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Rheumatoid arthritis and older age are associated with lower humoral and cellular immune response to primary series COVID-19 mRNA vaccine

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A B S T R A C T

Objective: People with autoimmune disease have worse COVID-19 infection-related outcomes, lower antibody responses to COVID-19 vaccine, and higher rates of breakthrough infection. Immunosuppressive medications used to treat rheumatoid arthritis (RA) are associated with lower COVID-19 vaccine responses, though independent contributions of comorbidities, T-cell immunity, and age are less clear. We sought to test the hypothesis that RA, immunosuppressive medications used to treat RA, and older age, contribute to reduced B and T cell response to COVID-19 vaccine.

Methods: We evaluated serum samples, taken the day of 1st vaccine dose, the day of 2nd dose, 2–6 weeks after 2nd dose, 7–12 weeks after 2nd dose, 13–24 weeks after 2nd dose, and 2–6 weeks after the 3rd dose, for anti-spike IgG and neutralizing antibody levels to Wuhan and Omicron BA.1 and peripheral blood mononuclear cells (PBMC) for spike-specific IFN-γ and IL-2 production by ELISPOT assay in 46 RA and 101 non-autoimmune control participants before and after the primary series COVID-19 mRNA vaccination.

Results: RA participants had lower spike-specific IgG and Wuhan-strain neutralizing antibody levels 2–6 weeks compared to controls after the second dose of primary vaccine series. Neutralizing antibody levels against Omicron BA.1 were low in both groups. IFN-γ production correlated with Wuhan neutralizing antibody levels, while older age negatively correlated with spike-specific IL-2, IFN-γ and IgG. Lower antibody levels were associated with older age, RA status, and medication usage, while lower T cell responses were associated primarily with older age.

Conclusions: These data indicate lower COVID-19 mRNA vaccine-induced antibody levels in persons with RA compared to individuals without RA, likely partially attributable to immune suppressive medications. At the same time, older age is associated with lower antibody and cellular immune response to COVID-19 vaccines.

1. Introduction

Persons with Rheumatoid Arthritis (RA) have higher rates of severe infection and reduced response to pneumococcal and influenza vaccines [1]. They also have a 1.2 fold higher incidence of COVID-19 infection and are at greater risk of hospitalization and/or death with SARS CoV-2 infection [2,3]. COVID-19 vaccination, especially the mRNA vaccines, stimulate robust antibody response to spike protein in the general population and reduces the incidence of infection and severity of disease [4,5]. To control infection, it is necessary for the vaccine to induce both antibodies and T cells that recognize the virus [6,7]. While Wuhan monovalent vaccine induced antibody response may be less effective against viral variants, the T cell response remains effective against variants of concern that have arisen thus far [8]. People with RA were shown to have lower spike-specific antibody responses and higher rates of breakthrough infection after COVID-19 vaccine [9–11]. This is attributable, in part, to immunosuppressive medications used to treat RA, including Rituximab, steroids, Abatacept, and Methotrexate [12–14]. Less is known about T cell responses or antibody responses to variants in this population. Some studies have indicated a delayed T cell

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response after COVID-19 mRNA vaccination [15], though the degree and quality of T cell responses have not been well characterized. In regard to the latter, it appears that cellular and humoral immunity wanes significantly in healthy adults 6 months post-vaccination [15–17], though there is less data analyzing this in people with RA.

Additional factors that likely impact vaccine response and clinical outcome of COVID-19 include age and comorbid conditions (e.g., diabetes (DM), hypertension (HTN)). Understanding how these factors impact host response to COVID-19 vaccine in the setting of RA will help optimize vaccination strategies for RA patients with or without comorbidities.

In this study, we characterized the immune response to COVID-19 vaccination in participants with RA by analysis of spike-specific IgG, Wuhan and Omicron-specific neutralizing antibody levels, and spike-specific cellular production of IFN-γ and IL-2.

2. Materials and methods

2.1. Study participants

Study participants, or their legally authorized representative, gave informed consent according to protocols approved by the IRB at the Cleveland Veterans Affairs Medical Center, MetroHealth Medical Center, and under the WCG Institutional Review Board. Subjects were recruited February 2021 through January 2022. We recruited non-pregnant adult participants with and without RA who were vaccinated with a COVID-19 mRNA vaccine. We excluded those known to be previously infected with SARS-CoV-2 and participants who had a positive Spike antibody or nucleocapsid or membrane ELISPOT response at baseline. Patients with additional autoimmune diseases, active infections (e.g. HCV, HIV), or active cancer diagnoses, were excluded from the study. Participants in the RA group had an RA ICD9/10 diagnosis made in Rheumatology clinic. Medical records were reviewed for supporting information including American College of Rheumatology diagnostic criteria [18]. RA participants were queried for whether they held any dose of their immunosuppressive medication before or after vaccine doses (interpreted as windowing medication). Control participants were recruited from outpatient clinics or from a Health Care Worker vaccine clinic. They had no known autoimmune diagnosis and were not taking immunosuppressive medications. Peripheral blood samples from 46 RA and 101 control participants were collected at 6 timepoints: the day of 1st vaccine dose, the day of 2nd dose, 2–6 weeks after 2nd dose, 7–12 weeks after 2nd dose, 13–24 weeks after 2nd dose, and 2–6 weeks after the 3rd dose. While samples were not taken from each participant at every time point, most participants provided samples at multiple time points. Additionally, the majority of the control cohort did not have samples taken on the day of the 2nd dose or weeks 7–12 after the second dose, as they were enrolled under a separate protocol.

