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TITLE

Humoral and T-cell response to SARS-CoV-2 vaccination in patients with rheumatoid arthritis

SUBTITLE

Immune response to SARS-CoV-2 vaccination in RA

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Declarations

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Availability of data and material

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

Authors' contributions

All authors listed in this manuscript were involved in drafting or revising this article critically for important intellectual content, and all authors approved the final version to be published.

Ethics approval

This study was conducted in accordance with Good Clinical Practice (GCP) guidelines, the International Conference on Harmonization (ICH), and with the ethical principles established in the Declaration of Helsinki, law 3301/09, and local guidelines. Personal identification data was kept anonymous. An independent ethics committee approved the protocol and informed consent form (*Comité Institucional de Evaluación de la Facultad de Ciencias Biomédicas de la Universidad Austral, number CIE P21-009*).

Abstract

Objective: To assess the SARS-CoV-2-specific humoral and T-cell response after a two-dose regimen of SARS-CoV-2 vaccine in patients with rheumatoid arthritis (RA).

Methods: In this observational study, patients with RA, ≥18 years old, vaccinated for SARS-CoV-2 according to the Argentine National Health Ministry´s vaccination strategy were included. Anti-SARS-CoV-2 IgG antibodies (ELISA-COVIDAR test), neutralizing activity (cytotoxicity in VERO cells) and specific T-cell response (IFN-γ ELISpot Assay) were assessed after the first and second dose.

Results: A total of 120 RA patients were included. Mostly, homologous regimens were used, including Gam-COVID-Vac (27.5%), ChAdOx1 (24.2%) and BBIBP-CorV (22.5%). The most frequent combination was Gam-COVID-Vac/mRNA-1273 (21.7%). After the second dose 81.7% presented anti-SARS-CoV-2 antibodies, 70.0% neutralizing activity and 65.3% specific T-cell response. The use of BBIBP-CorV, treatment with abatacept (ABA) and rituximab (RTX) were associated with undetectable antibodies and no neutralizing activity after two doses. BBIBP-CorV was also associated with the absence of T-cell response. The total incidence of adverse events was 357.1 events/1000 doses, significantly lower with BBIBP-CorV (166.7 events/1000 doses, $p<0.02$).

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Conclusion: In this RA cohort vaccinated with homologous and heterologous regimens against COVID-19, two out of ten patients did not develop IgG anti-SARS-CoV-2, 70% presented neutralizing activity and 65% specific T-cell response. The use of BBIBP-CorV was associated with deficient humoral and cellular response, while treatment with ABA and RTX resulted in an impaired IgG anti-SARS-CoV-2 formation and neutralizing activity.

Key words: SARS-CoV-2, COVID-19, Vaccines, Rheumatoid Arthritis

Significance and Innovations

- In this study, we assessed humoral and T-cell immune response in patients with rheumatoid arthritis after two doses of COVID-19 vaccine. Additionally, different types of vaccines, including inactivated, vector-based and mRNA, and heterologous regimens were included.
- Patients taking rituximab and abatacept were less likely to develop a specific humoral response. Moreover, most patients on rituximab presented an adequate T-cell immune response, unlike patients on abatacept, who demonstrated an impaired response.
- The use of the inactivated vaccine BBIBP-CorV was associated with lower efficacy, as assessed by a lower frequency of antibody and cellular responses when compared with other vaccines. On the contrary, those receiving heterologous regimens performed better.

Introduction

Patients with rheumatic diseases have been associated with increased risk of viral infections, including SARS-CoV-2, both due to the intrinsic risk associated with the disease and the effect of the treatments used [1,2]. In Argentina, data from the SAR-COVID National Registry showed that besides well-known risk factors, such as age, gender and the presence of comorbidities, high disease activity and treatment with glucocorticoids or rituximab had poorer outcomes [3]. Additionally, patients with immune-mediated diseases achieve a lower seropositivity rate to COVID-19 vaccine and deficient T-cell response compared to healthy controls, particularly those treated with glucocorticoids, rituximab (RTX), mycophenolate mofetil, abatacept (ABA) and methotrexate [4-7]. Moreover, 20% of patients with rheumatoid arthritis (RA) do not seroconvert after two doses [4]. In this context and considering the increased risk of severe COVID-19 of this population, the different scientific societies agree on the importance of vaccinating patients with immune-mediated diseases, including RA [8-10].

In Argentina, SARS-CoV-2 vaccination is organized according to a voluntary, strategic vaccination plan, guaranteeing equitable access. Although vaccination of patients with immune-mediated diseases was initially contraindicated due to lack of data supporting this indication, in June 2021, the Ministry of Health issued a statement authorizing the vaccination of this target population [11]. To date, six vaccines are available in our country. Gam-COVID-Vac, ChAdOx1 and BBIBP-CorV were the first to be approved, followed by BNT162b2, mRNA-1273 and AD5-nCOV [12], causing the latter to be mostly used to complete the vaccination as mixed regimens.

A coordinated innate and adaptive immune response is needed to control SARS-CoV-2 infection. The three fundamental components of the adaptive immune system are B-cells, CD4+ and CD8+ T-cells, which enable the elimination of intra and extracellular viruses. Vaccines aim to stimulate the development of long-lasting high titer neutralizing antibodies and memory T-cells to prevent SARS-CoV-2 infection, transmission and reduce disease severity[13,14]. Additionally, T-cell response has been associated with cross-recognition and protection against diverse variants [15].

Most information regarding SARS-CoV-2 vaccination immunogenicity in patients with rheumatic diseases is from cohorts in developed countries. However, important differences such as population characteristics and COVID-19 vaccine regimens might limit the generalizability of this data to developing countries. For example, ethnicity, poverty, living in developing countries, malnutrition and high body mass indexes have been associated with lower vaccine responses [16]. Particularly in Argentina, approximately 50% of the population is considered poor, more than 60% is overweight or obese and 37% has food insecurity [17,18]. On the other hand, Gam-COVID-Vac and BBIBP-CorV vaccines, which have been used in few countries in the word, were part of the initial vaccination plan in Argentina. Additionally, because of the shortage of vaccines, Argentina was one of the first countries to implement the use of heterologous primary 2-dose regimens [12]. Taking this scenario into account, patients from many Latin American countries and other regions of the world, including Argentina, cannot be compared with the populations assessed in most of the studies currently published from developed countries where ChAdOx1 and BNT162b2 were the most frequent vaccines used. Given the types of vaccines and regimens used, we hypothesize that the efficacy of COVID vaccination may not be the same in Argentina as in other countries. To the best of our knowledge, little has been published regarding the

efficacy and safety of all these types of vaccines and regimens in the setting described. Real world data is fundamental to improving patient management. The aim of this study was to assess the humoral and T-specific immune response after a two-dose regimen of SARS-CoV-2 vaccine in patients with RA, and to identify the factors associated with an impaired response.

