Humoral and cellular immunity in patients with rare autoimmune rheumatic diseases following SARS-CoV-2 vaccination

Leher Gumber[1], Nancy Gomez[2], Georgina Hopkins[2], Davis Tucis[2], Laura Bartlett[2], Kieran Ayling[3], Kavita Vedhara[3], Graham Steers[2], Mithun Chakravorty[4], Megan Rutter[4,5], Hannah Jackson[2], Patrick Tighe[2], Alastair Ferraro[6], Sheila Power[1], Marie-Josèphe Pradère[1], David Onion[2], Peter C. Lanyon[4,5,7], Fiona A. Pearce[4,5,7], Lucy Fairclough[2]

[1]Nottingham University Hospitals NHS Trust, Nottingham, UK
[2]School of Life Sciences, University of Nottingham, UK
[3]School of Medicine, University of Nottingham, UK
[4]Department of Rheumatology, Nottingham University Hospitals NHS Trust, Nottingham, UK
[5]Lifespan and Population Health, School of Medicine, University of Nottingham, UK
[6]Department of Nephrology, Nottingham University Hospitals NHS Trust, Nottingham, UK
[7]NIHR Nottingham Biomedical Research Centre

Corresponding author:
Dr Fiona Pearce
Department of Rheumatology, Nottingham University Hospitals NHS Trust, Nottingham, UK
fiona.pearce@nottingham.ac.uk

Keywords: rare autoimmune rheumatic diseases, SARS-CoV-2, vaccination, antibody, cell mediated, T cells

Abstract

Objectives: COVID-19 vaccine responses in rare autoimmune rheumatic diseases (RAIRD) remain poorly understood, in particular there is little known about whether people develop effective T-cell responses. We conducted an observational study to evaluate the short-term humoral and cell-mediated T-cell response after the second SARS-CoV-2 vaccination in RAIRD patients compared to healthy controls (HC).

Methods: Blood samples were collected after the second dose and anti-spike, anti-nucleocapsid antibody levels and SARS-CoV-2 specific T-cell responses were measured and compared with HC. Activation induced marker and deep phenotyping assays were used to identify differences in T cells between high and low/no antibody groups, followed by multi-dimensional clustering.

Results: 50 patients with RAIRD were included (31 with AAV, 4 with other systemic vasculitis, 9 with SLE and 6 with myositis). Median anti-spike levels were significantly lower in RAIRD compared to HC ($p<0.0001$). 15 (33%) patients had undetectable and 26 (57%) had lower levels than the lowest HC. Rituximab in the last 12 months ($p=0.003$) was associated with reduced immunogenicity compared to a longer pre-vaccination period. There was a significant difference in B cell percentages ($p=0.03$) and spike-specific CD4+ T cells ($p=0.02$) between no/low antibody vs. high antibody groups. Patients in the no/low antibody group had a higher percentage of terminally differentiated (exhausted) T cells.

Conclusions: Following two doses, most RAIRD patients have lower antibody levels than the lowest HC and lower anti-spike T cells. RAIRD patients with low/no antibodies have diminished numbers and poor quality of memory T cells which lack proliferative and functional capacities.

Key messages

- 57% of RAIRD patients had an insufficient antibody response (lower antibody levels than the lowest healthy control) following two vaccine doses.
- Patients with low or no antibodies also have significantly lower levels of memory T cells which lack both functional and proliferative capacities.
- Assessment of both serological and T cell responses is necessary to fully define responses to vaccination in immunosuppressed populations.

Introduction

The rapid development of vaccines and mass vaccination since the emergence of COVID-19 has helped control transmission and severity of SARS-CoV-2. Although these vaccines have a good efficacy and safety profile in the general population[1,2], less is known about their effects in immunocompromised patients (ICPs). There is a particular gap in the literature related to people with rare autoimmune rheumatic diseases (RAIRD) such as systemic vasculitis who are thought to be at increased risk of severe poor outcomes and mortality from COVID-19 compared to the general population and compared to people with rheumatoid arthritis and other inflammatory arthritis[3–6]. Successful host protection from vaccination relies upon a functional immune system including humoral and cell-mediated responses which can be diminished in RAIRD secondary to immunosuppressive therapy[7,8]. Previous research has identified that high disease activity and high-dose glucocorticoids are associated with an increased risk of severe COVID-19 infection[9,10]. In particular, rituximab, a monoclonal anti-CD20 B cell depleting agent, has been shown to increase severity of infection[11,12], risk of COVID-19 related death[9] and reduce vaccine responsiveness[13]. Additionally, the time since last rituximab treatment has been shown to impact humoral response with the seven to nine month period prior to vaccine being the most significant predictor of impaired response[14,15]. B cell numbers also influence response in rituximab-treated patients with a minimum of 0.4% of circulating lymphocytes being required for seroconversion[16]. Methotrexate and glucocorticoids have also been shown to diminish immunogenicity of SARS-CoV-2 vaccines[7,17–19].

The effect of vaccination on cellular immunity in patients with stable disease on long-term immunosuppressive therapy is less well described. A recent study on vasculitis and autoimmune glomerulonephritis patients found T-cell responses in more than 80% of patients even in the absence of serological responses[14]. Another study, which aimed to characterise the phenotype of the T-cell response, found a higher proportion of TNF-α producing CD4 cells in seronegative autoimmune rheumatic disease patients[20]. However, both of these studies did not provide any data on memory T cells. As we know from previous research, memory T cells mediate a faster and more potent response upon repeat encounter with antigens and thereby underpin long-lasting immunity against infection[21]. In addition, some questions remain unanswered, including the short and medium-term immune response to vaccination and vaccine response in different types of RAIRD.

To address these research gaps, we conducted a prospective cohort study to evaluate the humoral and cell-mediated response to SARS-CoV-2 vaccination in patients with RAIRD compared to healthy controls (HC). Here we present the findings of the short-term response to two doses of SARS-CoV-2 vaccination with a focus on memory T-cells, which have not been well-described in previous studies.

Methods

Study design and population

We conducted a prospective, single-centre longitudinal cohort study in individuals with RAIRD recruited from Nottingham University Hospitals NHS Trust in the UK from April to June 2021.

Individuals were recruited through outpatient rheumatology and renal clinics either during clinic appointments or via email, letter or telephone between appointments. Eligible individuals were adults aged ≥18 years with a diagnosis of RAIRD (vasculitis, systemic lupus erythematosus, myositis, scleroderma and Sjogren's syndrome), and eligible to receive SARS-CoV-2 vaccinations. People were not eligible if they were <18 years old, ineligible to receive SARS-CoV-2 vaccinations, unable to provide blood samples, unable to travel to the hospital for study visits, unable to consent or had low English proficiency. HC were invited from a related study and were age and sex-matched prior to invitation using a 1:1 ratio for comparison[22]. HC who were invited were matched with the RAIRD group who were invited. More HC (especially of older ages) did not wish to participate hence the differences in numbers and ages. Based on previous similar research, a sample size of 50 per group was deemed sufficient to detect any significant differences in responses. A total of 102 RAIRD patients were identified of whom 29 were ineligible and 21 declined to participate. A total of 52 RAIRD patients participated in the study of whom 50 gave a blood sample 4 weeks or 3 months after their second vaccine. 34 HC agreed to participate of which 2 were excluded as they were taking immunosuppressants for rheumatoid arthritis, leaving 32 eligible to participate. All participants provided written informed consent.