2.2. ELISPOT

IFN-γ/IL-2 double-color ELISPOT assays and IFN-γ single color ELISPOT assays were performed with cryopreserved PBMC, thawed and plated at 300,000 cells/well in 96 well plates precoated with capture antibody for IFN-γ and IL-2 (Cellular Technology Limited, Cleveland OH), or anti-IFN-γ capture antibody (TG1, Thermo Fisher Scientific) at 1 ug/ml, followed by IFN-γ monoclonal biotinylated detection antibody at 1 ug/ml (Thermo Fisher Scientific) for IFN-γ single cytokine detection assay. We used peptide pools of 15-mer length with a 11 amino acid overlap representing the Wuhan strain spike (Peptivator_S, Peptivator_S1, Peptivator_S+), nucleocapsid (Peptavitor_N), and membrane (Peptavitor_M)(Miltenyi) proteins. PBMC were stimulated overnight with media, overlapping peptide pools spanning Wuhan spike protein (described above) (1ug/mL), Cytomegalovirus/Epstein-Barr virus/Influenza CD8 immunodominant epitope pool (CEF 1 ug/ml NIH HIV Reagent Program), tetanus toxoid protein (3 ug/ml Wyeth, New York, NY), or phytohemagglutinin (PHA) (10 ug/ml, Fisher Scientific). Spots (red, blue and double color) were counted on the CTL Immunospot S5 Analyzer. For IFN-γ and IL-2 double-color assays > 6 spot- forming units (sfu)/300,000 PBMC above media response were considered positive.

2.3. Anti-spike assay

IgG responses to the vaccine were assessed by bead-multiplex immunoassay using the Wuhan strain protein as previously described [19]. Stabilized full-length spike protein (aa 16–1230, with furin site mutated) were conjugated to magnetic microbeads (Luminex) and assessed by the Magpix assay system (BioRad, Inc). The mean fluorescent index was recorded after detection of antigen-specific IgG in participant serum using phycoerythrin-conjugated donkey F(ab)2 anti-human IgG (Jackson Immunological). A secondary standard from the Frederick National Laboratory calibrated to the WHO standard 20/136 was used to quantitate antibodies to the spike protein expressed as binding arbitrary units (BAU) per milliliter. A response >5 BAU/ml was considered a positive response.

2.4. SARS-CoV-2 pseudovirus neutralization assay

To determine the neutralizing activity of vaccine recipients' sera against coronaviruses, we produced lentiviral particles pseudotyped with spike protein based on the Wuhan and Omicron BA.1 strains as previously described [20]. Briefly, neutralization assays were performed using a Fluent 780 liquid handler (Tecan) in 384-well plates (Grenier). Three-fold serial dilutions that ranged from 1:12 to 1:8748 were performed and added to 50–250 infectious units of pseudovirus for 1 h. pNT50 values were calculated by taking the inverse of the 50 % inhibitory concentration value for all samples with a pseudovirus neutralization value of 80 % or higher at the highest concentration of serum. The lower limit of detection (LLD) of this assay is 1:12 dilution.

2.5. Flow cytometry

PBMCs were labeled with anti-CD3-PerCP, CD27-BUV805, HLA-DR-PE, CD19 -BUV395, CD21-BV711, (BD Biosciences, San Jose, CA) CD4 -Pac blue, CD8 –APC-Cy7, CD45RA –PECy-7, CD38 – APC, CD14- FITC, CD16 -BV786, CD20-BV605, CD28-PE/Dazzle 594, (BioLegend). Compensation was performed using CompBeads (BD Biosciences San Jose, CA) for cell surface markers. Live/Dead Aqua (Invitrogen) was used to define live cells. Live cells were then gated for B cells (CD3-CD19+) and T cells (CD3 + CD19-). CD3+ T cells were gated for CD4+ and CD8+ then differentiated further by CD27 and CD45RA into naïve (CD27 + CD45RA+), central memory (CD27 + CD45RA-), effector memory (CD27-CD45RA-) and terminally differentiated subsets (CD27-CD45RA+). These subsets were also analyzed for HLA-DR and CD38 co-expression. Naïve CD4+ and CD8+ subsets were additionally analyzed as CD28+ or CD28- based on isotype gating [21]. CD19+ B cells were defined using CD38+ for plasmablasts and CD21 + CD27- for naïve B cells. Live cells were also gated for monocyte subsets using CD16 and CD14. Monocytes were divided into classical (CD14++ CD16-), intermediate (CD14++, CD16+), and nonclassical (CD14 + CD16+). Analysis was performed on a BD Fortessa flow cytometer. Results were analyzed using FlowJo.version10.8.

2.6. Statistical analysis

Demographic differences between groups were compared using a *t*-test for age and Chi-Squared tests for gender, race, and ethnicity. Simulated p-values were used when small cells numbers were present. To compare vaccine responses between RA status groups at a single time point, while adjusting for age differences between the groups, we estimated ordinary least-squares regression models predicting log-transformed assay levels with age, RA status, and their interaction.

Gender was included in these models as a covariate. To compare vaccine responses between the groups across time, we estimated a mixed-effects model to adjust for repeated measures within subjects. In this model, the interaction of vaccine time points and group was the effect of interest while adjusting for age and gender. Post-hoc contrasts were estimated to characterize this interaction, comparing the differences in estimated marginal means between RA status groups at each time point.. Spearman rank correlations were performed to assess bivariate associations combining the vaccine groups in the absence of a detected group effect, and for flow cytometry subset data shown in Supplemental Fig. 2. Models were estimated using R version 4.2.2 and nlme and emmeans packages. Simple univariate analysis comparing RA participants on vs. not on particular medications was performed by Independent-Samples Mann Whitney *U* test (GraphPad prism version 9.4.1).