Methods

Study design

Observational, multicenter study, which included RA patients according to ACR/EULAR 2010 [19] criteria, from two rheumatology centers, one private and one public from the Buenos Aires Metropolitan Area, who received SARS-CoV-2 vaccination according to the national strategic vaccination plan. The recruitment period took place between April 28 and July 2, 2021.

Study variables

Sociodemographic data (sex, date of birth, place of residence, ethnicity, health coverage, education, sociodemographic level according to the Graffar scale [20], occupation), presence of comorbidities, date of RA diagnosis, treatment and disease activity before vaccination were recorded. The latter was categorized according to the treating physician in remission, low, moderate, or high disease activity. Date, place and type of vaccine applied were identified. The development of adverse events (AE) was registered by the treating physician who performed the causality assessment using the Naranjo algorithm [21]. This algorithm allows, through a 10-item questionnaire, to identify the degree of association (doubtful, possible, probable, definitive) between the AE and the intervention.

Immune response assessment

Blood samples were taken between 21 and 45 days after the application of the first and second doses. In each case, 20 ml of peripheral venous blood were collected and stored in EDTA tubes. Anti-SARS-CoV-2 IgG antibodies were tested using ELISA-COVIDAR test with a cut-off value of OD450nm of 0.35+1SD. Results were expressed semi-quantitatively according to absorbance using the following categories: reactive (>0.35+1SD), indeterminate (0.35±1SD), and nonreactive (<0.35-1SD). In addition, the unit of normalized optical density (NOD) evaluated at 450 nm was established, which was normalized to the cut-off point and the positive control of the assay. To evaluate neutralizing activity, Vero-E6 cells and SARS-CoV-2 strain hCoV-19/Argentina/PAIS-G0001/2020 were cultured in patients´ plasma. The neutralization titer was calculated as the inverse of the highest plasma dilution showing 80%

inhibition of the cytopathic effect. Detection of SARSCoV-2 specific T-cells was conducted via an IFN-γ ELISpot Assay (BD Biosciences) using SARS-CoV-2 RBD from Spike protein (kindly provided by Dr. A. Gamarnik, Leloir Institute) and a Spike peptide pool (BEI Resources, NIAID, NIH. NR-52402, and NR-52404), which was previously titrated. A positive response was considered as ≥5 spot forming units (SFUs) above the negative control.

Ethical Considerations

This study was approved by an independent ethic committee (Comité Institucional de Evaluación de la Facultad de Ciencias Biomédicas de la Universidad Austral, number CIE P21-009) and was conducted in accordance with Good Clinical Practice (GCP) guidelines, the International Conference on Harmonization (ICH), the ethical principles established in the Declaration of Helsinki, law 3301/09, and the guidelines of the local ethics committee. Personal identification data was kept anonymous and protected according to international and national regulations in order to guarantee confidentiality, in accordance with the law on protection of personal data No. 25.326/2000. To participate in this study, all subjects signed the corresponding informed consent.

Statistical analysis

Descriptive analysis of sociodemographic and clinical data was carried out. Continuous variables were presented as mean and standard deviation (SD) for normal distributions, otherwise as median and quartiles. Categorical variables were summarized as frequencies and percentages. To assess the association between the presence of IgG anti-SARS-CoV-2, and neutralizing activity, neutralizing antibody titers and the presence of specific T-cell response with clinical variables and RA treatments, Chi-squared or Fisher's exact test and T-test or Mann-Whitney U-test were used, as appropriate. All variables with a p-value less than 0.10 in the univariate analysis were included in multiple logistic regression models, using each outcome as a dependent variable, to identify the factors associated to the development of humoral and cellular response after vaccination, adjusting for confounders. Later, variable selection was made using a stepwise method.

The incidence of AE and its 95% confidence interval was calculated as the number of events per 1000 doses of each of the most frequent types of vaccines. Comparisons between them were performed assessing the relative risk and its 95% confidence interval.

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

Results

A total of 120 RA patients vaccinated for COVID-19 were included, 90% females with a median age of 61.6 years (Q1, Q3 50.2, 69.6) and a median disease duration of 12.5 years (Q1, Q3 7.0, 19.0). History of SARS-CoV-2 infection was reported by 7 (5.8%)

patients. Sociodemographic, clinical characteristics and treatments received at vaccination are described in table 1. Mostly, homologous regimens were used, particularly Gam-COVID-Vac (27.5%), ChAdOx1 (24.2%), BBIBP-CorV (22.5%). Median time between doses was 81 days (Q1, Q3 57.0, 108.0). It was significantly lower for homologous BBIBP-CorV regimen compared to the rest of the schemes (median 32.0 days, Q1, Q3 28.5, 55.0 vs median 92.5 days Q1, Q3 71.0, 111.0, p<0.001). In most cases vaccination was indicated by the treating rheumatologist (66.7%) and 30.8% of the patients were vaccinated without prior consultation.

Anti-SARS-CoV-2 antibody response

After the first dose only 34.2% of the patients presented detectable anti-SARS-CoV-2 IgG, increasing to 81.7% (p<0.001) after the second dose. All the patients with a positive test after the first dose showed the same result after the second. Similarly, the absorbance was higher after completing the primary vaccination regimen (Figure 1A and B). All male patients (n=12) and those with SARS-CoV-2 infection before vaccination presented a reactive result after two doses. Most of the patients who received a mixed regimen (28/30, 93.3%) presented a reactive result (p=0.102) (Table 1). The use of BBIBP-CorV was significantly associated with a non-reactive or indeterminate result (OR 0.11, 95%CI 0.02-0.4, p=0.002) (Figure 1C). Regarding DMARDs, none of the patients receiving RTX and only 5/11 (45.5%) of those treated with ABA presented detectable anti-SARS-CoV-2 IgG after a two-dose scheme, p<0.005 in both cases (Table 1) (Figure 1D).

