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Immune Response to SARS-CoV-2 Third Vaccine in Patients With Rheumatoid Arthritis Who Had No Seroconversion After Primary 2-Dose Regimen With Inactivated or Vector-Based Vaccines

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ABSTRACT. ***Objective.*** The aim of this study was to assess the immune response after a third dose of SARS-CoV-2 vaccine in patients with rheumatoid arthritis (RA) with undetectable antibody titers after the primary regimen of 2 doses.

Methods. Patients with RA with no seroconversion after 2 doses of SARS-CoV-2 vaccine and who received a third dose of either an mRNA or vector-based vaccine were included. Anti-SARS-CoV-2 IgG antibodies, neutralizing activity, and T cell responses were assessed after the third dose.

Results. A total of 21 nonresponder patients were included. At the time of vaccination, 29% were receiving glucocorticoids and 85% biologic disease-modifying antirheumatic drugs (including 6 taking abatacept [ABA] and 4 taking rituximab [RTX]). The majority (95%) received the BNT162b2 vaccine and only one of them received the ChAdOx1 nCoV-19 vaccine. After the third dose, 91% of the patients presented detectable anti-SARS-CoV-2 IgG and 76% showed neutralizing activity. Compared to other treatments, ABA and RTX were associated with the absence of neutralizing activity in 4 out of 5 (80%) patients and lower titers of neutralizing antibodies (median 3, IQR 0-20 vs 8, IQR 4-128; $P = 0.20$). Specific T cell response was detected in 41% of all patients after the second dose, increasing to 71% after the third dose. The use of ABA was associated with a lower frequency of T cell response (33% vs 87%, $P = 0.03$).

Conclusion. In this RA cohort, 91% of patients who failed to seroconvert after 2 doses of SARS-CoV-2 vaccine presented detectable anti-SARS-CoV-2 IgG after a third dose. The use of ABA was associated with a lower frequency of specific T cell response.

Key Indexing Terms: Argentina, COVID-19, rheumatoid arthritis, SARS-CoV-2, vaccines

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A coordinated innate and adaptive immune response is needed to control SARS-CoV-2 infection. The 3 fundamental components of the adaptive immune system are B cells, CD4+ T cells, and CD8+ T cells, which facilitate the elimination of intra- and extracellular viruses. To prevent SARS-CoV-2 infection and transmission, as well as reduce disease severity, vaccines aim to stimulate the development of long-lasting high-titer neutralizing antibodies and memory T cell.[1,2] In addition, T cell response has been associated with cross-recognition and defense against diverse variants.[3]

Immunosuppressed patients, including those with immune-mediated diseases and organ transplants, achieve a lower seropositivity rate to COVID-19 vaccines and a deficient T cell response compared to healthy controls, particularly those treated with glucocorticoids, rituximab (RTX), mycophenolate mofetil, abatacept (ABA), and methotrexate.[4-9] Moreover, 20% of patients with rheumatoid arthritis (RA) did not seroconvert after 2 doses.[4] For this reason, a third dose has been included in this population as a primary regimen. Currently, scarce data about this strategy are available.

The aim of this study was to assess the immune response after a third dose of SARS-CoV-2 vaccine in patients with RA with undetectable antibody titers after the primary regimen of 2 doses.

METHODS

Patients aged ≥ 18 years with RA (according to the American College of Rheumatology/European Alliance of Associations for Rheumatology [ACR/EULAR] 2010 criteria; https://doi.org/10.1002/art.27584) from 2 rheumatology centers in Argentina were included. They all had no seroconversion (optical density [OD] 450 nm < 0.35 + 1 SD) after 2 doses of SARS-CoV-2 vaccine and had received a third dose of either mRNA or vector-based vaccine.

