA - rheumatoid arthritis
RBD - receptor-binding-domain
ROC - receiver operating characteristic
RTX - rituximab
SD - standard deviation
SDAI - simplified disease activity index
Declarations

Ethics approval and consent to participate

The study was performed in accordance with the principles of the Declaration of Helsinki and approved by the research ethics committees of the four participating medical centers: Tel Aviv Medical Center (TLV-1055-20), Carmel Medical Center (CMC-0238-20), Hadassah Medical Center (HMO-0025-21), and Rambam Medical Health Care Campus (RMB-417-20). All study participants signed an informed consent on recruitment into the study.

Consent for publication: not applicable

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request

Competing interests

The authors declare that they have no competing interests.

Funding

The study was funded by the department’s fund of each medical center participating in the study.

Authors' contributions

The study was designed, directed and coordinated by OE, the principle investigator. VF, TE, DZ, HP, YB, the sub-investigators, were in charge of the study conducted at all stages. All the MD co-authors recruited participants into the study. GS and OS performed the serology tests. SP and SN served as main study coordinators and questioned the study participants regarding the adverse events of vaccination.

OE, VF, and TE had full access to the study’s data and drafted the article, which was critically reviewed by DP, DZ, HP, YB.

Acknowledgements

We thank Mr Yoram Neufeld for his assistance with data management and Mr Yishai Friedlander, MPH, for performing the statistical analysis.
1. Polack FP, Thomas SJ, Kitchin N, Absalon J, Gurtman A, Lockhart S, et al. Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine. N Engl J Med. 2020 Dec 31;383(27):2603–15.

2. Baden LR, El Sahly HM, Essink B, Kotloff K, Frey S, Novak R, et al. Efficacy and Safety of the mRNA-1273 SARS-CoV-2 Vaccine. N Engl J Med. 2021 Feb 4;384(5):403–16.

3. Williamson EJ, Walker AJ, Bhaskaran K, Bacon S, Bates C, Morton CE, et al. Factors associated with COVID-19-related death using OpenSAFELY. Nature. 2020 Jul 8;584(7821):430–6.

4. D’Silva KM, Jorge A, Cohen A, McCormick N, Zhang Y, Wallace ZS, et al. COVID-19 Outcomes in Patients with Systemic Autoimmune Rheumatic Diseases (SARDs) Compared to the General Population: A US Multi-Center Comparative Cohort Study. Arthritis Rheumatol. 2020 Dec 10;

5. Curtis JR, Johnson SR, Anthony DD, Arasaratnam RJ, Baden LR, Bass AR, et al. American College of Rheumatology Guidance for COVID-19 Vaccination in Patients With Rheumatic and Musculoskeletal Diseases: Version 2. Arthritis Rheumatol. 2021 Jun 15;

6. Geisen UM, Berner DK, Tran F, Sümbül M, Vullriede L, Ciripoi M, et al. Immunogenicity and safety of anti-SARS-CoV-2 mRNA vaccines in patients with chronic inflammatory conditions and immunosuppressive therapy in a monocentric cohort. Ann Rheum Dis. 2021 Mar 24;

7. Haberman RH, Herati R, Simon D, Samanovic M, Blank RB, Tuen M, et al. Methotrexate hampers immunogenicity to BNT162b2 mRNA COVID-19 vaccine in immune-mediated inflammatory disease. Ann Rheum Dis. 2021 May 25;

8. Furer V, Eviatar T, Zisman D, Peleg H, Paran D, Levartovsky D, et al. Immunogenicity and safety of the BNT162b2 mRNA COVID-19 vaccine in adult patients with autoimmune inflammatory rheumatic diseases and in the general population: a multicentre study. Ann Rheum Dis. 2021 Jun 14;

9. Seyahi E, Bakhdiyarli G, Oztas M, Kuskucu MA, Tok Y, Sut N, et al. Antibody response to inactivated COVID-19 vaccine (CoronaVac) in immune-mediated diseases: a controlled study among hospital workers and elderly. Rheumatol Int. 2021 Aug;41(8):1429–40.