In the multivariable analysis, after adjusting for clinical and sociodemographic variables, patients treated with ABA (OR 0.13, 95%CI 0.03-0.63, p=0.012) and those who received the inactivated BBIBP-CorV vaccine (OR 0.13, 95%CI 0.02-0.51, p=0.007) presented less frequently detectable anti-SARS-CoV-2 IgG. Although no patient receiving RTX presented a reactive result, the number was insufficient in order to perform multivariate analysis (Table 2).

Anti-SARS-CoV-2 neutralizing activity

A total of 84 (70.0%) presented neutralizing activity after two doses of COVID-19 vaccine, with a median titer of 1/64 (Q1, Q3 1/16, 1/128). All of them also had detectable anti-SARS-CoV-2 IgG, while 14 (11.7%) patients had a reactive result but no neutralizing activity (p<0.001). Higher NOD was observed after the first and the second vaccine in those with a positive response (Figure 2A).

Most male patients (10/12; 11.9% vs 5.6%, p= 0.507) and all patients with a history of a previous SARS-CoV-2 infection (p=0.095) had neutralizing activity. Comorbidities were more frequent among those without neutralizing activity (77.8% vs 60.7%, p=0.110), no patient with a history of cancer developed neutralizing activity (n=4; p=0.014). Moreover, 77.8% of the patients receiving BBIBP-CorV vaccine had no neutralizing activity (OR 0.13, 95%CI 0.04-0.41, p<0.001) (Figure 2B). Heterologous regimens were more frequent among those with a positive response compared to those with a negative test (32.1% vs 8.3%, p=0.011).

None of the patients treated with rituximab developed neutralizing activity (p= 0.007), neither 72.7% of those receiving abatacept (p= 0.003) and 63.6% of the IL-6 inhibitors users (Figure 2C). No effect on neutralizing activity was observed according to the use

of glucocorticoids (with/without glucocorticoids 69.0% vs 70.3%, p=1.000) and methotrexate (with/without methotrexate 72.2% vs 65.9%, p=0.614).

In multivariable analysis, the use of BBIBP-CorV vaccine (OR 0.15, 95%CI 0.04-0.51, p=0.004) and treatment with ABA (OR 0.11, 95%CI 0.02-0.51, p=0.007) and IL-6 inhibitors (OR 0.14, 95%CI 0.03-0.58, p=0.008) remained significantly associated with the absence of neutralizing activity (Table 2).

Specific anti-SARS-CoV-2 T-cell response

Determination of specific T-cell response after the second dose of COVID-19 vaccine was available for only 101 patients. After thawing some samples, the peripheral blood mononuclear cells (PBMC) did not show adequate viability to be able to evaluate the specific response capacity. Patients without results were similar to those in which the IFN-γ ELISpot Assay was possible, except for the most frequent use of BBIBP-CorV vaccine (57.9% vs 15.8%, p= 0.013) and homologous regimen (94.7% vs 71.3%, p=0.040) (Supplementary table 1). A total of 66 patients (65.3%) developed a specific T-cell response after completing the two-dose vaccination scheme. They presented detectable anti-SARS-CoV-2 IgG more frequently (89.4% vs 68.3%, p=0.024), had higher NOD (median 1.2, Q1, Q3 0.8, 1.5 vs median 0.6, Q1, Q3 -0.003, 1.4, p= 0.019) and higher prevalence of neutralizing activity (80.3% vs 57.1%, p=0.025). Moreover, 11 patients with a humoral non-reactive or indeterminate result had a specific T-cell response.

Those with a positive specific T-cell response were more frequently males (10.6% vs 2.9%, p=0.256) and significantly younger (median 58.8 years old, Q1, Q3 45.3, 67.1 vs median 63.5 years old, Q1, Q3 56.6, 70.6, p=0.018). Patients with a history of cancer (n=3, p=0.049), one of which also had a prior SARS-CoV-2 infection (p=0.417) did not develop a specific T-cell response. Only 5 (n=17, 31.3%) patients who received a homologous regimen of BBIBP-CorV vaccine presented SARS-CoV-2 specific T-cell response (OR 0.24, 95%CI 0.06- 0.87, p=0.036) (Figure 3A). Although patients with detectable T-cell response most frequently received heterologous schemes (34.8% vs 17.1%, p=0.101), the difference was not significant.

The frequency of T-cell response was comparable among treatment groups (Figure 3B). Those who were and were not using glucocorticoids and methotrexate had similar responses (58.6% vs 53.8%, p=0.668; 58.2% vs 48.8%, p=0.187, respectively).

In the multivariable analysis, only older age was associated with a lower probability of developing a SARS-CoV-2 specific T-cell response (OR 0.96, 95%CI 0.93-1.00, p=0.042, per extra year of age) (Table 2).

Safety

After the first vaccine dose, 42.5% of the patients reported at least one AE, and 28.6% after the second dose. Most of them were definitely or probably associated with vaccination according to the Naranjo algorithm, 55.3% and 29.4% respectively. Flu-like syndrome and local hypersensitivity were the most frequent (Figure 3). None reported seizures or anaphylaxis. All AE were mild or moderate and no patient was hospitalized. Total incidence of AE was 357.1 events/1000 doses, significantly lower with BBIBP-CorV (166.7 events/1000 doses, p<0.02) (Table 3). The development of anti-SARS-CoV-2 IgG was similar between those with or without AE (88.2% vs 79.8,

p=0.634). However neutralizing antibodies titers and the presence of specific T-cell response was higher among those who reported AE, median 1/64 (Q1, Q3 1/16, 1/256) vs 1/32 (Q1, Q3 1/2, 1/64), p=0.049 and 70.6% vs 47.6%, p=0.039, respectively.

Five patients (4.2%) reported a disease flare, all characterized by arthritis and RA treatment modification. No new immune-mediated manifestations were reported in this cohort.