Patient blood samples were taken between 21 and 40 days after the third dose (mean 28.3 days, SD 3.4). Anti-SARS-CoV-2 IgG antibodies were tested using the ELISA-COVIDAR test (Laboratorio Lemos) with a cut-off OD 450 nm value of 0.35 + 1 SD. To evaluate neutralizing activity, Vero-E6 cells and SARS-CoV-2 strain hCoV-19/Argentina/PAIS-G0001/2020 were cultured in the patients' plasma. The neutralization titer was calculated as the inverse of the highest plasma dilution showing 80% inhibition of the cytopathic effect. The detection of SARS-CoV-2–specific T cells was conducted by an interferon (IFN)-γ ELISpot Assay (BD Biosciences) using SARS-CoV-2 receptor-binding domain from spike protein (kindly provided by Dr. A. Gamarnik, Leloir Institute) and a spike peptide pool (BEI Resources, National Institute of Allergy and Infectious Diseases, National Institutes of Health; NR-52402 and NR-52404), previously titrated. A positive response was considered as ≥ 5 spot forming units above the negative control.

Sociodemographic data, comorbidities, disease activity, treatments, vaccine applied, and the presence of adverse events (AEs) were recorded.

Statistical analysis. Descriptive analysis of sociodemographic and clinical data was carried out. To assess the association between seroconversion, neutralizing activity, neutralizing antibody titers, and the presence of T cell response with clinical variables and RA treatments, chi-square or Fisher exact test, and *t* test or Mann-Whitney *U* test were used.

A $P < 0.05$ was considered significant. All statistical analyses were performed with R version 4.0.0 (Free Software Foundation, Inc.).

Patient and public involvement. Patients and the public were not involved in the design, conduct, reporting, or dissemination plans of our research.

Availability of data and material. All data and materials generated and analyzed during the current study belong to the SAR-CoVAC-3D registry, Argentine Society of Rheumatology. They are available from the corresponding author on reasonable request. The authors declare that all relevant data are included in the article and its Supplementary Files (available from the authors upon request). More information about the registry is available at https://www.unisar.reumatologia.org.ar/registros_sarcovac.php.

Ethics approval. This study was conducted in accordance with Good Clinical Practice guidelines, the International Conference on Harmonization, and with the ethical principles established in the Declaration of Helsinki, Argentina law 3301/09, and local guidelines. Personal identification data were kept anonymous. An independent ethics committee approved the protocol and the informed consent form (ACTA-2021-27563857-GDEBA-CECMSALGP).

Consent for participation and publication. All patients signed the corresponding informed consent form to participate in this registry. Individuals provided signed consent for the publication of their data.

RESULTS

A total of 21 nonresponder patients with RA were included, all of them females, with a median age of 64.0 years (IQR 60.1-72.0) and median disease duration of 14.0 years (IQR 10.5-20.0). Most of them (81%) reported comorbidities; the most frequent were arterial hypertension, obesity, and dyslipidemia. At the time of vaccination, 6 (29%) were receiving glucocorticoids (3 of them receiving ≥ 10 mg/day), 17 (81%) conventional disease-modifying antirheumatic drugs (DMARDs; 57% receiving methotrexate), and 18 (85%) biologic DMARDs (including 6 receiving ABA and 4 receiving RTX; Table). Time between the last infusion of RTX and the application of the third dose was between 11.4 to14.0 months. Regarding the primary vaccination regimen, patients received 2 doses of BBIBP-CorV (62%), Gam-COVID-Vac (14%), ChAdOx1 nCoV-19 (14%), or a mixed regimen of Gam-COVID-Vac/mRNA-1273 (10%). As the third dose, the majority (95%) received the BNT162b2 vaccine and only one of them received the ChAdOx1 nCoV-19 vaccine, with a median time between the second and third dose of 180.0 days (IQR 126.5-181.5).

After the third dose, 91% of the patients presented detectable anti-SARS-CoV-2 IgG. The 2 patients who did not present detectable antibodies were obese and received BBIBP-CorV during the primary regimen and BNT162b2 as the third dose; one of them was taking methotrexate and ABA and the other one was taking RTX (Figure 1A). They were older (median [IQR] 66.6 [IQR 61.6-66.6] yrs vs 64.0 [IQR 59.4-72.3] yrs; $P = 0.73$) and had lower disease duration (median [IQR] 11.5 [IQR 11.0-11.5] yrs vs 16.0 [IQR 10.0-20.0] yrs; $P = 0.44$), although these differences were not statistically significant. Moreover, 76% presented neutralizing activity and the median of neutralizing antibody titer was 12 (IQR 7-48). Compared to other treatments, ABA and RTX were associated with the absence of neutralizing activity and lower titers of neutralizing antibodies (Figures 1B,C).