10. Deepak P, Kim W, Paley MA, Yang M, Carvidi AB, Demissie EG, et al. Effect of Immunosuppression on the Immunogenicity of mRNA Vaccines to SARS-CoV-2: A Prospective Cohort Study. Ann Intern Med. 2021 Aug 31;

11. Moor MB, Suter-Riniker F, Horn MP, Aeberli D, Amsler J, Möller B, et al. Humoral and cellular responses to mRNA vaccines against SARS-CoV-2 in patients with a history of CD20 B-cell-depleting therapy (RituxiVac): an investigator-initiated, single-centre, open-label study. Lancet Rheumatol. 2021 Sep 7;

12. Prendecki M, Clarke C, Edwards H, McIntyre S, Mortimer P, Gleeson S, et al. Humoral and T-cell responses to SARS-CoV-2 vaccination in patients receiving immunosuppression. Ann Rheum Dis. 2021 Aug 5;

13. Braun-Moscovici Y, Kaplan M, Braun M, Markovits D, Giryes S, Toledano K, et al. Disease activity and humoral response in patients with inflammatory rheumatic diseases after two doses of the Pfizer mRNA vaccine against SARS-CoV-2. Ann Rheum Dis. 2021 Jun 18;
14. Connolly CM, Koenig D, Ravi SN, Azar A, Kant S, Dalal M, et al. Correspondence on “SARS-CoV-2 vaccination in rituximab-treated patients: evidence for impaired humoral but inducible cellular immune response” by Bonelli et al. Ann Rheum Dis. 2021 Aug 2;

15. Spiera R, Jinich S, Jannat-Khah D. Rituximab, but not other antirheumatic therapies, is associated with impaired serological response to SARS-CoV-2 vaccination in patients with rheumatic diseases. Ann Rheum Dis. 2021 May 11;

16. Mrak D, Tobudic S, Koblishcke M, Graninger M, Radner H, Sieghart D, et al. SARS-CoV-2 vaccination in rituximab-treated patients: B cells promote humoral immune responses in the presence of T-cell-mediated immunity. Ann Rheum Dis. 2021 Jul 20;

17. Boekel L, Steenhuis M, Hooijberg F, Besten YR, van Kempen ZLE, Kummer LY, et al. Antibody development after COVID-19 vaccination in patients with autoimmune diseases in the Netherlands: a substudy of data from two prospective cohort studies. Lancet Rheumatol. 2021 Aug 6;

18. Ammitzbøll C, Bartels LE, Bøgh Andersen J, Risbøl Vils S, Elbaek Mistegård C, Dahl Johanssen A, et al. Impaired antibody response to the bnt162b2 messenger RNA coronavirus disease 2019 vaccine in patients with systemic lupus erythematosus and rheumatoid arthritis. ACR Open Rheumatol. 2021 Jul 17;

19. Rondaan C, Furer V, Heijstek MW, Agmon-Levin N, Bijl M, Breedveld FC, et al. Efficacy, immunogenicity and safety of vaccination in adult patients with autoimmune inflammatory rheumatic diseases: a systematic literature review for the 2019 update of EULAR recommendations. RMD Open. 2019 Sep 9;5(2):e001035.

20. Strangfeld A, Schäfer M, Gianfrancesco MA, Lawson-Tovey S, Liew JW, Ljung L, et al. Factors associated with COVID-19-related death in people with rheumatic diseases: results from the COVID-19 Global Rheumatology Alliance physician-reported registry. Ann Rheum Dis. 2021 Jan 27;

21. Schulze-Koops H, Krueger K, Vallbracht I, Hasseli R, Skapenko A. Increased risk for severe COVID-19 in patients with inflammatory rheumatic diseases treated with rituximab. Ann Rheum Dis. 2020 Jun 26;

22. FAI2R /SFR/SNFMI/SOFREMIP/CRI/IMIDiate consortium and contributors. Severity of COVID-19 and survival in patients with rheumatic and inflammatory diseases: data from the French RMD COVID-19 cohort of 694 patients. Ann Rheum Dis. 2020 Dec 2;

23. Avouac J, Drumez E, Hachulla E, Seror R, Georgin-Lavialle S, El Mahou S, et al. COVID-19 outcomes in patients with inflammatory rheumatic and musculoskeletal diseases treated with rituximab: a cohort study. Lancet Rheumatol. 2021 Jun;3(6):e419–26.

24. Sparks JA, Wallace ZS, Seet AM, Gianfrancesco MA, Izadi Z, Hyrich KL, et al. Associations of baseline use of biologic or targeted synthetic DMARDs with COVID-19 severity in rheumatoid arthritis: Results from the COVID-19 Global Rheumatology Alliance physician registry. Ann Rheum Dis. 2021 Sep;80(9):1137–46.

25. Bonelli MM, Mrak D, Perkman T, Haslacher H, Aletaha D. SARS-CoV-2 vaccination in rituximab-treated patients: evidence for impaired humoral but inducible cellular immune response. Ann Rheum Dis. 2021 Aug 2;
26. Aletaha D, Neogi T, Silman AJ, Funovits J, Felson DT, Bingham CO, et al. 2010 Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. Arthritis Rheum. 2010 Sep;62(9):2569–81.