Discussion

In this RA cohort who received 2 doses of COVID-19 vaccine, according to the Argentine strategic vaccination plan which included homologous and heterologous regimens, 2 out of 10 did not develop IgG anti-SARS-CoV-2, 70% presented neutralizing activity and 65.3% specific T-cell response. The use of the inactivated vaccine BBIBP-CorV was associated with deficient humoral and cellular response, while treatment with ABA and RTX resulted in an impaired IgG anti-SARS-CoV-2 formation and neutralizing activity.

The effect of some immunosuppressants, particularly RTX, on vaccine immunogenicity is well known [22,23]. Since RTX interferes with B-cell function, it might be expected a lower frequency of anti-SARS-CoV-2 antibodies after vaccination. The association between RTX and lower seroconversion rates has already been described in previous studies [4,24-26]. However, it has been demonstrated that RTX-treated patients can develop SARS-CoV-2-specific T-cell immunity in response to vaccination [27,28]. Like in our cohort, Mrak D, et al [28] showed that T-cell-mediated immune response occurs independently of seroconversion status. In our study four patients treated with RTX were included. While no humoral response was observed, two of them achieved specific T-cell response after the second dose of vaccine.

On the contrary, ABA has a dual effect on B and T-cells. This drug has been associated with lower IgG anti-SARS-CoV-2 titers and a significant reduction in the number of memory B-cells as well as an impaired T-cell response.[6,7,29] Although the frequency of positive T-cell response in patients taking ABA in our study was comparable to patients receiving other treatments, an impaired antibody formation was detected. Similarly, Le Moine, et al[29] described a 67% seropositivity rate in RA patients taking ABA. Regarding IL-6 inhibitors, only a few studies have reported the immunogenicity of SARS-CoV-2 vaccines in patients treated with these drugs. According to this data, they do not afect seroconversion rates after a double dose of COVID-19 vaccine. [4, 30,31] Although we found that the majority developed IgG antibodies, only 4 showed neutralizing activity. It is important to note that 3 out of the 11 patients under treatment with IL-6 inhibitors received the BBIBP-CorV vaccine, and most of them (81.2%) a homologous regimen. However, after adjusting for confounders, this association remained significant.

A lower efficacy of inactivated SARS-CoV-2 vaccines in eliciting humoral and cellular immune response has already been described.[32,33] These vaccines not only target the spike protein, but also the nucleocapsid and membrane proteins. In contrast, mRNA and adenoviral vaccines can elicit a much narrower response that only targets spike protein epitopes.[34] In our multivariable analysis considering the three different outcomes, the 2-dose BBIBP-CorV homologous vaccine regimen was consistently associated with poor efficacy in all these outcomes; only 48.1% developed IgG anti-SARS-CoV-2 antibodies and almost one third neutralizing activity and T-cell response.

Moreover, the association between this regimen and lower immunogenicity was independent of other variables, such as age, comorbidities and immunosuppressive treatments. Although we do not have a control group, similarities were found when indirectly comparing our data with that published from the general Argentine population. The use of BBIBP-CorV, both as a homologous and heterologous regimen, has been shown to present lower immunogenicity compared to the rest of the schemes.[35-36] They even demonstrated a higher percentage of patients without neutralizing activity [35] and lower efficacy in preventing hospitalization and death due to SARS-CoV-2 infection.[37] These data are of great interest in countries like ours, where vaccine availability differs from that in developed countries. Particularly in Argentina, although inactivated vaccines are no longer applied, approximately 30% of the population received a primary regimen of BBIBP-CorV. Therefore, it is important to strongly encourage this group to receive additional and booster doses. Furthermore, comparable to what has been shown in the general population [35] and in dialysis patients from Argentina [38], over 90% and 80% of the patients receiving heterologous schemes developed a humoral and cellular response, respectively.

Although some studies proved that environmental and sociodemographic factors affect other vaccines´immunogenicity [16], to the best of our knowledge, this has not been assessed with COVID-19 vaccines. In our study, we found no association between the cellular and humoral response triggered by the COVID-19 vaccine and some of these factors, including ethnicity, socioeconomic level, and education. Additionally, differences in immune and cellular responses derived from poverty are mostly related to malnutrition. Although some of our cohort was recruited from a public center, none of the patients were in this situation. Likewise, despite the particular sociodemographic, economic, and environmental characteristics of our country, we have detected similar responses to those reported in studies conducted in other countries, considering the different types of vaccines and schedules used [26,27,34].

This study has strengths and limitations. To the best of our knowledge, this is the first cohort to assess the anti-SARS-CoV-2 humoral and T-cell response after two doses of SARS-CoV-2 vaccine in Argentina. Unlike other cohorts from around the world, where primary homologous regimens prevailed, we describe the results observed with all available vaccine types, both as homologous and heterologous primary schemes. Additionally, considering that RA patients are treated with different immunosuppressive drugs, causing diverse impact, the presence of anti-SARS-CoV-2 IgG and specific T-cell responses were assessed. To determine the latter, a whole-blood test quantifying the IFN-γ response to spike peptides was evaluated, considering that these are the most potent immunogenic stimuli and have shown high sensitivity and specificity [39,40.] However, T-cell response could not be assessed in all patients due to inadequate PBMC viability after thawing some samples. Unfortunately, no control group was included, however indirect comparisons with current published data from the general population in our country was carried out. Additionally, data on treatment management during the vaccination period was not collected and we know that this might have impacted on the achieved immune response. Disease activity as well as disease treatment was assessed at the time of the first dose, changes in these two variables during the second dose could have affected our results. However, it should be noted that the median time between doses was 81 days and that during that period only 1.7% of the patients modified their treatment schedule. Moreover, the number of previous biologic and targeted synthetic treatments was not assessed. It should be noted that some drugs, special rituximab, are frequently used as second or

third line and those patients are usually considered to have a more severe disease, use higher doses of glucocorticoids and have more comorbidities. Finally, at the time of this analysis, follow-up information was unavailable, therefore, the association between immune response and SARS-CoV-2 infection after vaccination could not be established. This data will be collected and reported in the future.