Specific SARS-CoV-2 spike T cell response was present in 41% of all patients after the second dose, increasing to 71% with the third dose. The use of ABA was associated with a lower frequency of T cell response (33% vs 87%, $P = 0.03$). Further, of the 6 patients taking ABA, 2 (33%) did not present neutralizing

Table. Characteristics of patients with RA and anti-SARS-CoV-2 immune response after the application of the third vaccine dose.

Patient	3rd Vaccine Dose	CS (PDN, mg/d)	DMARD	Anti-SARS-CoV-2 IgG	Neutralizing Antibody Titer	T Cell Response
1	BNT162b2	–	MTX + TNFi	R	4	+
2	BNT162b2	2.5	MTX + TNFi	R	8	+
3	BNT162b2	10	AZA + ABA	R	64	-
4	BNT162b2	–	RTX	R	2	+
5	BNT162b2	–	MTX + IL-6i	R	4	+
6	BNT162b2	–	LEF + IL-6i	R	8	+
7	BNT162b2	7.5	TNFi	R	8	-
8	BNT162b2	–	MTX	R	8	+
9	BNT162b2	5	LEF + ABA	R	16	+
10	BNT162b2	–	MTX + ABA	R	16	+
11	BNT162b2	–	ABA	R	32	-
12	BNT162b2	–	MTX	R	128	+
13	BNT162b2	–	MTX + TNFi	R	128	+
14	BNT162b2	–	MTX + ABA	NR	–	-
15	BNT162b2	15	MTX + ABA	R	–	-
16	BNT162b2	–	MTX + RTX	R	4	+
17	BNT162b2	10	MTX + IL-6i	R	16	+
18	BNT162b2	–	HCQ + RTX	R	–	+
19	ChAdOx1 nCoV-19	–	MTX + IL-6i	R	–	-
20	BNT162b2	–	RTX	NR	–	+
21	BNT162b2	–	LEF	R	512	+

ABA: abatacept; AZA: azathioprine; CS: corticosteroid; DMARD: disease-modifying antirheumatic drugs; HCQ: hydroxychloroquine; IL-6i: interleukin 6 inhibitor; LEF: leflunomide; MTX: methotrexate: NR: nonreactive; PDN: prednisone; R: reactive; RA: rheumatoid arthritis; RTX: rituximab; TNFi: tumor necrosis factor inhibitor.

activity or T cell response. Conversely, all the patients treated with RTX, even those who had no neutralizing antibodies, developed a T cell response.

There was no association between cellular and humoral immune responses and the presence of comorbidities or disease activity.

Sixteen (76%) patients reported ≥ 1 AE after the third dose; the most frequent AE was injection site reaction (67%), followed by flu-like syndrome (25%). Additionally, 3 flares were reported, all of them mild.

DISCUSSION

In this RA cohort, 91% of patients who failed to seroconvert after 2 doses of SARS-CoV-2 vaccine presented detectable anti-SARS-CoV-2 IgG after a third dose. Further, 76% of these patients achieved neutralizing activity and 71% achieved antiviral-specific T cell response. The use of ABA was associated with lower titers of neutralizing antibodies and lower frequency of T cell response.

Simon et al[10] studied patients with immune-mediated inflammatory diseases (IMIDs), of whom 45% had RA and half of them had been treated with RTX. Almost half of the patients seroconverted with the administration of the third dose, but if those treated with RTX were excluded, this frequency rose to 79%. Overall, 50% developed neutralizing antibodies; 80% were in the non-RTX–exposed group and 22% were in the RTX group. A total of 73% achieved a specific T cell response; in RTX-treated patients, the frequency was 87%.[10] In our cohort, if RTX-treated patients were excluded, 94% seroconverted and 82% achieved neutralizing activity.