Data and sample collection

A baseline questionnaire was administered to collect information on demographics, clinical factors (previous COVID-19 infection and tests), diagnosis, current and/or recent immunosuppressive medications, recent glucocorticoid use and vaccination details. Whole blood samples were collected 4 weeks after the second dose of the COVID-19 vaccine. In cases where the 4-week target could not be met due to appointment unavailability, blood samples

were collected three months after the second dose (n=14). Samples were taken at hospital sites and stored in accordance with the Human Tissue Authority and NHS guidelines. HC had the same blood sample collections.

Patient involvement

Patients and members of the public were involved at all stages of the study design and conduct. The study proposal was peer reviewed by people with vasculitis and other RAIRD and their feedback was incorporated into the study design. Study findings will be disseminated to patients and public through the Vasculitis UK website and newsletters.

Antibody response

Heparinized whole blood was centrifuged to separate the plasma. Plasma was tested for nucleocapsid and spike specific antibodies in two separate ELISAs. Briefly, 384 well Maxisorp (NUNC) assay plates were coated with 20µL per well of 1µgmL-1 of either Wuhan strain SARS-CoV-2 full-length spike protein or Wuhan strain SARS-CoV-2 nucleocapsid protein. Plates were sealed, incubated overnight and serially diluted as per WHO standards. Antibody titres were defined as positive if the value was greater than 10 BAU. An antibody response was defined as sufficient if the IgG level was higher than that of the lowest HC. Further details are provided in the supplementary methods available at *Rheumatology* online.

T cell response

We examined the percentages of both T and B cells in 10 patients with low/no anti-spike IgG and 10 patients with high anti-spike IgG. Cryopreserved PBMCS were thawed and stimulated with SARS COV-2 derived peptide pools (Supplementary Table S1, available at *Rheumatology*

online). An Activation Induced Marker (AIM) Assay was used to identify total CD4+ and CD8+ T cells to spike and nucleocapsid and a Deep Phenotyping assay used to determine cytokine responses and memory T cells (Supplementary Table S2 and S3, available at *Rheumatology* online). Data analysis for flow cytometry was performed using Kaluza (V2.2) and further multi-dimensional clustering analysis (FlowSOM) was then utilised to characterise the major phenotype of cells. Further details are provided in the supplementary methods.

Statistical analysis

Antibody responses were compared between individuals with RAIRD and HC using Stata version 14. Differences between demographic and clinical characteristics and humoral immunogenicity were tested for significance using the chi-squared test. For outcome variables with low frequencies (<5), we used Fisher's exact test. All other outcome variables were incorporated into the multivariable logistic regression analysis to determine the influence of RAIRD on the magnitude of response to the second dose of the vaccine. It has previously been suggested that age, sex, and rituximab can influence antibody levels[18,23,24] and hence we adjusted for these as a priori confounders during the analysis. A 5% α level was used to determine significance level. Only patients with complete outcome data were included in the models. Missing data were assumed as missing at random and no imputations were performed.

Study outcomes

The primary outcomes were the antibody and T cell responses to two doses of SARS-CoV-2 vaccination. Secondary outcomes included a comprehensive analysis of T cell activation, cytokine production and generation of memory T cells.

Ethical approval

The study was approved by the West Midlands - Black Country Research Ethics Committee (REC reference: 21/WM/0097). The controls were obtained from a related study (REC reference: 21/NW/0048).

Results

Patient characteristics

The demographics and clinical characteristics of the patients with RAIRD (n = 50) and HC (n = 32) are shown in Table 1. The median age of the RAIRD cohort was 53 (IQR 42 - 61). The majority were female (n=35, 70%) and White Caucasian (n=45, 90%). The HC group also comprised of predominantly females (n=23, 72%) and White Caucasians (n=25, 78%) and had a median age of 51 (IQR 42 – 62). The most common RAIRD was ANCA-associated vasculitis (n=31, 62%), followed by systemic lupus erythematosus (n=9, 18%), myositis (n=6, 12%) and other systemic vasculitis (n=4, 8%). 17 patients were taking glucocorticoids daily of which seven (14%) were on high doses (≥10mg per day of prednisolone equivalent). 22 (44%) patients had rituximab in the 12 months prior to the first vaccination and 40 (80%) patients had a prior history of rituximab. One of these patients was taking a different anti-CD20 drug due to rituximab allergy. 10 (20%) were currently taking immunosuppressive medications other than steroids and rituximab. Five (6%) patients had hypogammaglobulinemia (IgG <5.3g/L) and four of these patients had recently received immunoglobulin replacement therapy, thus, their data was excluded from the antibody analysis. Half of the RAIRD cohort

received the Pfizer-BioNTech vaccine and the other half received the Oxford-AstraZeneca vaccine.

Antibody responses

The median anti-spike IgG antibody response was significantly lower in RAIRD (median=34; IQR 3-687) compared to HC (median=1453; IQR 733-3405) (χ^2 = 21.2, p<0.001). Furthermore, 15 (33%) RAIRD patients had undetectable antibodies (Supplementary Table S4, available at *Rheumatology* online) and only 20 (43%) patients had a sufficient antibody response (IgG higher than the lowest HC) (Figure 1a). Both RAIRD and HC groups had virtually undetectable anti-nucleocapsid IgG responses (Figure 1b), which is consistent with any previous antibody response to infection no longer being detectable and the responses observed being due to vaccination only. In general, older adults, males, patients with myositis and those on immunosuppressive or steroid treatment were more likely to have insufficient antibody responses (IgG levels lower than the lowest HC) as shown in Table 2. Additionally, none of the patients who had rituximab in the 6 months prior to the first vaccine and only 3 (16%) who had rituximab in the last 12 months had a sufficient antibody response. In the univariate analyses we found a significant inverse correlation between sufficient humoral response (Table 2) and rituximab therapy in the 12 months prior to receiving the first dose of the SARS-COV-2 vaccination (p=0.003). There was also a strong association between the diagnosis (ANCA-associated vasculitis, other systemic vasculitis, myositis or SLE) and humoral response, however this did not reach significance level. In the multivariable analyses, rituximab in the last 12 months was associated with insufficient humoral response (OR 0.11, 95% CI 0.03 – 0.48; p=0.003). Age, gender and ethnicity did not have an influence on the humoral response which reached statistical significance.

T and B cell responses in anti-spike antibody high and antibody low RAIRD patients

There was no significant difference in either total T cells (Figure 2a), CD4+ T cells (Figure 2b) or CD8+ T cells (Figure 2c), but there was a significant difference in B cell percentages between the antibody low/no and high (p=0.0359; Figure 2d). Interestingly, two patients in the antibody high group had no detectable B cells at the time of sampling which is likely because both had treatment with rituximab after their second vaccination and before the blood sample was collected.