To conclude, in this cohort of patients with RA who received 2 doses of COVID-19 vaccine, two out of ten did not develop IgG anti-SARS-CoV-2, 70% presented neutralizing activity and 65% had a specific T-cell response. The use of BBIBP-CorV in comparison with other vaccine regimens available in Argentina during the pandemic was associated with deficient humoral and cellular response, while treatment with ABA and RTX affected the development of IgG anti-SARS-CoV-2 and neutralizing activity.

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Figure legends

Figure 1. Humoral response after the second COVID-19 vaccine dose. (A) Anti-SARS-CoV-2 IgG after the first and second dose; (B) Anti-SARS-CoV-2 IgG after the first and second dose for each patient; (C) Anti-SARS-CoV-2 IgG after the 2nd dose according to the COVID-19 vaccination regimen and (D) RA treatment.

Figure 2. Neutralizing activity after the second COVID-19 vaccine dose. (A) Neutralizing antibodies titres among patients with neutralizing activity (n=84). (B) Anti-SARS-CoV-2 IgG after the first and second dose according to the presence of neutralizing activity. (C) Neutralizing activity according to the COVID-19 vaccination regimen and (D) RA treatment.

Figure 3. Specific anti-SARS-CoV-2 T-cell response after 2 dose regimen of COVID-19 vaccination. (A) according to the COVID-19 vaccination regimen and (B) RA treatment.

Table 1. Comparison of sociodemographic and clinical characteristics and type of vaccine applied according to IgG anti SARS-CoV-2 response in the total cohort.

Variables	**IgG anti-SARS-CoV-2 positive after two doses** n= 98	**IgG anti-SARS-CoV-2 negative after two doses** n= 22	**p**	**Total** n=120
Female sex, n (%)	86 (87.8)	22 (100.0)	0.120	108 (90.0)
Age (years), m (Q1, Q3)	61.6 (48.8, 68.9)	62.3 (59.2, 71.0)	0.266	61.6 (50.2, 69.6)
Ethnicity, n (%)				
Caucasian	44 (44.9)	10 (45.5)		54 (45.0)
Mestizo	50 (51.0)	12 (54.5)	1.000	62 (51.7)
Other	4 (4.1)	0 (0)		4 (3.3)
Socioeconomic status, n (%)				
High or medium/high	27 (27.6)	4 (18.2)		31 (25.8)
Medium	44 (44.9)	13 (59.0)	0.158	57 (47.5)
Low or medium/low	25 (25.5)	4 (18.2)		29 (24.2)
Unknown	2 (2.0)	1 (4.6)		3 (2.5)
Education (years), m (Q1, Q3)	7.0 (5.0, 11.0)	10.0 (6.8, 12.3)	0.109	7.0 (5.0, 11.0)
Comorbidities, n (%)	61 (62.2)	18 (81.8)	0.133	79 (65.8)
Interstitial lung disease	6 (9.8)	0 (0)	0.328	6 (7.6)
Obstructive lung disease	7 (11.5)	0 (0)	0.341	7 (8.9)
Diabetes	11 (18.0)	3 (16.7)	1.000	14 (17.7)
Obesity	10 (16.4)	5 (27.8)	0.313	15 (19.0)
Arterial hypertension	30 (49.2)	9 (50.0)	1.000	39 (49.4)
Dyslipidemia	17 (27.9)	6 (33.3)	0.878	23 (29.1)
Cancer	2 (3.3)	2 (11.1)	0.222	4 (5.1)
History of SARS-CoV-2 infection, n (%)	7 (7.1)	0 (0)	0.347	7 (5.8)
Disease duration (years), m (Q1, Q3)	12.0 (6.3, 19)	14.0 (10.0, 18.8)	0.323	12.5 (7.0, 19.0)
RA treatments, n (%)				
Glucocorticoids	23 (23.5)	6 (27.3)	0.920	29 (24.2)
Methotrexate	67 (68.4)	12 (54.5)	0.324	79 (65.8)
Leflunomide	23 (23.5)	3 (13.6)	0.400	26 (21.7)
Hydroxychloroquine	3 (3.1)	1 (4.6)	0.560	4 (3.3)
Azathioprine	1 (1.0)	1 (4.6)	0.334	2 (1.7)
TNF inhibitors	31 (31.6)	4 (18.2)	0.320	35 (29.2)
IL-6 inhibitors	7 (7.1)	4 (18.2)	0.116	11 (9.2)
Abatacept	5 (5.1)	6 (27.3)	0.005	11 (9.2)
Rituximab #	0 (0)	4 (18.2)	<0.001	4 (3.3)
JAK inhibitors	15 (15.3)	1 (4.6)	0.299	16 (13.3)
b- or ts-DMARD monotherapy, n (%)	14/58 (24.1)	5/19 (26.3)	0.687	20/77 (26.0)
Disease activity, n (%)				
Remission	34 (34.7)	10 (45.5)		44 (36.7)
Low disease activity	36 (36.7)	5 (22.7)	0.608	41 (34.2)
Moderate disease activity	19 (19.4)	5 (22.7)		24 (20.0)
High disease activity	9 (9.2)	2 (9.1)		11 (9.1)
Two-dose regimen, n (%)				
ChAdOx1/ChAdOx1	26 (26.5)	3 (13.6)		29 (24.2)
ChAdOx1/ BNT162b2	1 (1.0)	0 (0)		1 (0.8)
BNT162b2/BNT162b2	1 (1.0)	0 (0)	<0.001	1 (0.8)

BBIBP-CorV/BBIBP-CorV Gam-COVID-Vac/ChAdOx1 Gam-COVID-Vac/mRNA-1273 Gam-COVID-Vac/Gam-COVID-Vac	13 (13.3) 3 (3.1) 24 (24.5) 30 (30.6)	14 (63.6) 0 (0) 2 (9.09) 3 (13.6)		27 (22.5) 3 (2.5) 26 (21.7) 33 (27.5)
Heterologous regimen, n (%)	28 (28.6)	2 (9.1)	0.102	30 (25.0)
Time between 1st and 2nd dose (days), m (Q1, Q3)	91.0 (66.0, 110.0)	51.5 (30.3, 69.8)	<0.001	81.0 (57.0, 108.0)

*IgG: immunoglobulin G; n: number; m: median; Q: quartile; RA: rheumatoid arthritis; TNF: tumor necrosis factor; IL: interleukin; JAK: Janus kinase; b-: biologic; ts-: targeted synthetic; DMARD: disease modifying antirheumatic drug.