Since RTX interferes with B cell functions, a lower frequency of anti-SARS-CoV-2 antibodies after vaccination is expected. The association between RTX and lower seroconversion rates has already been described in previous studies.[4,11] However, it has been demonstrated that RTX-treated patients can develop SARS-CoV-2–specific T cell immunity in response to vaccination.[12,13] Like in our cohort, Mrak et al[13] showed that T cell–mediated immune response occurred independently of seroconversion status. In our study, 4 patients treated with RTX were included, and the time between the last infusion and the application of the third dose was 11.4 to 14.0 months. Two patients achieved specific T cell response after the second vaccine and the other 2 patients after the third vaccine. Moreover, 3 patients developed IgG anti-SARS-CoV-2 and 2 developed neutralizing activity. This improvement could be explained by the reinforcement effect of the third dose on the immune system, but also by a longer drug-vaccine interval.

Conversely, ABA was associated with a deficient humoral and cellular response. Even after a third dose, 33% and 66% of the patients did not develop neutralizing antibodies or specific T cell response, respectively. Moreover, 2 patients achieved neither humoral nor T cell response. Although there are no data from patients with 3 doses, after a primary regimen of 2 doses, patients with RA using ABA included in a cohort from Belgium had a seropositivity rate of 67%.[14] Out of the 11 patients treated with ABA who were originally recruited in our cohort, 5 (45%) of