T cell responses to spike and nucleocapsid peptides in antibody high and antibody low/no RAIRD patients

In the antibody high group there were significantly more spike specific CD4+ T cells than in the antibody low/no group (p=0.0217) (Figure 3ai). There were no detectable nucleocapsid-specific CD4+ T cells in either group consistent with no residual response to any prior infection with SARS-CoV2 if exposed at all (Figure 3aii). In addition, there were no significant differences in total cytokine specific-CD4+ T cells to spike (Figure 3bi) between the antibody low/no and antibody high groups and no nucleocapsid-specific cytokine secreting CD4+ T cells (Figure 3bii). Analysis of cytokine patterns in spike-specific CD4+ T cells showed this was mainly IFN-γ for both the antibody low/no and antibody high group, with some cells also producing TNF-α in combination with IFN-γ in the low/no group and TNF-α alone in the antibody high group (Figure 3ci). There was no detectable cytokine production by CD4+ T cells in response to nucleocapsid (Figure 3cii).

We then examined CD8+ T cell responses and found no significant differences between antibody high and antibody low/no for spike specific CD8+ T cells (Figures 3di and 3ei). Furthermore, there were very minimal CD8+ T cell responses to nucleocapsid (Figures 3dii and 3eii). Analysis of cytokine patterns by spike-specific CD8+ T cells highlighted cytokine production in the antibody low/no group was primarily IL-2, whereas IFN-γ was the predominant cytokine in the antibody high group (Figure 3fi). There was minimal cytokine production in response to nucleocapsid (Figure 3fii).

The multi-dimensional clustering analysis using t-SNE showed a total of 27 clusters with different expression markers (Figure 4a and 4b). Although not statistically significant, there was a higher percentage of Clusters 9 and 12 in the low/no antibody group compared to the antibody high group (Figures 4c, 4d and 4f). These clusters are associated with two terminally differentiated populations of T cells: effector memory 3 (EM3) and effector TEMRA (Figure 4e).

Discussion

Our study highlights several important findings about immunological effects of COVID-19 vaccination in patients with RAIRD. We found that that antibody responses were completely undetectable in 33% of RAIRD patients and insufficient in a further 24% of RAIRD patients after two doses of SARS-CoV-2 vaccines compared to HC. Additionally, there were no statistically significant differences in antibody response between different types of RAIRD. However, we had small numbers and it is notable that none of the six people with myositis had a sufficient antibody response (IgG levels above that of the lowest healthy control).

Interestingly, five of these six (83%) individuals had a prior history of rituximab treatment so we hypothesise that this may have in part contributed to their diminished response.

Corticosteroids are commonly used in the treatment of RAIRD particularly during relapses. Our results suggested that concurrent use of corticosteroids does not significantly affect humoral response in RAIRD. However, it is important to highlight that only seven RAIRD patients were on ≥10mg steroids/day so our sample may have been too small to make a firm conclusion. Previous studies have revealed conflicting evidence about the role of corticosteroids in immunogenicity. Observational studies have identified a decrease in serological response to pneumococcal and hepatitis vaccines with long-term steroid use[25,26]. The effect of corticosteroids on COVID-19 vaccines has not been thoroughly investigated. A recent study suggested that short-term use of low-dose steroids may not hinder antibody responses to COVID-19 vaccination[27]. However, this study was restricted to healthcare workers who did not have any significant comorbidities.

With the data from our cohort, we were able to demonstrate that antibody responses were significantly lower in RAIRD patients compared to HC. This is similar to a recent Dutch study on patients with immune-mediated inflammatory disorders on concurrent immunosuppression. The authors identified that patients receiving rituximab, mycophenolate mofetil combination treatments and sphingosine 1-phosphate receptor modulators had lower rates of seroconversion following the second vaccine. These rates did improve after the third vaccine for all groups except rituximab[28]. Our results also showed that antibody response was diminished in patients receiving rituximab (anti-CD20), and the interval between the administration of rituximab and vaccination was critical in predicting response.

We found that all patients who received rituximab in the last 6 months prior to the first dose had insufficient antibody responses, and 89% of those who had rituximab in the 12 months prior to first vaccination had insufficient antibody responses. These findings correlate with previous studies which also demonstrate the negative impact of B-cell depleting therapies on response to vaccines[7,11,24,29–32]. Conversely, some studies have shown that even individuals with low numbers of B cells secondary to rituximab treatment were able to mount a significant antibody response to COVID-19 vaccination provided T-cell mediated immunity was intact[33,34]. Recent literature has suggested that the optimal timing for vaccination in rituximab-treated patients should be at least 9 months after the last infusion to maximise response[33,35]. However, our study shows that even patients who had been treated with rituximab in the 12 months prior to first vaccination did not mount a sufficient response.

Cellular immune responses are essential in providing long lasting immunity and underpin vaccine efficacy. Most current vaccines rely on the delivery of spike protein and as a consequence the generation of spike-specific T cell response in order to maintain immune memory after antibodies have waned[36]. Previous studies have shown that in healthy individuals two doses of vaccination is sufficient to generate a similar T cell response to those after natural infection[37,38]. However, our study importantly revealed that RAIRD patients with low/no antibody response had significantly fewer spike specific CD4+ T cells which are essential in coordinating and regulating antiviral immunity. Our results are in line with a recent study in kidney transplant recipients on immunosuppression which also found a weak T-cell response and positive SARS-CoV-2 antibodies in only 5-10% of patients following the first and second vaccine doses[39,40]. However, this serological response improved to 36% after administration of the third dose[41]. This augmented response suggests that repeated

booster strategies could provide more long-term immunity in ICPs and warrants further research.

Our study also brings to light to new findings about the function of memory T cells in people with RAIRD. We observed the importance of an IFN-γ predominant CD8+ T cell response in RAIRD patients with high antibodies in coordinating the adaptive immune system. We also noted that this response was lower and predominantly IL-2 related in patients with low/no antibodies. This suggests that whilst CD8+ T cells may be activated, the main effector cytokines for sustaining the antiviral response are not produced in RAIRD patients in the absence of antibodies. We also observed increased levels of clusters 9 and 12 encoding for EM3 ($CD27^{-}CD28^{-}$) and effector TEMRA cells in RAIRD patients with low/no antibodies. These cells lack expression of CD27 and CD28 suggesting immunosenescence and incompetence to vaccination [42,43] and therefore increased susceptibility and greater probability of more severe SARS-CoV-2 infection.

The strengths of this study include: the broad inclusion criteria, patients with a variety of RAIRD diagnoses and the use of age-matched HC which increases the generalisability of our findings. In addition, we evaluated both the humoral and cellular response to two doses of SARS-CoV-2 vaccines. The limitations of this study include a small sample size and demographic differences between the RAIRD and control groups, some of which were adjusted for during our analysis. Furthermore, 14 patients were not able to have a blood sample taken four weeks post-vaccination and in these cases, we were only able to analyse their 3-month post-vaccination sample (however in patients with both samples, we found no significant differences in the titres of antibodies). Additionally, some of the outcome variables

had low frequencies and hence we could not adjust for these as potential confounders in our multivariate analysis.