Mean time between the last infusion of rituximab and the first vaccine dose was 181 days (SD 16.8) and with the second dose was 258 days (SD 30.0).

Table 2. Sociodemographic and clinical variables associated with the presence of detectable anti-SARS-CoV-2 IgG, neutralizing activity and the presence of specific anti-SARS-CoV-2 T-cell response after a two-dose regimen of COVID vaccine.

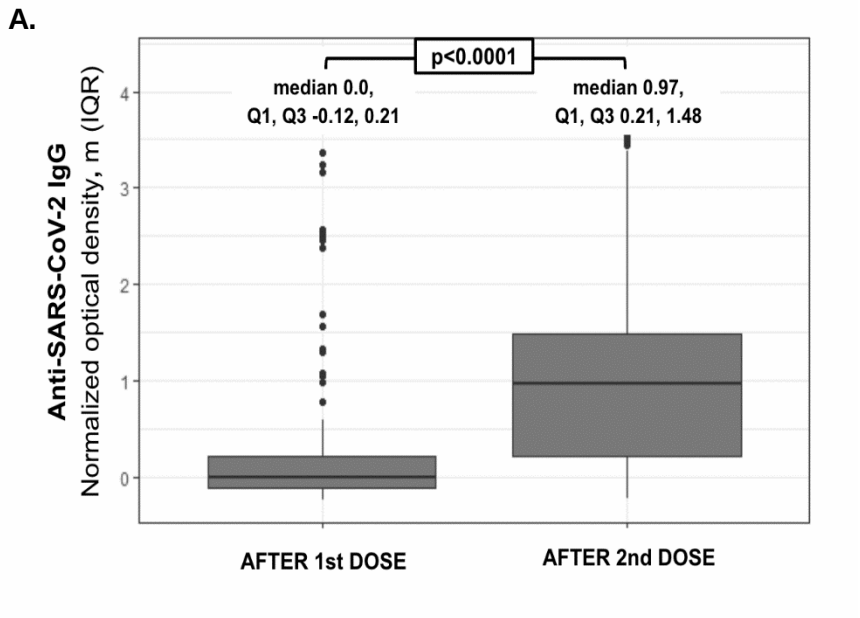
	Detectable anti-SARS-CoV-2 IgG				Detectable neutralizing activity				Specific anti-SARS-CoV-2 T-cell response			
	Unadjusted analysis		Adjusted analysis †		Unadjusted analysis		Adjusted analysis †		Unadjusted analysis		Adjusted analysis †	
Variables	**OR (95%CI)**	**p**	**OR (95%CI)**	**p**	**OR (95%CI)**	**p**	**OR (95%CI)**	**p**	**OR (95% CI)**	**p**	**OR (95% CI)**	**p**
Age	0.98 (0.94-1.01)	0.200			0.98 (0.95-1.01)	0.300			0.96 (0.93-1.00)	0.032	0.96 (0.93-1.00)	0.042
COVID-19 vaccine regimen (ref. ChAdOx1/ChAdOx1)												
BBIBP-CorV/ BBIBP-CorV	0.11 (0.02-0.40)	0.002	0.13 (0.02-0.51)	0.007	0.13 (0.04-0.41)	<0.001	0.15 (0.04-0.51)	0.004	0.24 (0.06-0.87)	0.036	0.29 (0.07-1.10)	0.075
Gam-COVID-Vac/mRNA-1273	1.38 (0.21-11.2)	0.700	1.68 (0.24-14.6)	0.600	2.00 (0.47-10.4)	0.400	2.62 (0.55-15.4)	0.200	2.12 (0.61-8.05)	0.200	2.58 (0.72-10.2)	0.200
Gam-COVID-Vac/Gam-COVID-Vac	1.15 (0.20-6.71)	0.900	1.58 (0.25-10.1)	0.600	0.70 (0.20-2.24)	0.500	0.90 (0.24-3.26)	0.900	1.39 (0.44-4.46)	0.600	1.52 (0.47-5.10)	0.500
Abatacept	0.14 (0.04-0.53)	0.003	0.13 (0.03-0.63)	0.012	0.13 (0.03-0.48)	0.004	0.11 (0.02-0.51)	0.007	1.07 (0.26-5.32)	0.900		
IL-6 inhibitors	0.35 (0.09-1.43)	0.120	0.22 (0.04-1.13)	0.061	0.21 (0.05_0.74)	0.018	0.14 (0.03-0.58)	0.008	0.64 (0.16-2.72)	0.500		
TNF inhibitors	2.08 (0.71-7.66)	0.200			2.64 (1.04-7.67)	0.054			0.77 (0.32-1.89)	0.600		
JAK inhibitors	3.80 (0.71-70.5)	0.200			0.93 (0.31-3.17)	0.912			0.71 (0.21-2.58)	0.600		

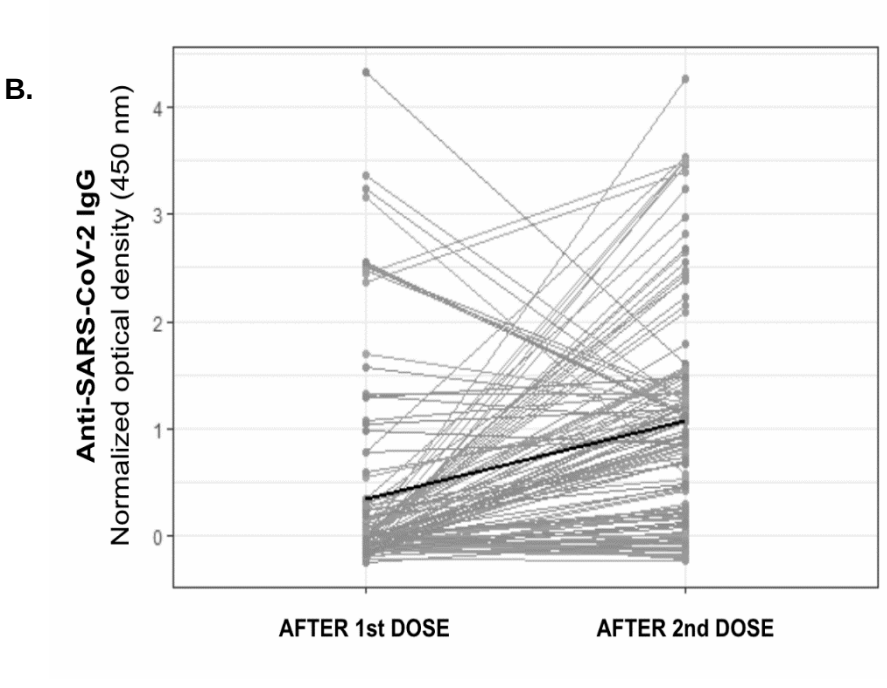
* OR: odds ratio; CI: confidence interval; ref: reference; IL: interleukin; TNF: tumor necrosis factor; JAK: Janus kinase. † The models were adjusted for: sex, age, ethnicity, education, comorbidities, disease duration, RA activity, glucocorticoid, conventional, biological and targeted synthetic DMARD use, vaccine regimen and time between doses. The variables use of rituximab and history of SARS-CoV-2 infection could not be included in these models because of the low number of patients in those groups.

