EPIDEMIOLOGICAL SCIENCE

SARS-CoV-2 Omicron escapes mRNA vaccine booster-induced antibody neutralisation in patients with autoimmune rheumatic diseases: an observational cohort study

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ABSTRACT

Objectives This study investigates whether COVID-19 vaccines can elicit cross-reactive antibody responses against the Omicron variant in patients with autoimmune rheumatic diseases (ARDs).

Methods This observational cohort study comprised 149 patients with ARDs and 94 healthcare workers (HCWs). Blood samples were obtained at enrolment, a median of 15 weeks after the second vaccine dose or 8 weeks after the third dose. The functional cross-neutralisation capacity of sera was measured using the Omicron variant receptor-binding domain-ACE2 binding inhibition assay. We assessed the incidence of breakthrough infections and the potential correlation with neutralising responses in participants after receiving third doses. The association of time-from-vaccine and neutralising responses in sera was predicted using linear regression analysis.

Results The mean cross-neutralising responses against the Omicron variant developed after the second dose was 11.5% in patients with ARDs and 18.1% in HCWs (p=0.007). These responses were significantly lower in patients with ARDs than in HCWs after the third dose (26.8% vs 50.3%, p<0.0001). Only 39.2% of the patient sera showed functional neutralisation capacity to the Omicron variant and cross-neutralising responses were shown to be poorly correlated with anti-spike immunoglobulin G titres. Within 6 weeks of immunological assessments, significantly lower Omicron-neutralising responses were detected in sera from patients with ARDs who developed breakthrough infections compared with those who did not (p=0.018). Additionally, a relative decline was implied in neutralising responses against the Omicron variant as a reference to the wild-type virus during 120 days since the third vaccination, with a predicted decay rate of −0.351%/day (95% CI, −0.559 to −0.144, p=0.001).

Conclusions Striking antibody evasion manifested by the Omicron variant in patients with ARDs and current vaccine-induced immunity may not confer broad protection from Omicron breakthrough infection, highlighting the need for further research on vaccine effectiveness in patients with immune dysfunctions.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Antibody neutralisation of the Omicron variant of SARS-CoV-2 was potently induced by the third dose of an mRNA vaccine in the general population. However, real-world data evaluating the impact of the SARS-CoV-2 Omicron variant on vaccine-induced immunity in patients with autoimmune rheumatic diseases are sparse.

WHAT THIS STUDY ADDS

⇒ This study shows that, while the third dose of an mRNA vaccine is immunogenic in patients with autoimmune rheumatic diseases, at least half of the patients with measurable neutralising responses against the wild-type virus failed to generate cross-neutralising responses against the Omicron variant. Further, sera from vaccinated patients with confirmed breakthrough infections showed lower cross-neutralising responses, suggesting a significant correlation between the functional cross-variant neutralisation capacity and protection from breakthrough infection.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Given the limited correlation between cross-neutralising responses against the Omicron variant and the ancestral anti-spike immunoglobulin G titres elicited by the third dose of an mRNA vaccine in patients with autoimmune rheumatic diseases, quantifying the functional cross-variant neutralisation capacity may be a precise approach for determining the immunological benefit conferred to them by booster immunisations.

INTRODUCTION

SARS-CoV-2—the aetiological agent of COVID-19—has caused substantial morbidity and mortality in patients with autoimmune rheumatic diseases (ARDs).[1 2] Rapid development of successful vaccines has enabled their widespread administration.[3] Nevertheless, some patients with ARDs reportedly have higher breakthrough infection rates.[4] Given the absence of a definitive immune correlates indicating the clinical benefits of COVID-19 vaccines, neutralising antibody titres remain highly predictive of protection from symptomatic SARS-CoV-2 infection.[5] After the initial