3. Results

3.1. Study population characteristics

Study participants included 46 individuals with RA and 101 adults without autoimmune diseases (Table 1). RA medications prescribed included the dihydrofolate reductase inhibitor methotrexate (MTX) (n = 23), dihydroorotate dehydrogenase inhibitor Leflunomide (LEF) (n = 5), tumor necrosis factor (TNF) blocker (etanercept, infliximab, or adalimumab) (n = 14), Janus Kinase (JAK) inhibitor (tofacitinib) (n = 2), B cell depletion agent Rituximab (n = 3), T cell costimulation inhibiting agent Abatacept (n = 5), hydroxychloroquine (n = 13), sulfasalazine (n = 3), and prednisone (n = 6). None of the controls were on immune suppressive medications. Three RA participants were taking no immune suppressive medications, 21 on a single medication type, 15 on 2 different, and 7 on 3 or more different medication types. Rheumatoid factor (RF) and cyclic citrullinated peptide antibody (CCP) status within the RA group included 28 participants seropositive for both RF and CCP, 6 positive for only RF, 3 positive for only CCP, and 9 were seronegative. RA and control groups differed by age (mean 66.8 vs. 52.2, respectively, Welch 2 Sample $p < 0.001$) and gender (80 % vs. 57 % male, Chi-squared $p = 0.01$), but not race (Table 1). Most participants received the Pfizer vaccine, with only 7 receiving the Moderna vaccine. RA participants commonly had hypertension (74.5 %), diabetes (31.9 %), coronary artery disease (29.8 %), and were with a mean body mass index of 29.09).

3.2. Participants with RA and those with older age have lower SARS-CoV-2 spike-specific IgG levels and Wuhan neutralizing antibody levels 2–6 weeks following COVID-19 mRNA vaccination

Both RA and control groups had increases in spike-specific IgG levels after the second dose of mRNA vaccine (Repeated measures analysis p=<0.001, Fig. 1**a** and not shown) and this difference over time remained after adjusting for age and gender ($p < 0.001$ for each). We observed an interaction between time and groups and this interaction effect remained after adjusting for age and gender. Comparing the groups at each timepoint using post hoc model contrasts, we found that this group difference was localized to 2–6 weeks post vaccine, where antibody levels were lower in the RA group compared to controls (median 143.3 BAU/mL vs 5598 BAU/mL, p=<0.0001, Fig. 1**b**). Notably, within the RA group there was substantial variability in antibody level, with about 40 % of individuals having very low (<100 BAU/ml) or undetectable antibody levels. At 13–24 weeks the antibody levels were comparable between RA and control groups (Fig. 1**b**). Two to six weeks after the 3rd dose of vaccine antibody were similar comparing control and RA groups (model contrast $p = 0.33$). These data indicate that participants with RA have significantly lower spike specific IgG 2–6 weeks after vaccination compared to controls, even after adjusting for age and gender. At the same time 3rd dose boosting appears to bridge this gap.

Additionally, in this dataset age was negatively correlated with antibody level (Fig. 1**C**). In a regression model predicting log-transformed antibody level with age, gender and RA group status, and the interaction of age and RA group status variables, both age and RA group status, but not gender, were independently associated with antibody level ($p < 0.001$ for each), while there was no detected interaction between age and RA group status ($p = 0.83$). At the post boost time point, antibody level varied by age ($p = 0.048$), but not by group status (as discussed above). These data indicate both RA group and older age are associated with lower antibody response to the primary series of COVID-19 vaccination.

Neutralizing antibody levels to the Wuhan and Omicron BA.1 strain spike proteins were measured at 2–6 weeks and 13–24 weeks after receiving the second dose of the COVID-19 vaccine. Similar to total spike antibody levels we observed Wuhan neutralizing antibody levels to be higher in controls compared to RA at 2–6 weeks post-vaccine ($p < 0.0001$, Fig. 2a). Additionally, Omicron BA.1 neutralizing antibody levels were lower than Wuhan neutralizing antibody levels in controls at 2–6 weeks post-vaccine (Fig. 2b $p < 0.0001$) and in RA at 13–24 weeks post-vaccine ($p = 0.05$, Fig. 2c). Furthermore, Wuhan spike neutralizing antibody levels correlated with total Wuhan spike IgG levels for the control and RA populations combined ($r = 0.44$, $p < 0.001$, Fig. 2d). Finally, age tended to negatively correlate with Wuhan neutralizing antibody level ($r = -0.20$, $p = 0.09$, not shown). These data indicate Wuhan neutralizing antibody levels correlate with total antibody levels and tend to correlate with age, while Omicron BA.1 variant neutralizing antibody levels are low in response to the primary Wuhan mRNA vaccine series.

3.3. Relation between cellular immune response, age, and antibody response: older age correlates with lower levels of spike-specific IFN-γ, and spike specific IFN-γ correlates with neutralizing antibody levels

IFN-γ and IL-2 SARS-CoV-2 antigen-specific T cell responses were measured by ELISpot assay that detects IFN-γ and IL-2 secretion separately or simultaneously, using a 2-color marking system, quantified as spot forming units/300,000 plated PBMC over the course of COVID-19 vaccine (Fig. 3**a and 3b** for RA group). Most spots were either IFN-γ (50.8–52.3 %) or IL-2 (42.3–43.8 %) single cytokine producing cells, and IFN-γ production was strongly correlated with IL-2 production ($r = 0.87$, $p < 0.0001$, not shown). PBMCs producing both IFN-γ and IL-2 were at lower frequencies (average of 6 % of total spots) but rose and declined in proportion to the IFN-γ and IL-2 single producing cell frequencies over time (not shown).

Participants with RA tended to have lower spike-specific IFN-γ sfu than the control group at 2–6 weeks post-vaccination (7.8 vs. 39.0 sfu, $p = 0.05$ univariate non-parametric comparison). However, after adjusting for age and gender these differences did not hold in ordinary least-squares regression models, while age tended to associate with spike specific IFN-γ sfu ($p = 0.05$), RA group status did not ($p = 0.79$). Moreover, when both groups combined were analyzed, age itself correlated with IFN-γ (Fig. 3c, $p = 0.02$) and nearly IL-2 (Fig. 3d, p =

Table 1
Study participant clinical characteristics.