In summary, we identified that patients with RAIRD have significantly diminished antibody and T cell responses following two doses of SARS-CoV-2 vaccines. Receipt of rituximab in the last 12 months was associated with a reduced humoral response so, where possible, vaccination should precede treatment with rituximab, as per clinical guidance. This also justifies the need for the additional booster vaccine doses in line with national guidelines[44] and emphasises the importance of assessing B and T cell responses in ICPs. We also recommend that for individuals requiring maintenance rituximab, shared decision making and risk assessments should be conducted by clinicians to review the timing of rituximab for any future booster doses. It also raises questions about whether additional prophylactic measures such as antivirals may be required in addition to booster vaccine doses in individuals who do not mount a sufficient antibody response. Notably, some patients with low/no antibody response also have poor memory T cells which lack both proliferative and functional capacities and so future research is important to determine the long-term immune response to additional vaccine doses.

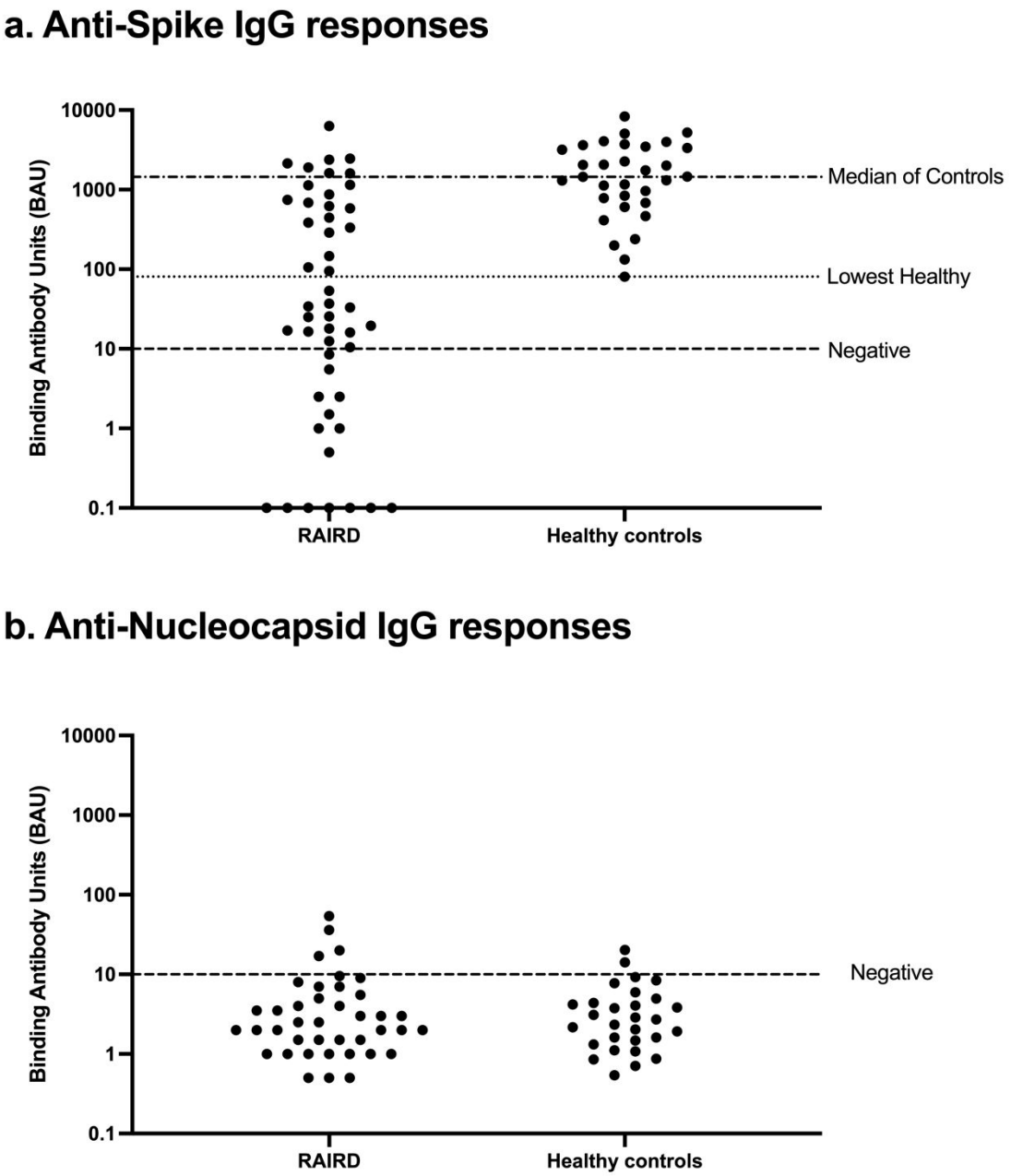
Figures and Tables

Table 1. Demographic and clinical characteristics of patients with rare autoimmune rheumatic diseases and healthy age-matched controls

	RAIRD (%) n=50	Controls (%) n=32
Age		
Median age (range)	53 (22 – 81)	53 (22 – 79)
18-49	20 (40.0)	14 (43.8)
50-64	19 (38.0)	14 (43.8)
≥65	11 (22.0)	4 (12.5)
Gender		
Female	35 (70.0)	23 (71.9)
Male	15 (30.0)	9 (28.1)
Ethnicity		
White	45 (90.0)	25 (78.1)
Non-white	5 (10.0)	7 (21.9)
Diagnosis		
ANCA-associated vasculitis	31 (62.0)	
SLE	9 (18.0)	
Other systemic vasculitis	4 (8.0)	
Myositis	6 (12.0)	
Current immunosuppression		
Methotrexate	4 (8.0)	
Mycophenolate	4 (8.0)	
Belimumab	2 (4.0)	
Previous rituximab	40 (80.0)	
≤6 months	17 (34.0)	
≤12 months	22 (44.0)	
Current glucocorticoids		
≥10mg per day	7 (14.0)	
<10mg per day	10 (20.0)	
No steroids	33 (66.0)	
Hypogammaglobulinemia	5 (6.0)	
Recent immunoglobulin therapy*	4 (8.0)	
Vaccine type		
Oxford-AstraZeneca	25 (50.0)	8 (23.5)
Pfizer-BioNTech	25 (50.0)	26 (76.5)

*Excluded from analysis on antibody response to vaccination

Figure 1. IgG responses to SARS-CoV-2 between RAIRD (vasculitis) patients and healthy controls.



(A) Anti-spike IgG antibody responses in RAIRD (vasculitis) patients compared to healthy controls. The dashed line (negative) represents the cut-off for the assay, the dotted line (lowest healthy) shows the binding antibody units of the lowest healthy control, and the semi-dashed line represents the median of the health controls. (B) Anti-nucleocapsid IgG responses

were mainly below the limit of detection of the assay in both RAIRD patients and healthy controls. The dashed line (negative) represents the cut-off for the assay.