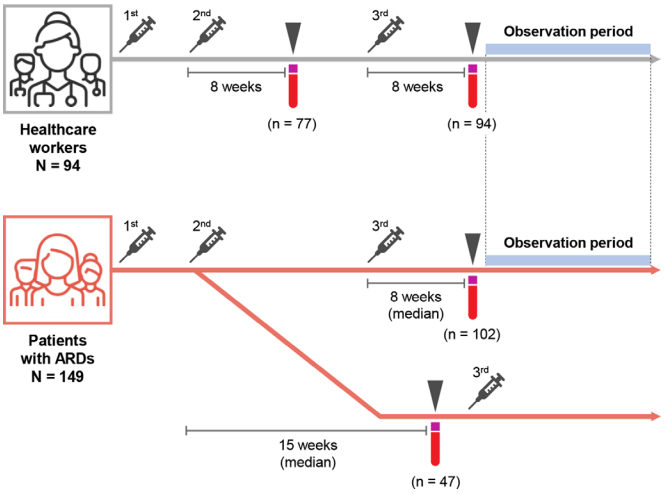

Figure 1 Schematic representation of the study flow diagram. SARS-CoV-2 spike-specific antibody concentrations and neutralisation responses against the wild-type virus and the Omicron variants were measured in serum samples from vaccinated healthcare workers and patients with autoimmune rheumatic diseases (ARDs). Grey triangles indicate the timing of sample collections for immunological assessments, and the blue shading illustrates the observation period for tracking breakthrough cases. The numbers in the brackets denote the number of participants in each group.

authorisation in Israel, many public health authorities stated that a third dose of the vaccine must be mandatory. This was under the presumption that recall responses led by booster doses increase the neutralising antibody responses and consequently induce protective immunity.[6–9] Unfortunately, patients with ARDs undergoing immunomodulatory therapies are excluded from COVID-19 vaccination trials, and there is limited data on immunogenicity of vaccines for the circulating SARS-CoV-2 variants of concern (VOCs).[10]

The highly mutated SARS-CoV-2 Omicron (B.1.1.529) variant has rapidly replaced the Delta strain and virtually all the circulating strains in the community.[11] Omicron's spike mutations are concentrated in the receptor-binding domain (RBD), which results in the variant escaping from vaccine-induced antibody neutralisation,[12–16] while vaccines elicit highly conserved cellular immunity between the Omicron and ancestral spikes.[17–20] To this end, a large-scale epidemiologic study suggested that the third dose of an mRNA vaccine provides exceptional protection from symptomatic SARS-CoV-2 infection, despite lesser protection against the Omicron variant.[21] In immunocompetent individuals, three consecutive exposures with spike antigen resulted in the maturation of antibody responses required to increase avidity, which may be critical for highly potent neutralisation for counteracting VOCs with immune evasion capabilities such as SARS-CoV-2 Omicron.[22–24] However, the susceptibility of the Omicron variant to vaccine-elicited neutralisation in patients with ARDs employing a myriad of immunomodulators remain unresolved.

The primary objective of this study was to provide a deeper understanding of the cross-neutralising antibody responses in patients with ARDs induced by third COVID-19 vaccine doses and whether the magnitude of neutralisation would be comparable to that observed in healthy recipients. To this end, we measured ancestral spike-specific binding antibody and neutralising antibody titres against the Omicron variant as well as the wild-type virus in a coordinated manner. The secondary objective was to determine the incidence of COVID-19 breakthrough infection and to further elucidate the relationship between the functional neutralisation capacity and the protection from COVID-19 in patients with ARDs.

METHODS

Study design

In January 2022, we initiated the study at the beginning of the unprecedented COVID-19 pandemic surge caused by the Omicron variant in Korea, which peaked on 16 March (online supplemental figure S1). Patients with ARDs (including systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), ankylosing spondylitis (AS), Behçet's disease (BD), adult-onset Still's disease (AOSD), antineutrophil cytoplasmic antibody-associated vasculitis, systemic sclerosis, IgG_4-related disease) were asked to participate in the study during their regular outpatient visits if they had received a second or third dose of COVID-19 vaccine at least 3 weeks prior. Individuals diagnosed with COVID-19 or those who had received anti-CD20 therapy or chemotherapy were excluded. Patients taking methotrexate, mycophenolate mofetil or Janus kinase inhibitors were instructed to withhold the drug for 1 week after the vaccination. Blood samples were collected at enrolment between 12 January 2022 and 11 March 2022, and the cohort was followed-up for the development of COVID-19 breakthrough infections until the study end date of 6 April.

Healthy control participants included in the study were voluntarily recruited from healthcare workers (HCWs) and were followed longitudinally to study the immune responses to COVID-19 vaccination. They were not treated with immunosuppressants for any indication. Samples for analysis in this study were assessed post hoc (after booster immunisation), and breakthrough cases were identified during the same observation period. All participants were aged 18 years or older and had been vaccinated with mRNA (BNT162b2 and mRNA1273) or viral vector (AZD1222 and Ad26.COV2.S) vaccines according to the approved schedules. All study participants provided written informed consent prior to enrolment.