	RA n = 46	Control n = 101	p=
Age mean (SD)	66.8 (8.9)	52.2 (13)	p < 0.001
Gender	37 (80 %) Male	58 (57 %) Male	p = 0.01
Race	White: 74 %	White: 73 %	p = 0.6
	African American: 22 %	African American: 22 %	
	Hispanic: 0 %	Hispanic: 3 %	
	Asian: 4 %	Other: 2 %	
RF+/CCP-	6 (12.7 %)	N/A	
RF-/CCP+	3 (6.3 %)	N/A	
RF+/CCP+	28 (59.6 %)	N/A	
RF-/CCP-	9 (21.3 %)	N/A	

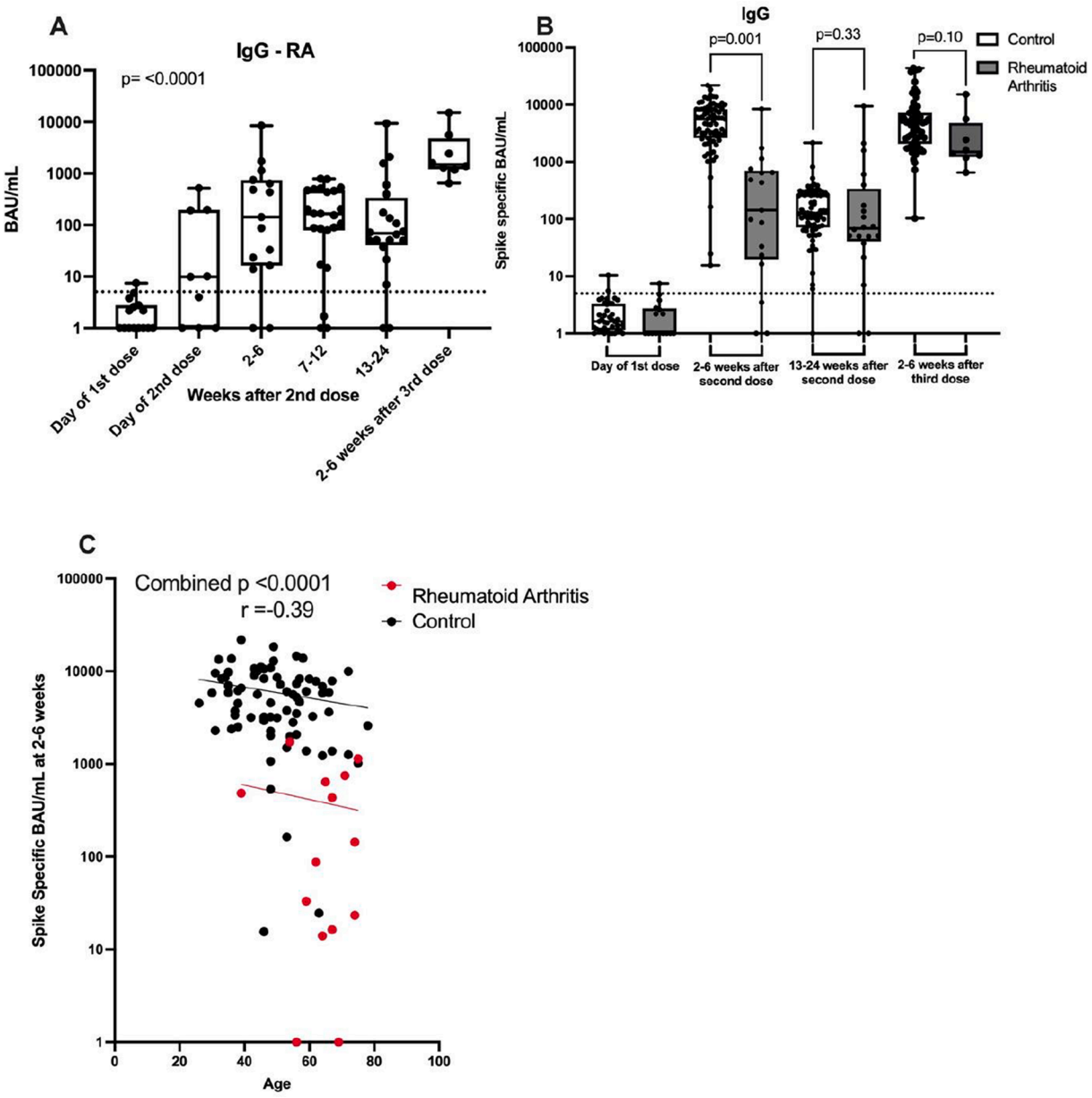

Fig. 1. Participants with RA as well as those with older age have lower SARS-CoV-2 spike specific IgG levels following COVID-19 vaccination. Panel A: SARS CoV-2 Wuhan Spike specific IgG levels over course of primary vaccine series in RA participants. **Panel B:** Comparison of RA and non-RA control spike specific IgG levels over the course of vaccine. **Panel C:** Correlation between spike IgG level and age for all participants. Trend line shown for both RA and control groups. Dotted lines on Panels A and B indicate pre-determined spike specific IgG positive response cutoff.

0.09) responses. Finally, IFN-γ levels correlated with neutralizing antibody levels for the control and RA populations combined (Fig. 3e, p = 0.001).

3.4. Relation between immunosuppressive medications, other clinical parameters and vaccine response

The sample size of our RA group overall is limited to directly compare medication treatment subgroups and many participants were on more than one medication, making it difficult to attribute variation to one specific medication (Supplemental Fig. 1). Notable standouts included spike-specific IgG levels that were negative in rituximab-treated participants (Supplemental Fig. 1**d**), and lower in those RA participants on methotrexate than those not on methotrexate in univariate analysis (Supplemental Fig. 1**d**, p = 0.03). In the case of TNF-blocker, no significant differences in antibody levels were observed comparing those on vs. not on TNF-blocker. We observed no significant associations between the presence or absence of hypertension, diabetes or coronary artery disease and antibody response in the RA group, or differences comparing those receiving Pfizer vs. Moderna vaccine. These data indicate variation in antibody response is associated with some RA medications.