27. Taylor W, Gladman D, Helliwell P, Marchesoni A, Mease P, Mielants H, et al. Classification criteria for psoriatic arthritis: development of new criteria from a large international study. Arthritis Rheum. 2006 Aug;54(8):2665–73.

28. Petri M, Orbai A-M, Alarcón GS, Gordon C, Merrill JT, Fortin PR, et al. Derivation and validation of the Systemic Lupus International Collaborating Clinics classification criteria for systemic lupus erythematosus. Arthritis Rheum. 2012 Aug;64(8):2677–86.

29. Jennette JC, Falk RJ, Andrassy K, Bacon PA, Churg J, Gross WL, et al. Nomenclature of systemic vasculitides. Proposal of an international consensus conference. Arthritis Rheum. 1994 Feb;37(2):187–92.

30. Lundberg IE, Tjärnlund A, Bottai M, Werth VP, Pilkington C, Visser M de, et al. 2017 European League Against Rheumatism/American College of Rheumatology classification criteria for adult and juvenile idiopathic inflammatory myopathies and their major subgroups. Ann Rheum Dis. 2017 Oct 27;76(12):1955–64.

31. Criscuolo E, Diotti RA, Strollo M, Rolla S, Ambrosi A, Locatelli M, et al. Weak correlation between antibody titers and neutralizing activity in sera from SARS-CoV-2 infected subjects. J Med Virol. 2021 Apr;93(4):2160–7.

32. Jung K, Shin S, Nam M, Hong YJ, Roh EY, Park KU, et al. Performance evaluation of three automated quantitative immunoassays and their correlation with a surrogate virus neutralization test in coronavirus disease 19 patients and pre-pandemic controls. J Clin Lab Anal. 2021 Sep;35(9):e23921.

33. Perkmann T, Perkmann-Nagele N, Koller T, Mucher P, Radakovics A, Marculescu R, et al. Anti-Spike Protein Assays to Determine SARS-CoV-2 Antibody Levels: a Head-to-Head Comparison of Five Quantitative Assays. Microbiol Spectr. 2021 Sep 3;9(1):e0024721.

34. Benucci M, Damiani A, Infantino M, Manfredi M, Grossi V, Lari B, et al. Correspondence on “SARS-CoV-2 vaccination in rituximab-treated patients: evidence for impaired humoral but inducible cellular immune response”by Bonelli et al. Ann Rheum Dis. 2021 Aug 2;

35. Thiel J, Rizzi M, Engesser M, Dufner A-K, Troilo A, Lorenzetti R, et al. B cell repopulation kinetics after rituximab treatment in ANCA-associated vasculitides compared to rheumatoid arthritis, and connective tissue diseases: a longitudinal observational study on 120 patients. Arthritis Res Ther. 2017 May 18;19(1):101.

36. Salviani C, Jeannin G, Cancarini G, Gregorini G. 346. factors influencing b cell repopulation after remission induction with rituximab in newly diagnosed, treatment–naïve patients with anca-associated vasculitis. Rheumatology. 2019 Mar 1;58(Supplement_2).

37. Bergwerk M, Gonen T, Lustig Y, Amit S, Lipsitch M, Cohen C, et al. Covid-19 Breakthrough Infections in Vaccinated Health Care Workers. N Engl J Med. 2021 Oct 14;385(16):1474–84.
Supplementary Files

Supplementary Files are not available with this version.

Figures

Figure 1

Scatter plots showing a correlation between immunoglobulin G (IgG) levels prior to last rituximab course (panel a) and time interval between last rituximab course in days (panel b) and anti-S1/S2 antibody titer after BNT162b2 mRNA vaccination.

Legend: Ab, Antibody; BAU, binding antibody units; IgG, immunoglobulin G.

\[
\frac{\exp(-2.682 + 1.441 \cdot SLE - 1.665 \cdot CTD - 1.564 \cdot ANCA - 0.738 \cdot Vasculitis + 0.002 \cdot IgG - 0.135 \cdot total\ courses + 0.007 \cdot Days)}{1 + \exp(-2.682 + 1.441 \cdot SLE - 1.665 \cdot CTD - 1.564 \cdot ANCA - 0.738 \cdot Vasculitis + 0.002 \cdot IgG - 0.135 \cdot total\ courses + 0.007 \cdot Days)}
\]

Figure 2

Equation of the predicting model. Legend: AAV, ANCA-associated vasculitis; IIM, idiopathic inflammatory myopathy; IgG, immunoglobulin G, mg/dl; SLE, systemic lupus erythematosus. Vasculitis includes non-AAV types of vasculitis. Days indicate the interval between last RTX course and vaccination.
Figure 3

ROC curve for determining the predicting calculator optimal cut-off for a seropositive immunogenic response to the BNT162b2 mRNA vaccination in rituximab treated AIIRD patients.