Assessment of SARS-CoV-2 spike-specific IgG

We performed the Euroimmun (Lübeck, Germany) anti-SARS-CoV-2 ELISA intended for the detection of the ancestral anti-spike IgG antibodies in all serum samples obtained from patients with ARDs and HCWs (figure 1), as previously described.[25 26] The microplate wells were coated with recombinant S1 domain of SARS-CoV-2 spike protein and the results were evaluated by measuring optical density (OD) at 450 nm, with responses expressed as arbitrary units per millilitre (AU/mL). Antibody titres greater than 1.1 AU/mL were considered to be seropositive.

Examination of virus neutralisation response

We used the GenScript (Piscataway, New Jersey, USA) cPass surrogate virus neutralisation test to specifically detect neutralising antibodies, which was granted emergency use authorisation by the US Food and Drug Administration and has been applied in several published studies.[27–31] This test mimics the interaction between the virus and host cell by using the recombinant components of the RBD of the SARS-CoV-2 spike protein and human ACE2 receptors. Assays are typically ELISA-based, and the percentage neutralisation can be calculated as (1 − OD of sample/OD of negative control)×100. The test has been validated for high sensitivity and specificity (with a recommended positive threshold of 30%), and strongly correlated with the

plaque reduction neutralisation test and the focus reduction neutralisation test.[32 33] The test was modified to detect SARS-CoV-2 neutralising antibodies against the Omicron RBD by replacing the horseradish peroxidase-conjugated recombinant RBD fragment according to the manufacturer's specifications.

Assessment of SARS-CoV-2 spike-specific cellular responses

We determined SARS-CoV-2–specific T cell responses by measuring interferon-gamma (IFN-γ) production on stimulation with SARS-CoV-2 S1 peptide pool using the Euroimmun Interferon Gamma Release Assay (IGRA). The response was defined as IFN-γ concentration in peptide stimulated minus that in unstimulated, in international units per millilitre (IU/mL). IFN-γ responses above 200 mIU/mL were interpreted as positive, according to the manufacturer's recommendations. This test has been proven useful in identifying individuals with post-vaccination cellular immunity.[34 35] SARS-CoV-2 IGRA test was conducted in the first and second weeks and the sixth and seventh weeks during the sampling period.

Identification of breakthrough infections

South Korea has conducted rigorous and extensive epidemiological field investigations regarding COVID-19. This process includes active, population-based surveillance of COVID-19-like illnesses and case-based contact tracing regardless of the symptoms. All suspected cases are confirmed by a reverse transcriptase-PCR (RT-PCR) assay. As part of the Korean government's COVID-19 response, rapid antigen tests were conducted by medical personnel and symptomatic individuals who tested positive for the period starting on 14 March were considered COVID-19 cases. Semi-structured, in-depth telephonic interviews conducted on 6 and 7 April were used for the identification of breakthrough cases among patients with ARDs during the observation period in the study. In parallel, all HCWs with compatible symptoms or exposure to confirmed cases were tested for COVID-19 using an RT-PCR assay as per the hospital's infection control policies.

Statistical analysis

The demographics of the study participants are summarised as medians with IQRs for quantitative variables and were compared using the Mann-Whitney U test or as percentages for qualitative variables and compared using the χ^2 test or Fisher's exact test. For virus neutralisation responses, the inhibition percentages are displayed and were compared using paired or non-paired t-tests when appropriate. Differences in the proportion of participants were evaluated using the chi-square test or Fisher's exact test. Anti-spike antibody titres were $\log_{10}$-transformed for visualisation and modelling. Linear regression models were applied to assess the potential decay in neutralising responses against the wild-type virus and the Omicron variant in immune sera as a factor of time elapsed from the third dose. Because of the small sample size, the IGRA results were expressed as medians with IQRs and compared using the Mann-Whitney test. Statistical tests were two tailed, and values of $p<0.05$ were considered significant. All analyses were performed using the GraphPad Prism V.9.0. and SPSS Statistics V.26.

RESULTS

Cohorts of vaccinated individuals

To characterise COVID-19 vaccine-induced immune responses on the domination of the SARS-CoV-2 Omicron variant, 149 patients with ARDs and 94 HCWs participated in this study (figure 1). Among the enrolled patients, 102 (68.5%) received the third dose of an mRNA vaccine (BNT162b2 or mRNA-1273) before enrolment. The median time from the date of the third vaccination to the date of sampling was 7.9 weeks (IQR, 5.6–9.8). Details of the patient characteristics are shown in table 1. The enrolled HCWs ranged from 24 to 64 years old (median: 38.5 years) and composed of both males (29.8%) and females (70.2%), with a similar sex distribution to the enrolled patients ($p=0.535$).