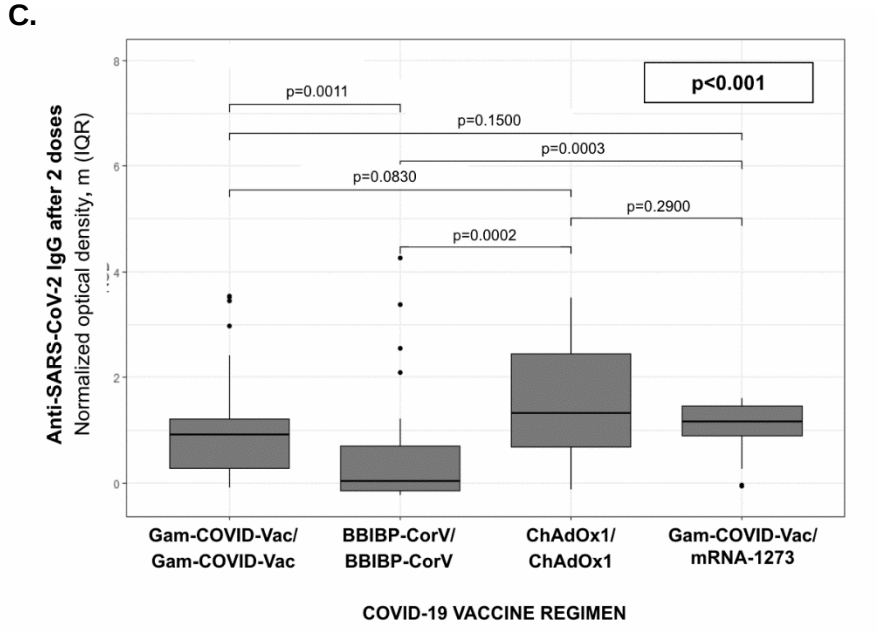
Table 3. Incidence of adverse events associated with COVID vaccines

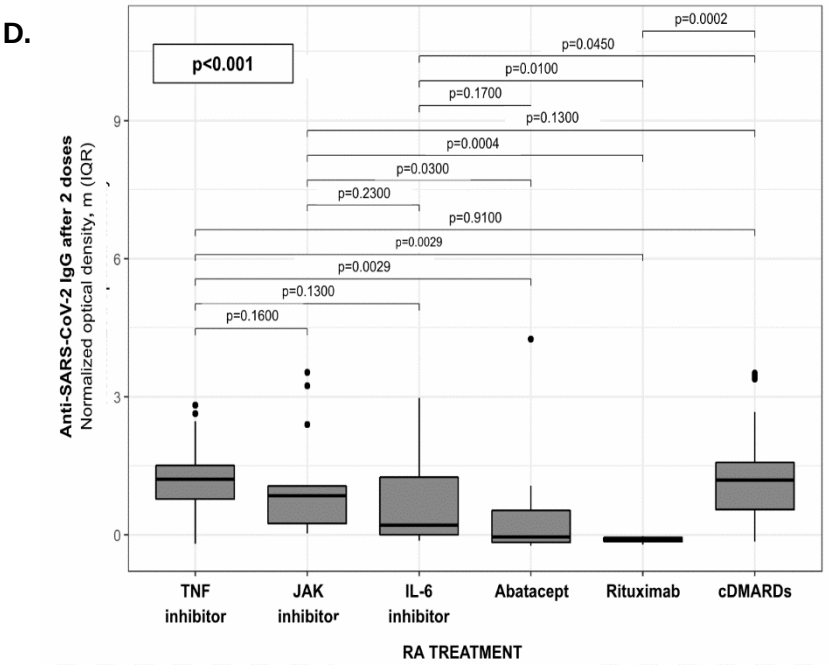
Variables	Gam-COVID-Vac	ChAdOx1	BBIBP-CorV	mRNA-1273
Number of doses	95	62	54	24
Number of AE	40	24	9	11
Incidence of AE (every 1000 doses, 95% CI)	**421.1 (300.8-573.4)**	**387.1 (248.0-575.97)**	**166.7 (76.2-316.4)**	**458.33 (228.8-820.1)**
Relative risk #				
Gam-COVID-Vac		1.08 (0.73 to 1.61)	2.52 (1.33 to 4.79)	0.91 (0.46 to 1.98)
ChAdOx1			2.32 (1.18 to 4.55)	0.84 (0.49 to 1.44)
BBIBP-CorV				0.36 (0.17 to 0.76)
mRNA-1273				

*AE: adverse events; CI: confidence interval
Relative risk was calculated using the type of vaccine expressed in the file as numerator and the one expressed in the column as denominator.