Table 2. Univariate and multivariate logistic regression analyses of sufficient antibody response following the second dose of vaccine in RAIRD patients†

Variables	**Sufficient antibody response† (%)** n=20	**Insufficient antibody response (%)** n=26	**Univariate analyses** OR (95% CI) or Two-sided P value	**Multivariate analyses** OR (95% CI)	P value
Age (for each additional year)			0.99 (0.95 – 1.03)	1.00 (0.95 – 1.04)	0.833
18-49	8 (50.0)	8 (50.0)			
50-64	8 (42.1)	11 (57.9)			
≥65	4 (36.4)	7 (63.6)			
Gender					
Female	15 (46.7)	17 (53.1)	1 (reference)	1 (reference)	
Male	5 (35.7)	9 (64.3)	0.63 (0.17 –2.30)	0.58 (0.14 – 2.52)	0.471
Ethnicity					0.369■
White	19 (46.3)	22 (53.7)			
Non-white	1 (20.0)	4 (80.0)			
Diagnosis					0.084■
ANCA-associated vasculitis	13 (46.4)	15 (53.6)			
SLE	4 (50.0)	4 (50.0)			
Other systemic vasculitis	3 (75.0)	1 (25.0)			
Myositis	0	6 (100.0)			
Current immunosuppression					
Yes	5 (38.5)	8 (61.5)	0.75 (0.20 – 2.78)		
No	15 (45.5)	18 (54.6)	1 (reference)		
Current glucocorticoids					
Yes	7 (41.2)	10 (58.8)	0.86 (0.26 – 2.90)		
No	13 (44.8)	16 (55.2)	1 (reference)		
Previous rituximab					
≤6 months					
Yes	0	15 (100.0)	Omitted		
No	20 (64.5)	11 (35.5)	1 (reference)		
≤12 months					
Yes	3 (15.8)	16 (84.2)	0.11 (0.03 – 0.47)*	0.11 (0.03 – 0.48)	0.003*
No	17 (63.0)	10 (37.0)	1 (reference)	1 (reference)	
Rituximab ever					
Yes	14 (37.8)	23 (62.2)	0.30 (0.65 – 1.42)		
No	6 (66.7)	3 (33.3)	1 (reference)		

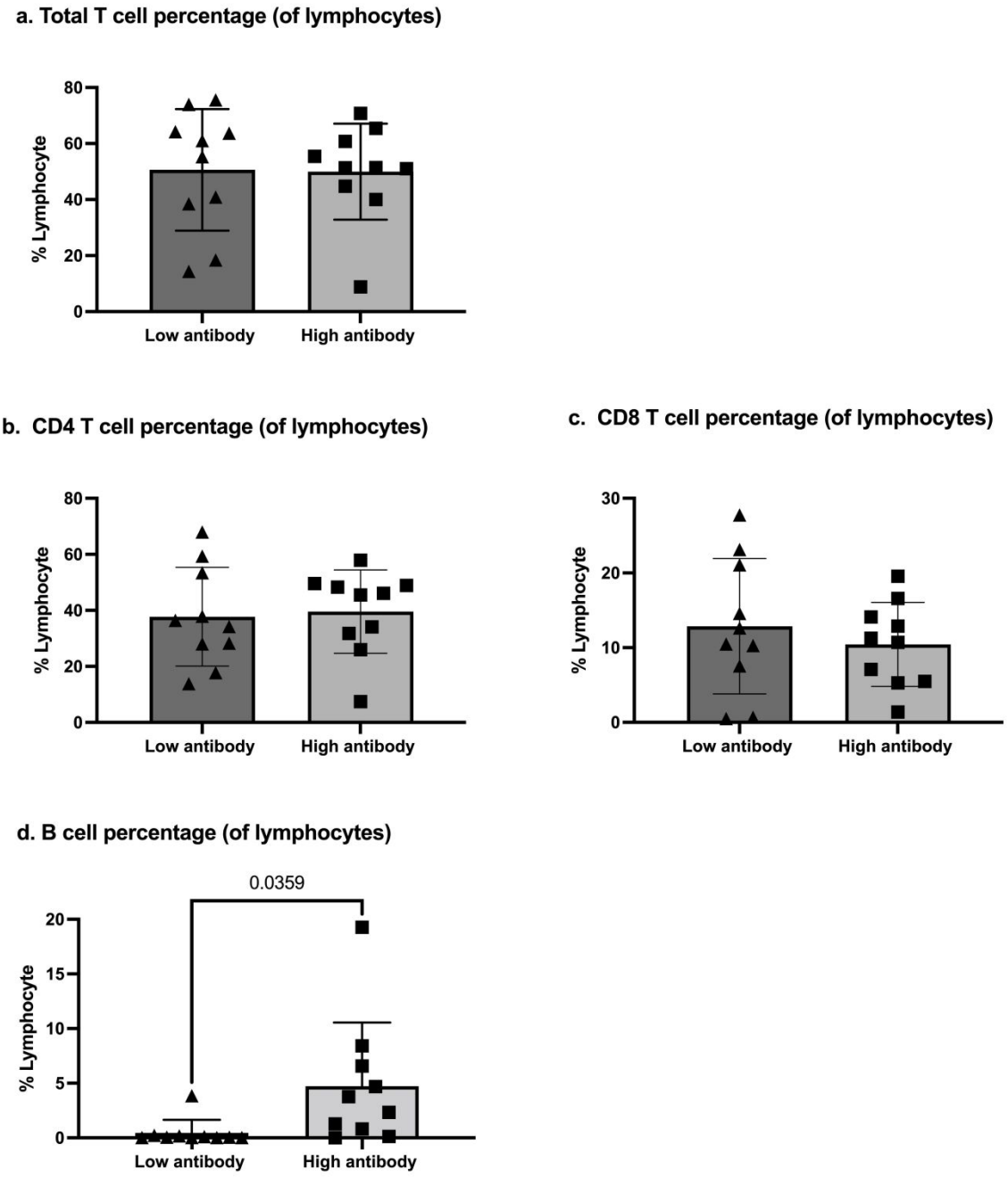
†An antibody response was defined as sufficient if IgG levels were above that of the lowest healthy control

‡Patients on immunoglobulin therapy were excluded from analysis on antibody response to vaccination