Vaccine-induced neutralisation responses

Neutralising antibody responses were quantified by testing the serum against purified RBD from the wild-type virus and the Omicron variant.[36] We found that two doses of COVID-19 vaccines induced strong neutralising responses against the wild-type virus in both HCWs and patients with ARDs (72.1% and 76.2%, respectively; $p=0.329$; figure 2A). However, the mean neutralising response against the Omicron variant was 18.1% in HCWs and 11.5% in patients with ARDs ($p=0.007$). Following administration of the third dose of an mRNA vaccine, HCWs developed a mean of 97.2% wild-type virus-specific neutralising responses, which decreased to 88.1% in patients with ARDs ($p<0.0001$, figure 2B). Meanwhile, the third dose elicited a mean of 50.3% cross-neutralising responses to the Omicron variant in HCWs, with a majority (72.3%) of sera demonstrating Omicron-neutralisation capacity (neutralising response ≥30%). By comparison, a significantly lower mean cross-neutralising response of 26.8% was observed in patients with ARDs ($p<0.0001$), and only 39.2% of sera were capable of neutralising the Omicron variant, despite a significant increase in responses compared with that in two-dose recipients ($p<0.001$). Specifically, patients with ARDs had intrinsically diminished neutralisation capacity against the Omicron variant, as indicated by the relative ratio of the Omicron- over the wild-type virus-neutralising response of 0.29, which was significantly lower than the 0.52 observed in HCWs ($p<0.0001$, figure 2C).

Correlation between Omicron-neutralisation and anti-spike IgG

The seropositivity rate regarding the ancestral anti-spike IgG (≥1.1 AU/ml) was 94.8% and 87.2% after the second dose in HCWs and patients with ARDs, respectively, which increased to 100% and 96.1% after the third dose. Following the third vaccination, a positive correlation between the ancestral anti-spike IgG titres and the Omicron-neutralising responses was identified by linear regression analysis for the HCWs (figure 2D, blue line), with a calculated slope of 122 (95% CI 64.3 to 180, $p<0.0001$, $R^2=0.160$). However, this association was far less relevant in patients with ARDs, with a slope of 24.3 (95% CI 8.43 to 40.2, $p=0.003$, $R^2=0.085$; figure 2D, red line). Indeed, only 40.8% of sera from IgG seropositive patients showed neutralisation capacity against the Omicron variant, and 93.5% of patients who did not demonstrate serum neutralisation of the Omicron variant were seropositive.

Differential neutralisation capacity against omicron variant

We subsequently evaluated the functional neutralisation capacity against the Omicron variant stratified by clinical and biological profiles. Among the third-dose recipients, 52.0% of individuals with SLE, 25.0% with RA, 37.5% with AS, and 33.3% with BD, while 100% with AOSD had measurable Omicron-neutralisation capacity in their sera (figure 3A,B). Sera from a fraction of SLE patients solely on hydroxychloroquine (70.0%)

Table 1 Characteristics of vaccinated patients according to neutralisation against the SARS-CoV-2 Omicron*