Similar to findings with antibody response, there was wide variability in IFN-γ (Supplemental Fig. 1**B**), IL-2 (Supplemental Fig. 1**C**) or double color (not shown) sfu across RA treatment subgroups. In contrast to antibody response, production of IFN-γ and IL-2 showed no observable differences in the rituximab subgroup compared to all other RA participants (Supplemental Fig. 1**e**). Also, when comparing participants on all types of medication who windowed their RA treatment (held any dose of treatment medication) at time of vaccine vs. those that did not window, we observed greater spike-specific IFN-γ sfu at week 2–6 post-vaccination in those who windowed medication (n = 4) compared to

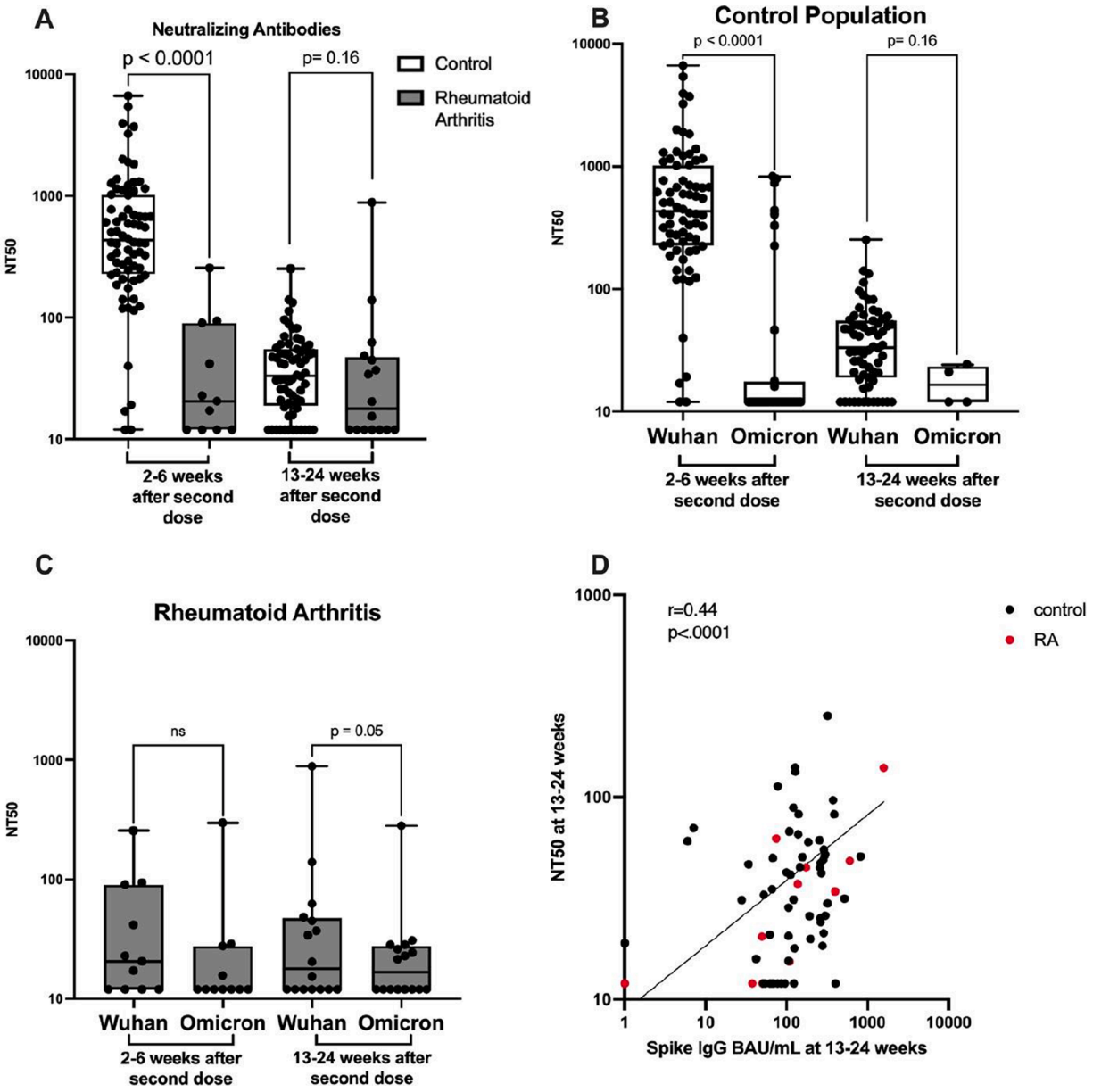

Fig. 2. Wuhan neutralizing ab is lower in RA as well as with older age and correlates with total IgG while Omicron is lower in all groups at all timepoints. SARS Cov2 Neutralizing capacity. **Panel A**: Comparison of RA and non-RA control neutralizing titers. **Panel B**: Control group comparison of Wuhan vs. Omicron BA.1 neutralizing titers. **Panel C:** RA group comparison of Wuhan vs. Omicron BA.1 neutralizing titers. **Panel D:** Correlation between Spike specific IgG level and neutralizing antibody level for all participants combined.

those who did not (n = 20) (Supplemental Fig. 1**e**, p = 0.02). We additionally observed variation in cellular immune response across the spectrum in persons with hypertension, diabetes and coronary artery disease and there were no significant differences in response that associated with these comorbidities, or by Pfizer vs. Moderna vaccine type in univariate analyses.