*Statistically significant p value

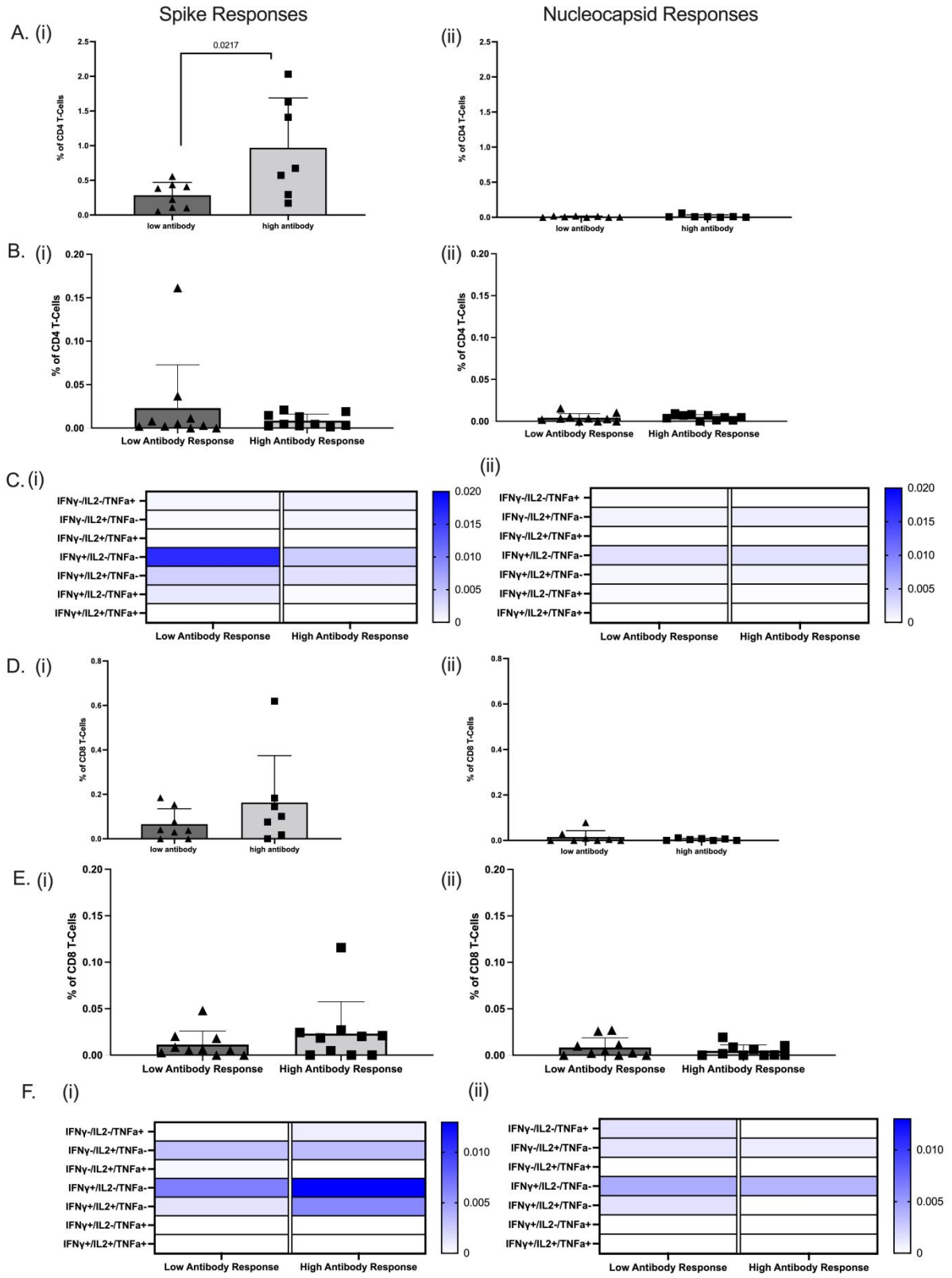
■P value obtained from two-sided Fisher's exact test

Figure 2. T and B cell responses in anti-spike antibody high and antibody low RAIRD patients.



(A) Total percentage of T lymphocytes in anti-spike antibody high and antibody low/no RAIRD patients. (B) CD4 T cell percentage and (C) CD8 T cell percentages of lymphocytes in anti-spike antibody high and antibody low/no RAIRD patients. (D) B cell percentage of lymphocytes was significantly lower in anti-spike antibody low/no RAIRD patients compared to anti-spike antibody high RAIRD patients.

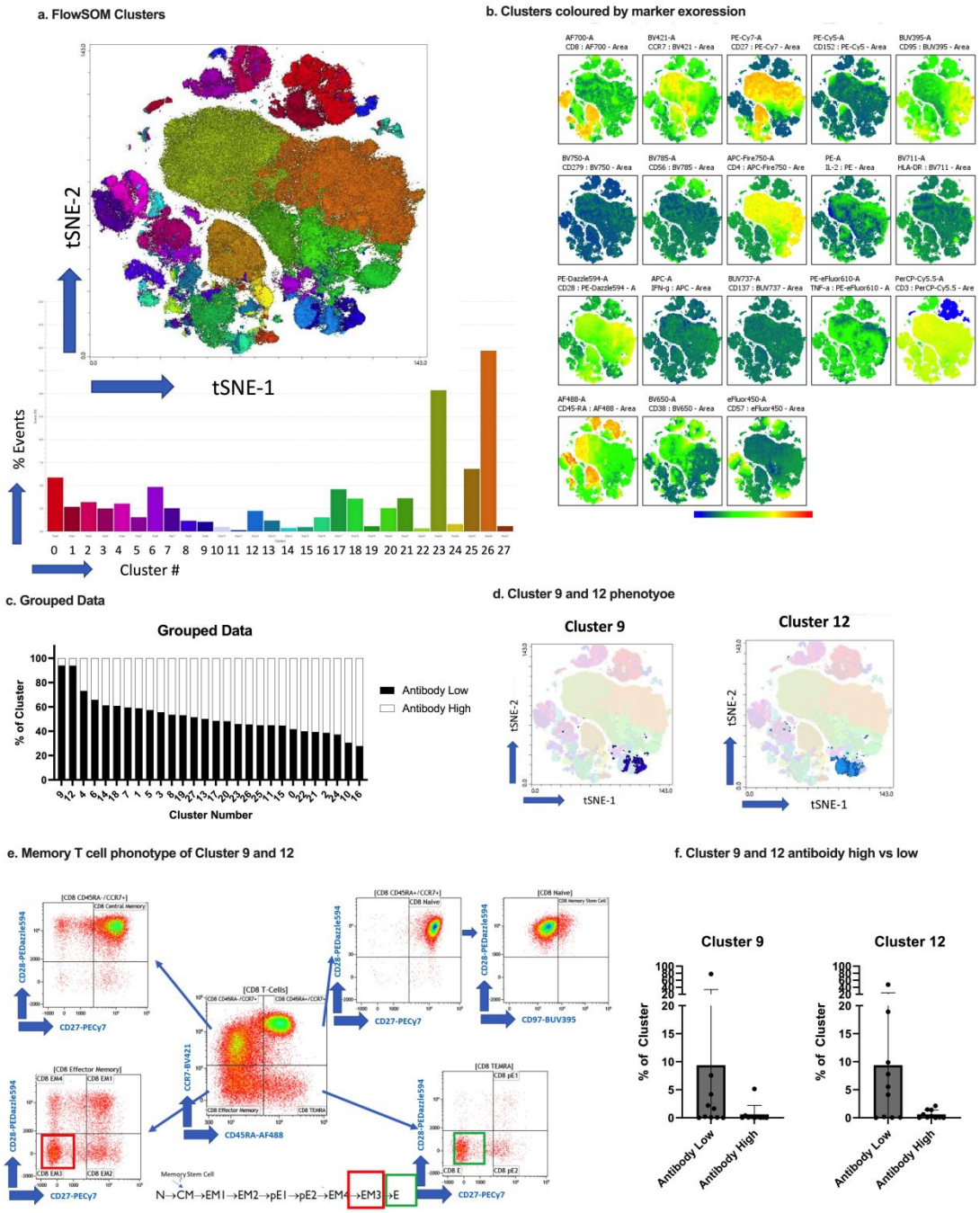
Figure 3. CD4+ and CD8+ T cell responses to SARS-CoV-2 peptides in antibody high and antibody low/no RAIRD patients.



(A) Spike-specific CD4+ T cells was significantly higher in the antibody high patients and there

were no detectable nucleocapsid-specific CD4+ T cells. (B) No difference in total cytokine-positive spike-specific CD4+ T cells. (C) Cytokine patterns expressed by spike and nucleocapsid-specific CD4+ T cells. (D) Higher spike-specific CD8+ T cells in antibody high patients. (E) No difference in total cytokine-positive spike-specific CD8+ T cells. (F) Cytokine patterns expressed by spike and nucleocapsid-specific CD8+ T cells.