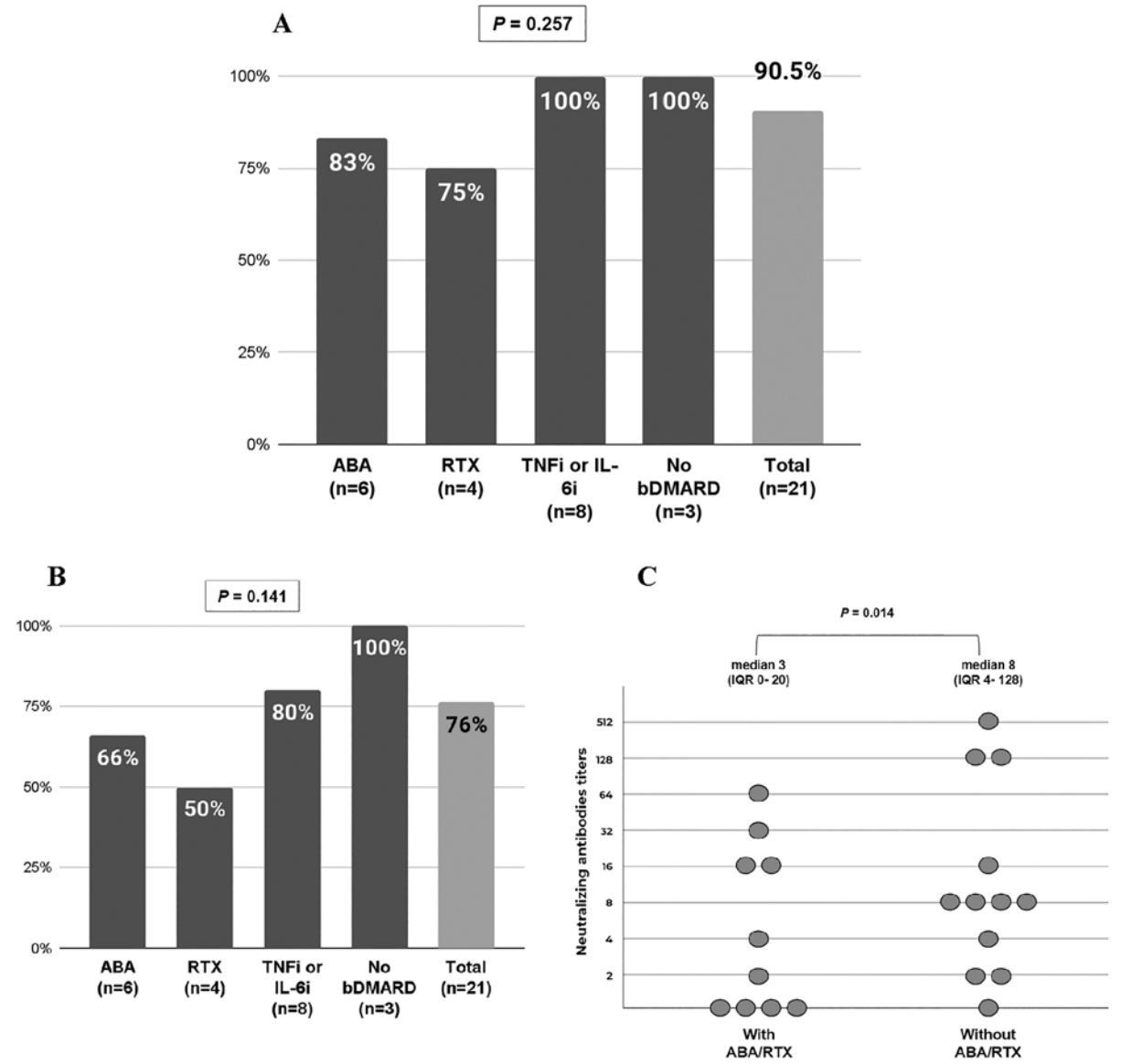

Figure 1. Anti-SARS-CoV-2 IgG, neutralizing antibodies seropositivity, and neutralizing antibody titers after a third vaccine dose in RA patients according to their baseline treatment. Patients taking ABA or RTX presented (A) lower frequency of detectable anti-SARS-CoV-2 IgG, (B) anti-SARS-CoV-2 neutralizing activity, and (C) lower titers of neutralizing antibodies after a third dose of vaccine. ABA: abatacept; bDMARD: biologic disease-modifying antirheumatic drugs; IL6-i: interleukin 6 inhibitor; RTX: rituximab; TNFi: tumor necrosis factor inhibitor.

whom seroconverted after 2 doses, a total of 90% developed anti-SARS-CoV-2 antibodies and 82% developed neutralizing activity after 3 doses. However, they developed these in lower titers when compared with other treatments. Additionally, ABA has been associated with lower IgG anti-SARS-CoV-2 titers and a significant reduction in the number of memory B cells compared to healthy controls 6 months after completing a 2-dose regimen with BNT162b2 mRNA vaccine.[7] Similarly, an immediate impaired SARS-CoV-2–specific T cell response has been described in patients with RA taking ABA and after 6 months of vaccination.[6,7]

It is worth noting that all patients received a heterologous third dose regimen. Likewise, Simon et al[10] showed that patients that received mix schemes achieved numerically higher frequencies of humoral and cellular responses. Nevertheless, data are scarce about this strategy in patients with IMIDs, and information from the general population is not conclusive. While some studies did not find differences between both regimens, others have shown higher efficacy of heterologous schemes in comparison with homologous ones.[15-17]

This study has its strengths and limitations. Even though only 21 patients with RA were included, this is one of the first cohorts to assess the immune response after a third dose of SARS-CoV-2 vaccine in patients with undetectable antibody titers after a primary regimen of 2 doses. Unlike other cohorts worldwide where homologous regimens were used, we describe the results observed with all available types of vaccines, both as homologous and heterologous schemes. Additionally, considering that the components of the immune system may be heterogeneously affected in patients with RA treated with different

immunosuppressive drugs, the presence of anti-SARS-CoV-2 IgG and specific T cell response were assessed. To determine the latter, a whole-blood test quantifying the IFN-γ response to spike peptides was evaluated, which are the most potent immunogenic stimuli and have shown high sensitivity and specificity.[18,19] Unfortunately, the number of patients included did not allow us to perform a multivariable analysis. However, univariable comparisons have shown that the use of RTX and ABA are associated with an impaired immune response, comparable to those reported by other cohorts.[4,6,7,11,14]

Finally, these data highlight the importance of a third vaccine dose in this group of patients, showing > 90% of RA patients who fail to develop anti-SARS-CoV-2 antibodies after a primary 2-dose regimen achieve seroconversion after a third dose. Further, three-quarters of these patients achieved neutralizing activity and a T cell response. Although more evidence is needed to determine the best time interval between doses, the third vaccine dose should be considered as part of the primary regimen in this population.

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