To further understand potential cellular immune factors that may contribute to altered immune responses in the RA group, we analyzed bulk T cell (CD3+), T cell subset (CD4, CD8, naive, central memory, effector memory), B cell (CD19+), and monocyte subset (CD14, CD16) distribution and phenotype (activation state assessed by HLA-DR or HLA-DR/CD38 co-expression) by flow cytometry. While the subset of participants analyzed here was small (n = 18 RA and n = 5 controls), in the RA group we observed correlations between greater age and lower naïve CD8 T cell frequency (r = −0.62, p = 0.04, Supplemental Fig. 2a), and lower CD28 expression on naïve CD8 cells (r = −0.83, p < 0.001, Supplemental Fig. 2b) and "total" B cell frequency and antibody response to vaccine (r = 0.79, p = 0.002)(Supplemental Fig. 2c). While comparisons between groups were difficult given the sample size, persons with RA had higher frequencies of plasmablasts (CD38 + CD20- B cells) at 6 months post-vaccine compared to controls (12.9 % vs.1.7 % p = 0.016, not shown). Total B cells and monocyte subsets (classical (CD14++ CD16-), intermediate (CD14++, CD16+), and nonclassical (CD14+, CD16+)) frequencies were not found to differ between the control and RA groups.

4. Discussion

This study aimed to analyze how RA impacts the ability of the immune system to mount antibody and cellular immune response to the COVID-19 mRNA vaccine. We found that in addition to lower spike-specific IgG levels, there were lower Wuhan spike neutralizing

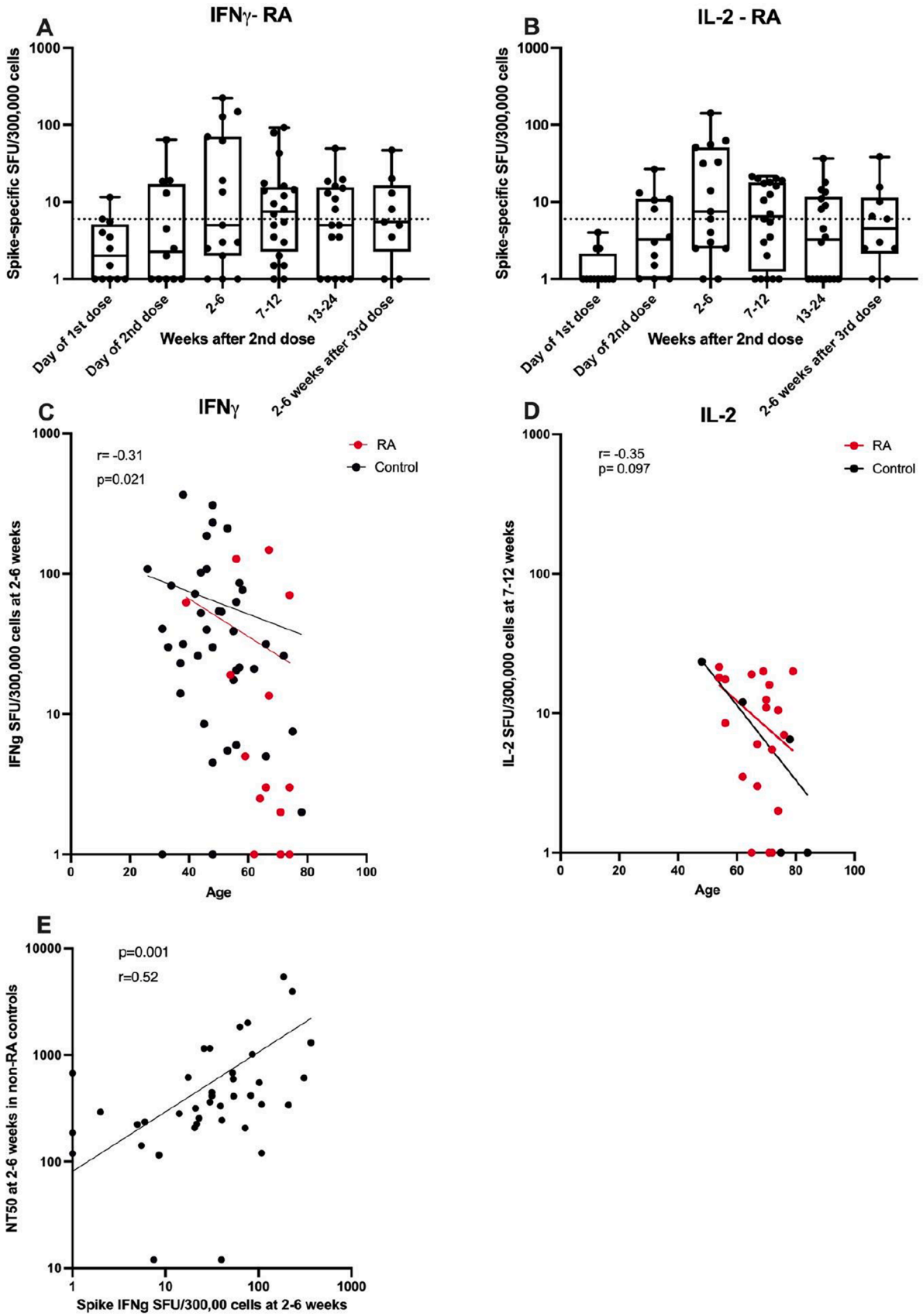

Fig. 3. Older age correlates with lower levels of spike-specific IFN-γ, and spike specific IFN-γ correlates with neutralizing antibody levels. Panel A: Spike specific IFN-γ sfu over the course of COVID-19 vaccine. **Panel B:** Spike specific IL-2 sfu over the course of COVID-19 vaccine. **Panel C:** Correlation between age and spike specific IFN-γ sfu. **Panel D:** Correlation between age and spike specific IL-2 sfu. **Panel E:** higher IFN-γ sfu correlates with higher neutralizing titers in the control population. Dotted line in Panels A-B indicate pre-determined spike specific positive response.