Figure 4. Multidimensional clustering analysis.



(A) FlowSOM identified 27 clusters of major cell types from both antibody high and antibody low/no groups which are colour-coded and displayed on a 2-dimensional t-SNE plot. (B) t-SNE visualisation colored according to marker expression. (C-E) Clusters 9 and 12 were identified in the antibody low/no group and were associated with two terminally differentiated populations of T cells: effector memory 3 (EM3) and effector TEMRA. (F) No significant

differences for clusters 9 and 12 were observed between the antibody low/no and antibody high group.

Funding

This study was funded by Vasculitis UK. MR, Versus Arthritis Clinical Research Fellow (Versus Arthritis 22727) is funded by Versus Arthritis. FAP, NIHR advanced fellow (NIHR300863) is funded by the NIHR for this research project. The views expressed in this publication are those of the author(s) and not necessarily those of the NIHR, NHS or the UK Department of Health and Social Care.

Competing interests

FAP and PCL are recipients of an investigator-led research award from Vifor pharma for another project unrelated to COVID-19 or vaccination.

Data availability

Due to the nature of the research and ethical restrictions, the data are not publicly available. Please contact the corresponding author should you wish to access the data.

References

1 Polack FP, Thomas SJ, Kitchin N, *et al.* Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine. *N Engl J Med* 2020;**383**:2603–15. doi:10.1056/NEJMoa2034577

2 Baden LR, El Sahly HM, Essink B, *et al.* Efficacy and Safety of the mRNA-1273 SARS-CoV-2 Vaccine. *N Engl J Med* 2021;**384**:403–16. doi:10.1056/NEJMoa2035389

3 Pablos JL, Galindo M, Carmona L, *et al.* Clinical outcomes of hospitalised patients with COVID-19 and chronic inflammatory and autoimmune rheumatic diseases: a multicentric matched cohort study. *Ann Rheum Dis* 2020;**79**:1544–9. doi:10.1136/annrheumdis-2020-218296

4 Akiyama S, Hamdeh S, Micic D, *et al.* Prevalence and clinical outcomes of COVID-19 in patients with autoimmune diseases: a systematic review and meta-analysis. *Ann Rheum Dis* 2021;**80**:384–91. doi:10.1136/annrheumdis-2020-218946

5 Peach E, Rutter M, Lanyon P, *et al.* Risk of death among people with rare autoimmune diseases compared with the general population in England during the 2020 COVID-19 pandemic. *Rheumatology* 2021;**60**:1902–9. doi:10.1093/rheumatology/keaa855

6 Freites Nuñez DD, Leon L, Mucientes A, *et al.* Risk factors for hospital admissions related to COVID-19 in patients with autoimmune inflammatory rheumatic diseases. *Ann Rheum Dis* 2020;**79**:1393. doi:10.1136/annrheumdis-2020-217984

7 Furer V, Eviatar T, Zisman 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. doi:10.1136/annrheumdis-2021-220647

8 Fagni F, Simon D, Tascilar K, *et al.* COVID-19 and immune-mediated inflammatory diseases: effect of disease and treatment on COVID-19 outcomes and vaccine responses. *Lancet Rheumatol* 2021;**3**:e724–36. doi:10.1016/S2665-9913(21)00247-2

9 Strangfeld A, Schäfer M, Gianfrancesco MA, *et al.* Factors associated with COVID-19-related death in people with rheumatic diseases: results from the COVID-19 Global Rheumatology Alliance physician-reported registry. *Ann Rheum Dis* 2021;**80**:930–42. doi:10.1136/annrheumdis-2020-219498

10 Felten R, Duret P-M, Bauer E, *et al.* B-cell targeted therapy is associated with severe COVID-19 among patients with inflammatory arthritides: a 1-year multicentre study in 1116 successive patients receiving intravenous biologics. *Ann Rheum Dis* 2022;**81**:143–5. doi:10.1136/annrheumdis-2021-220549

11 Mehta P, Porter JC, Chambers RC, *et al.* B-cell depletion with rituximab in the COVID-19 pandemic: where do we stand? *Lancet Rheumatol* 2020;**2**:e589–90. doi:10.1016/S2665-9913(20)30270-8

12 Schulze-Koops H, Krueger K, Vallbracht I, *et al.* Increased risk for severe COVID-19 in patients with inflammatory rheumatic diseases treated with rituximab. *Ann Rheum Dis* 2021;**80**:e67. doi:10.1136/annrheumdis-2020-218075

13 Shields AM, Venkatachalam S, Paneesha S, *et al.* Vaccine Efficacy after Rituximab Exposure: First Interim Analysis of Virtue Project on Behalf of West Midlands Research Consortium, UK. *Blood* 2021;**138**:196–196. doi:10.1182/blood-2021-150220

14 Prendecki M, Clarke C, Edwards H, *et al.* Humoral and T-cell responses to SARS-CoV-2 vaccination in patients receiving immunosuppression. *Ann Rheum Dis* 2021;**80**:1322–9. doi:10.1136/annrheumdis-2021-220626

15 Moor MB, Suter-Riniker F, Horn MP, *et al.* Humoral and cellular responses to mRNA vaccines against SARS-CoV-2 in patients with a history of CD20 B-cell-depleting therapy (RituxiVac): an investigator-initiated, single-centre, open-label study. *Lancet Rheumatol* 2021;**3**:e789–97. doi:10.1016/S2665-9913(21)00251-4

16 Stefanski A-L, Rincon-Arevalo H, Schrezenmeier E, *et al.* B Cell Numbers Predict Humoral and Cellular Response Upon SARS-CoV-2 Vaccination Among Patients Treated With Rituximab. *Arthritis Rheumatol Hoboken NJ* 2022;**74**:934–47. doi:10.1002/art.42060

17 Braun-Moscovici Y, Kaplan M, Braun M, *et al.* Disease activity and humoral response in patients with inflammatory rheumatic diseases after two doses of the Pfizer mRNA vaccine against SARS-CoV-2. *Ann Rheum Dis* 2021;**80**:1317. doi:10.1136/annrheumdis-2021-220503

18 Simon D, Tascilar K, Schmidt K, *et al.* Humoral and Cellular Immune Responses to SARS–CoV-2 Infection and Vaccination in Autoimmune Disease Patients With B Cell Depletion. *Arthritis Rheumatol* 2022;**74**:33–7. doi:10.1002/art.41914

19 Geisen UM, Berner DK, Tran F, *et al.* Immunogenicity and safety of anti-SARS-CoV-2 mRNA vaccines in patients with chronic inflammatory conditions and immunosuppressive therapy in a monocentric cohort. *Ann Rheum Dis* 2021;**80**:1306–11. doi:10.1136/annrheumdis-2021-220272

20 Szebeni GJ, Gémes N, Honfi D, *et al.* Humoral and Cellular Immunogenicity and Safety of Five Different SARS-CoV-2 Vaccines in Patients With Autoimmune Rheumatic and Musculoskeletal Diseases in Remission or With Low Disease Activity and in Healthy Controls: A Single Center Study. *Front Immunol* 2022;**13**:846248. doi:10.3389/fimmu.2022.846248

21 Tungland B. Role of Gut Microbiota in Immune Homeostasis. In: *Human Microbiota in Health and Disease*. Elsevier 2018. 135–54. doi:10.1016/B978-0-12-814649-1.00004-1

22 Vedhara K. Do Behaviours & Mood Affect How Well COVID19 vaccines work? [COVID-19]. Health Res. Auth. https://www.hra.nhs.uk/planning-and-improving-research/application-summaries/research-summaries/do-behaviours-mood-affect-how-well-covid19-vaccines-work-covid-19/ (accessed 10 May 2022).