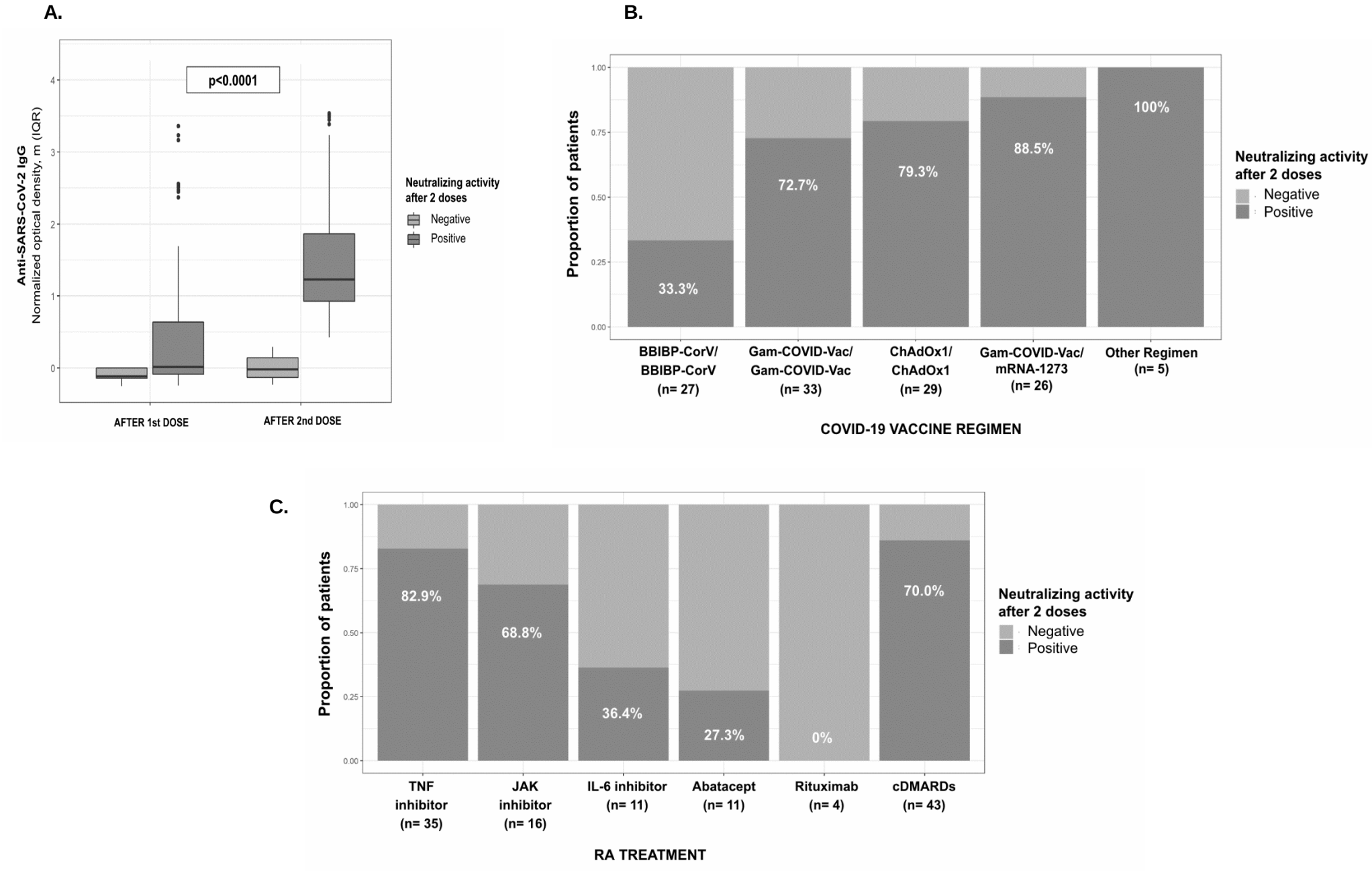
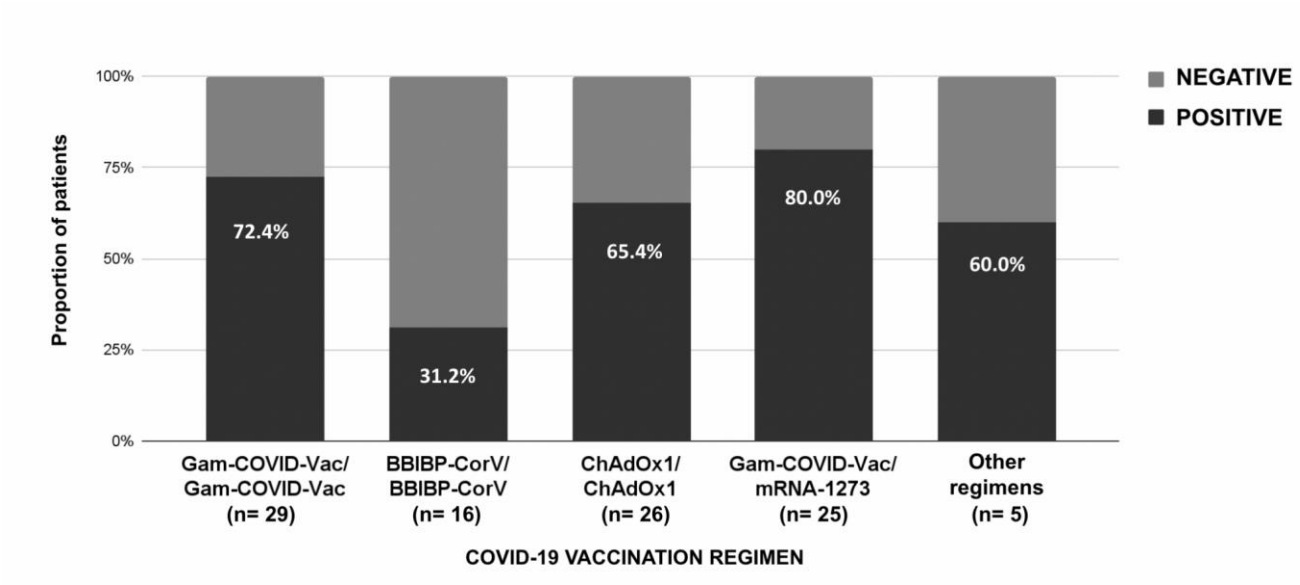


Figure 3. Specific anti SARS-CoV-2 T-cell response after vaccination in patients with rheumatoid arthritis

A.



B.