antibody levels at 2–6 weeks after the second dose of COVID-19 mRNA primary 2 dose vaccine series in persons with RA compared to controls. While differences between groups in antibody response remained after adjusting for age and gender, both cellular and humoral immunity were also associated with age, highlighting independent contributions of age and RA group status for antibody response and primarily a contribution of age for cellular immune response. After the 3rd dose of vaccine, antibody levels increased in both groups, and differences between control and RA groups no longer remained significantly different after adjusting for age. Additionally, while immune response appeared generally lower across all RA treatment medication subgroups, cellular immune response did tend to be higher in those few participants who windowed medication around the time of vaccine, while antibody response was greater in those not on methotrexate compared to those on methotrexate. Finally, neutralizing antibody levels for Omicron induced by this Wuhan primary COVID-19 vaccine series were generally low in all participants, while Wuhan strain neutralizing antibody levels strongly correlated with total spike antibody levels and also correlated with IFN-γ producing cellular immunity. Overall, these data are consistent with RA patients having an impaired humoral response to COVID-19 mRNA vaccine primary series, while older age and RA medication contributes to lower humoral and cellular immune response to vaccine.

Existing literature has indicated impaired antibody response to COVID-19 vaccine in the setting of RA, in some cases associated with use of immunosuppressive drugs such as rituximab, methotrexate or Abatacept [15,22,23]. Our data is consistent with existing literature indicating lower antibody response in the setting of RA, and lower spike-specific IgG levels in those persons receiving rituximab (an anti-CD20 B cell depleting agent) as well as methotrexate. Of particular interest, our data indicates that at more distal timepoints (13–24 weeks and later) the vaccine induced antibody response in the control population is similar to that in participants with RA. Also, medication associated variability in immune response observed at 13–24 weeks (discussed above) did not persist after the 3rd dose of the vaccine here. Other studies have indicated persistence of lower antibody response after 3rd dose with lower levels in persons on MTX, anti- TNFα, and Rituximab [24]. It may be that our sample size after 3rd dose is insufficient to appreciate such differences, though the overall magnitude of immune response after 3rd dose appears comparable to controls in our data set. Certainly, more data comparing individuals on immune suppressive medications after additional vaccine dosing can help clarify this issue.

IFN-γ has frequently been studied as a marker of cellular immune response. Regarding COVID-19 vaccination, our study suggests older age may play a larger role than RA group status. We also found that IL-2 production was lower than IFN-γ but followed a similar pattern of peaking at 2–6 weeks post vaccination then modestly waning after 7–24 weeks. Additionally, we found that 5.8–6.1 % of the spike-specific cellular immune response was in the category of IFN-γ/IL-2 dual producing cells, while the majority were either IFN-γ (50.8–52.3 %) or IL2 (42.3–43.8 %) single cytokine producing cells. Together, these findings suggest a coordinated IFN-γ/IL2 cellular immune response that is affected by age. Furthermore, the observed correlation between IFN-γ sfu and Wuhan spike neutralizing antibody levels also suggests coordination between cellular and humoral immunity, in agreement with prior literature [15]. In an effort to understand cellular compartments participating in this coordinated immune response we enumerated B and T cell subset frequencies by flow cytometry. While this sample set was very small, we observed a correlation between total B cell frequency and antibody response, and a higher plasmablast frequency in participants with RA. Others have observed lower IgG+ switched memory B cells in those with RA [15]. Certainly, B cell subset perturbations may contribute to a lower vaccine response in those with RA, and additional studies of bulk and antigen specific cell subsets in the context of vaccine response may help clarify mechanisms underlying impaired host response.

We find that neutralizing antibody responses to Omicron BA.1 are remarkably low in both controls and RA patients, while Wuhan neutralizing activity is robust in controls and greater than that in patients with RA. This is consistent with other literature [25]. While antibody response is remarkably low against the variants, T cell recognition of variants is reported to be similar to that of the Wuhan strain in individuals with and without immune diseases [8,25].

When specifically focusing on age, both cellular and humoral immune responses were observed to be lower in older persons. It has recently been shown in one study that older people have a lower response to COVID-19 vaccine [26]. In agreement, here we show that older age correlated with lower cellular and humoral response to the primary series COVID-19 mRNA vaccines. This could be due to lower naïve T cell frequency. Notably, we did not evaluate T follicular helper cells known to relate to antibody levels.

Limitations of this data set include the small sample size limiting our ability to identify associations between immune responses and the various combinations of immunosuppressive medications; a lack of immune function data at all time points for each participant due in part to the pandemic-related telehealth visit emphasis during the time period of this study; lack of health information on a subset of the controls enrolled through a health care worker protocol, and the small sample size of post 3rd dose booster data. However, these data both confirm other reported studies and extend RA host cellular immune function and neutralizing antibody findings to lend insight into the intersection between age and treated autoimmune host immunity to COVID-19 vaccine.

In conclusion, our data shows impaired humoral immune response to COVID-19 vaccination in persons with RA and impaired humoral and cellular immune response to COVID-19 vaccination in older persons. Selective medications used to treat RA likely additionally contribute to the magnitude of immune impairment. Finally, decline in humoral immunity following peak response may be altered in the setting of RA, and 3rd dose of vaccine may partially mitigate differences between controls and RA patients.

5. Funding

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2023.08.033.

References

[1] Chen C-M, Chen H-J, Chen W-S, Lin C-C, Hsu C-C, Hsu Y-H. Clinical effectiveness of influenza vaccination in patients with rheumatoid arthritis. Int J Rheum Dis 2018; 21:1246–53.
[2] Malek Mahdavi A, Varshochi M, Hajialilo M, Dastgiri S, Khabbazi R, Khabbazi A. Factors associated with COVID-19 and its outcome in patients with rheumatoid arthritis. Clin Rheumatol 2021;40:4527–31.
[3] Cordtz R, Lindhardsen J, Soussi BG, Vela J, Uhrenholt L, Westermann R, et al. Incidence and severeness of COVID-19 hospitalization in patients with inflammatory rheumatic disease: a nationwide cohort study from Denmark. Rheumatology 2021;60.