23 Walsh EE, Frenck RW, Falsey AR, *et al.* Safety and Immunogenicity of Two RNA-Based Covid-19 Vaccine Candidates. *N Engl J Med* 2020;**383**:2439–50. doi:10.1056/NEJMoa2027906

24 Bingham CO, Looney RJ, Deodhar A, *et al.* Immunization responses in rheumatoid arthritis patients treated with rituximab: Results from a controlled clinical trial. *Arthritis Rheum* 2010;**62**:64–74. doi:10.1002/art.25034

25 Yıldız N, Sever L, Kasapçopur Ö, *et al.* Hepatitis B virus vaccination in children with steroid sensitive nephrotic syndrome: Immunogenicity and safety? *Vaccine* 2013;**31**:3309–12. doi:10.1016/j.vaccine.2013.05.004

26 Fischer L, Gerstel PF, Poncet A, *et al.* Pneumococcal polysaccharide vaccination in adults undergoing immunosuppressive treatment for inflammatory diseases – a longitudinal study. *Arthritis Res Ther* 2015;**17**:151. doi:10.1186/s13075-015-0663-9

27 Yang J, Ko J-H, Baek JY, *et al.* Effects of Short-Term Corticosteroid Use on Reactogenicity and Immunogenicity of the First Dose of ChAdOx1 nCoV-19 Vaccine. *Front Immunol* 2021;**12**:744206. doi:10.3389/fimmu.2021.744206

28 Wieske L, van Dam KPJ, Steenhuis M, *et al.* Humoral responses after second and third SARS-CoV-2 vaccination in patients with immune-mediated inflammatory disorders on immunosuppressants: a cohort study. *Lancet Rheumatol* 2022;**4**:e338–50. doi:10.1016/S2665-9913(22)00034-0

29 Hua C, Barnetche T, Combe B, *et al.* Effect of Methotrexate, Anti-Tumor Necrosis Factor α, and Rituximab on the Immune Response to Influenza and Pneumococcal Vaccines in Patients With Rheumatoid Arthritis: A Systematic Review and Meta-Analysis: Immune Response to Influenza and Pneumococcal Vaccines in RA. *Arthritis Care Res* 2014;**66**:1016–26. doi:10.1002/acr.22246

30 Rondaan C, Furer V, Heijstek MW, *et al.* Efficacy, immunogenicity and safety of vaccination in adult patients with autoimmune inflammatory rheumatic diseases: a systematic literature review for the 2019 update of EULAR recommendations. *RMD Open* 2019;**5**:e001035. doi:10.1136/rmdopen-2019-001035

31 van Assen S, Holvast A, Benne CA, *et al.* Humoral responses after influenza vaccination are severely reduced in patients with rheumatoid arthritis treated with rituximab. *Arthritis Rheum* 2010;**62**:75–81. doi:10.1002/art.25033

32 Kearns P, Siebert S, willicombe michelle, *et al.* Examining the Immunological Effects of COVID-19 Vaccination in Patients with Conditions Potentially Leading to Diminished Immune Response Capacity – The OCTAVE Trial. *SSRN Electron J* Published Online First: 2021. doi:10.2139/ssrn.3910058

33 Jyssum I, Kared H, Tran TT, *et al.* Humoral and cellular immune responses to two and three doses of SARS-CoV-2 vaccines in rituximab-treated patients with rheumatoid arthritis: a prospective, cohort study. *Lancet Rheumatol* 2022;**4**:e177–87. doi:10.1016/S2665-9913(21)00394-5

34 Mrak D, Tobudic S, Koblischke M, *et al.* SARS-CoV-2 vaccination in rituximab-treated patients: B cells promote humoral immune responses in the presence of T-cell-mediated immunity. *Ann Rheum Dis* 2021;**80**:1345. doi:10.1136/annrheumdis-2021-220781

35 Bitoun S, Henry J, Desjardins D, *et al.* Rituximab Impairs B Cell Response But Not T Cell Response to COVID -19 Vaccine in Autoimmune Diseases. *Arthritis Rheumatol* 2022;:art.42058. doi:10.1002/art.42058

36 Tregoning JS, Flight KE, Higham SL, *et al.* Progress of the COVID-19 vaccine effort: viruses, vaccines and variants versus efficacy, effectiveness and escape. *Nat Rev Immunol* 2021;**21**:626–36. doi:10.1038/s41577-021-00592-1

37 Skelly DT, Harding AC, Gilbert-Jaramillo J, *et al.* Two doses of SARS-CoV-2 vaccination induce robust immune responses to emerging SARS-CoV-2 variants of concern. *Nat Commun* 2021;**12**:5061. doi:10.1038/s41467-021-25167-5

38 Moss P. The T cell immune response against SARS-CoV-2. *Nat Immunol* 2022;**23**:186–93. doi:10.1038/s41590-021-01122-w

39 Benotmane I, Gautier-Vargas G, Cognard N, *et al.* Weak anti-SARS-CoV-2 antibody response after the first injection of an mRNA COVID-19 vaccine in kidney transplant recipients. *Kidney Int* 2021;**99**:1487–9. doi:10.1016/j.kint.2021.03.014

40 Chavarot N, Ouedrani A, Marion O, *et al.* Poor Anti-SARS-CoV-2 Humoral and T-cell Responses After 2 Injections of mRNA Vaccine in Kidney Transplant Recipients Treated With Belatacept. *Transplantation* 2021;**105**:e94–5. doi:10.1097/TP.0000000000003784

41 Schrezenmeier E, Rincon-Arevalo H, Stefanski A-L, *et al.* B and T Cell Responses after a Third Dose of SARS-CoV-2 Vaccine in Kidney Transplant Recipients. *J Am Soc Nephrol* 2021;**32**:3027–33. doi:10.1681/ASN.2021070966

42 Romero P, Zippelius A, Kurth I, *et al.* Four Functionally Distinct Populations of Human Effector-Memory CD8 $^{+}$ T Lymphocytes. *J Immunol* 2007;**178**:4112–9. doi:10.4049/jimmunol.178.7.4112

43 Mojumdar K, Vajpayee M, Chauhan NK, *et al.* Altered T cell differentiation associated with loss of CD27 and CD28 in HIV infected Indian individuals. *Cytometry B Clin Cytom* 2012;**82B**:43–53. doi:10.1002/cyto.b.20610

44 Department of Health and Social Care. Joint Committee on Vaccination and Immunisation (JCVI) advice on third primary dose vaccination. GOV.UK. 2021.https://www.gov.uk/government/publications/third-primary-covid-19-vaccine-dose-for-people-who-are-immunosuppressed-jcvi-advice/joint-committee-on-vaccination-and-immunisation-jcvi-advice-on-third-primary-dose-vaccination (accessed 4 May 2022).