[4] 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;383:2603–15.
[5] 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;384:403–16.
[6] Sette A, Crotty S. Adaptive immunity to SARS-CoV-2 and COVID-19. Cell 2021;184:861–80.
[7] Goletti D, Petrone L, Manissero D, Bertoletti A, Rao S, Ndunda N, et al. The potential clinical utility of measuring severe acute respiratory syndrome coronavirus 2-specific T-cell responses. Clin Microbiol Infect 2021;27:1784–9.
[8] Tarke A, Coelho CH, Zhang Z, Dan JM, Yu ED, Methot N, et al. SARS-CoV-2 vaccination induces immunological T cell memory able to cross-recognize variants from Alpha to Omicron. Cell 2022;185:847–859.e11.
[9] Baker MC, Mallajosyula V, Davis MM, Boyd SD, Nadeau KC, Robinson WH. Effective viral vector response to SARS-CoV-2 booster vaccination in a patient with rheumatoid arthritis after initial ineffective response to messenger RNA vaccine. Arthritis Rheumatol 2022;74:541–2.
[10] Sun J, Zheng Q, Madhira V, Olex AL, Anzalone AJ, Vinson A, et al. Association between immune dysfunction and COVID-19 breakthrough infection after SARS-CoV-2 vaccination in the US. JAMA Intern Med 2022;182:153–62.
[11] Ammitzbøll C, Bartels LE, Bøgh Andersen J, Risbøl Vils S, Elbaek Mistegård C, Dahl Johannsen 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;3:622–8.
[12] van Assen S, Holvast A, Benne CA, Posthumus MD, van Leeuwen MA, Voskuyl AE, et al. Humoral responses after influenza vaccination are severely reduced in patients with rheumatoid arthritis treated with rituximab. Arthritis Rheum 2010;62:75–81.
[13] Mahil SK, Bechman K, Raharja A, Domingo-Vila C, Baudry D, Brown MA, et al. The effect of methotrexate and targeted immunosuppression on humoral and cellular immune responses to the COVID-19 vaccine BNT162b2: a cohort study. Lancet Rheumatol 2021;3. e627–37.
[14] Saleem B, Ross RL, Bissell L-A, Aslam A, Mankia K, Duquenne L, et al. Effectiveness of SARS-CoV-2 vaccination in patients with rheumatoid arthritis (RA) on DMARDs: as determined by antibody and T cell responses. RMD Open 2022:8. https://doi.org/10.1136/rmdopen-2021-002050.
[15] Farroni C, Picchianti-Diamanti A, Aiello A, Nicastri E, Laganà B, Agrati C, et al. Kinetics of the B- and T-cell immune responses after 6 months from SARS-CoV-2 mRNA vaccination in patients with rheumatoid arthritis. Front Immunol 2022;13:846753.
[16] Levin EG, Lustig Y, Cohen C, Fluss R, Indenbaum V, Amit S, et al. Waning immune humoral response to BNT162b2 covid-19 vaccine over 6 months. N Engl J Med 2021;385:e84.
[17] Dayam RM, Law JC, Goetgebuer RL, Chao GY, Abe KT, Sutton M, et al. Accelerated waning of immunity to SARS-CoV-2 mRNA vaccines in patients with immune mediated inflammatory diseases. JCI Insight 2022. https://doi.org/10.1172/jci.insight.159721.
[18] Aletaha D, Neogi T, Silman AJ, Funovits J, Felson DT, Bingham 3rd CO, et al. 2010 Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. Arthritis Rheum 2010;62:2569–81.
[19] Canaday DH, Carias L, Oyebanji OA, Keresztesy D, Wilk D, Payne M, et al. Reduced BNT162b2 mRNA vaccine response in SARS-CoV-2-naive nursing home residents. Clin Infect Dis 2021. https://doi.org/10.1093/cid/ciab447.
[20] Garcia-Beltran WF, St Denis KJ, Hoelzemer A, Lam EC, Nitido AD, Sheehan ML, et al. mRNA-based COVID-19 vaccine boosters induce neutralizing immunity against SARS-CoV-2 Omicron variant. Cell 2022;185:457–466.e4.
[21] Morimoto C, Rambotti SSFP, Lecture.. Human naive and memory T cells revisited: new markers (CD31 and CD27) that help define CD4+ T cell subsets. Clin Exp Rheumatol 1993;11:241–7.
[22] 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;80:1330–8.
[23] Jena A, Mishra S, Deepak P, Kumar-M P, Sharma A, Patel YI, et al. Response to SARS-CoV-2 vaccination in immune mediated inflammatory diseases: Systematic review and meta-analysis. Autoimmun Rev 2022;21:102927.
[24] Farroni C, Aiello A, Picchianti-Diamanti A, Laganà B, Petruccioli E, Agrati C, et al. Booster dose of SARS-CoV-2 messenger RNA vaccines strengthens the specific immune response of patients with rheumatoid arthritis: A prospective multicenter longitudinal study. Int J Infect Dis 2022;125:195–208.
[25] Petrone L, Picchianti-Diamanti A, Sebastiani GD, Aiello A, Laganà B, Cuzzi G, et al. Humoral and cellular responses to spike of δ SARS-CoV-2 variant in vaccinated patients with immune-mediated inflammatory diseases. Int J Infect Dis 2022;121:24–30.
[26] Zhao M, Slotkin R, Sheth AH, Pischel L, Kyriakides TC, Emu B, et al. Serum neutralizing antibody titers 12 months after coronavirus disease 2019 messenger RNA vaccination: Correlation to clinical variables in an adult. US Population Clin Infect Dis 2023;76. e391–9.