	2X vaccine (N=47)		3X vaccine (N=102)		
	Omicron neutralisation (+)	Omicron neutralisation (–)	Omicron neutralisation (+)	Omicron neutralisation (–)	
	N=3	N=44	N=40	N=62	P value†
Age (years)	62.0	45.5 (37.0; 56.3)	57.0 (46.0; 66.8)	62.0 (54.0; 69.5)	0.211
Male	1 (33.3)	10 (22.7)	7 (17.5)	20 (32.3)	0.099
Disease entities					
SLE (n=43)	–	18 (40.9)	13 (32.5)	12 (19.4)	0.019
RA (n=62)	2 (66.7)	16 (36.4)	11 (27.5)	33 (53.2)	
AS (n=11)	–	3 (6.8)	3 (7.5)	5 (8.1)	
BD (n=10)	1 (33.3)	–	3 (7.5)	6 (9.7)	
AOSD (n=6)	–	1 (2.3)	5 (12.5)	–	
Others (n=17)	–	6 (13.6)	5 (12.5)	6 (9.7)	
Comorbidities					
Asthma (n=5)	1 (33.3)	1 (2.3)	2 (5.0)	1 (1.6)	0.559
Cancer (n=18)	–	3 (6.8)	4 (10.0)	11 (17.7)	0.281
Cardiovascular disease (n=35)	1 (33.3)	10 (22.7)	7 (17.5)	17 (27.4)	0.249
Diabetes (n=21)	–	7 (15.9)	3 (7.5)	11 (17.7)	0.142
Thyroid disorder (n=17)	–	6 (13.6)	5 (12.5)	6 (9.7)	0.748
Immunomodulators					
Steroid (n=62)	1 (33.3)	20 (45.5)	14 (35.0)	27 (43.5)	0.39
Steroid dose (mg, prednisone equivalent)	2.5	5.0(1.6; 6.3)	3.8(2.5; 6.6)	5.0(2.5; 5.0)	0.683
Hydroxychloroquine (n=42)	–	17 (38.6)	14 (35.0)	11 (17.7)	0.048
Methotrexate (n=58)	2 (66.7)	16 (36.4)	12 (30.0)	28 (45.2)	0.126
Leflunomide (n=29)	–	8 (18.2)	6 (15.0)	15 (24.2)	0.262
Sulfasalazine (n=2)	–	1 (2.3)	1 (2.5)	–	0.392
Mycophenolate mofetil (n=17)	–	8 (18.2)	4 (10.0)	5 (8.1)	0.735
Calcineurin inhibitors (n=23)	1 (33.3)	8 (18.2)	9 (22.5)	5 (8.1)	0.039
Azathioprine (n=23)	1 (33.3)	5 (11.4)	6 (15.0)	11 (17.7)	0.717
Cyclophosphamide (n=2)	–	2 (4.5)	–	–	–
JAK inhibitors (n=3)	–	2 (4.5)	–	1 (1.6)	1
TNF inhibitors (n=17)	–	3 (6.8)	4 (10.0)	10 (16.1)	0.380
Tocilizumab (n=3)	–	2 (4.5)	–	1 (1.6)	1
Belimumab (n=1)	–	–	–	1 (1.6)	1
Laboratory tests					
Neutrophils (10^6 /L)	3972.0	3051.5(2267.8; 3893.3)	3037.5(2013.0; 3811.5)	3197.5(2589.0; 4222.0)	0.138
Lymphocytes (10^6 /L)	1289.0	1693.0 (1112.3; 2200.5)	1909.0 (1314.5; 2395.5)	1724.0(1271.8; 2392.8)	0.435
ESR (mm/hour)	30	22 (12; 31)	23 (11; 36)	25(11; 43)	0.676
CRP (mg/L)	1.7	0.9 (0.4; 2.3)	0.9(0.4; 2.7)	0.9(0.5; 3.3)	0.624
Creatinine (mg/dL)	0.76	0.68 (0.57; 0.84)	0.67(0.59; 0.80)	0.74(0.61; 0.90)	0.058
eGFR (mL/1.73 m^2)	103.4	98.6 (82.2; 123.9)	94.8 (78.1; 108.5)	90.4(73.0; 108.4)	0.215
Vaccine type					
mRNA-mRNA (n=38)	1 (33.3)	37 (84.1)			
Ad-Ad (n=6)	1 (33.3)	5 (11.4)			
Ad-mRNA (n=3)	1 (33.3)	2 (4.5)			
mRNA-mRNA-mRNA (n=57)			27 (67.5)	30 (48.4)	0.114
Ad-Ad-mRNA (n=43)			13 (32.5)	30 (48.4)	
Ad-mRNA-mRNA (n=2)			–	2 (3.2)	

*Neutralisation (+), neutralising response ≥ 30%; neutralisation (–), neutralising response <30%.
†Qualitative variables were compared using the $\chi 2$ or Fisher's exact test, and quantitative variables were compared using the Mann-Whitney U test. Statistical analyses for two-dose recipients are not provided because of the small number of participants with Omicron-neutralising capacity.
Ad, adenoviral vector; AOSD, adult-onset Still's disease; BD, Behçet's disease; CRP, C reactive protein; eGFR, estimated glomerular filtration rate; ESR, erythrocyte sedimentation rate; JAK, Janus kinase; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus.

and patients taking calcineurin inhibitors for various indications (64.3%) were most likely to exhibit functional cross-neutralising responses (figure 3C,D). We observed a significant reduction in the proportion of Omicron-neutralisation capacity with a neutrophil-to-lymphocyte ratio (NLR) greater than 2.0 (25.6 vs 47.6%, p=0.027; figure 3F). No difference in the proportion of Omicron-neutralisation capacity was detected between those previously immunised with one or more doses of the viral vector vaccine and those without prior exposure to the viral vector vaccine (figure 3G). There was a significant interaction with time elapsed since the third dose (p=0.012), which raised questions regarding the durability of cross-neutralising

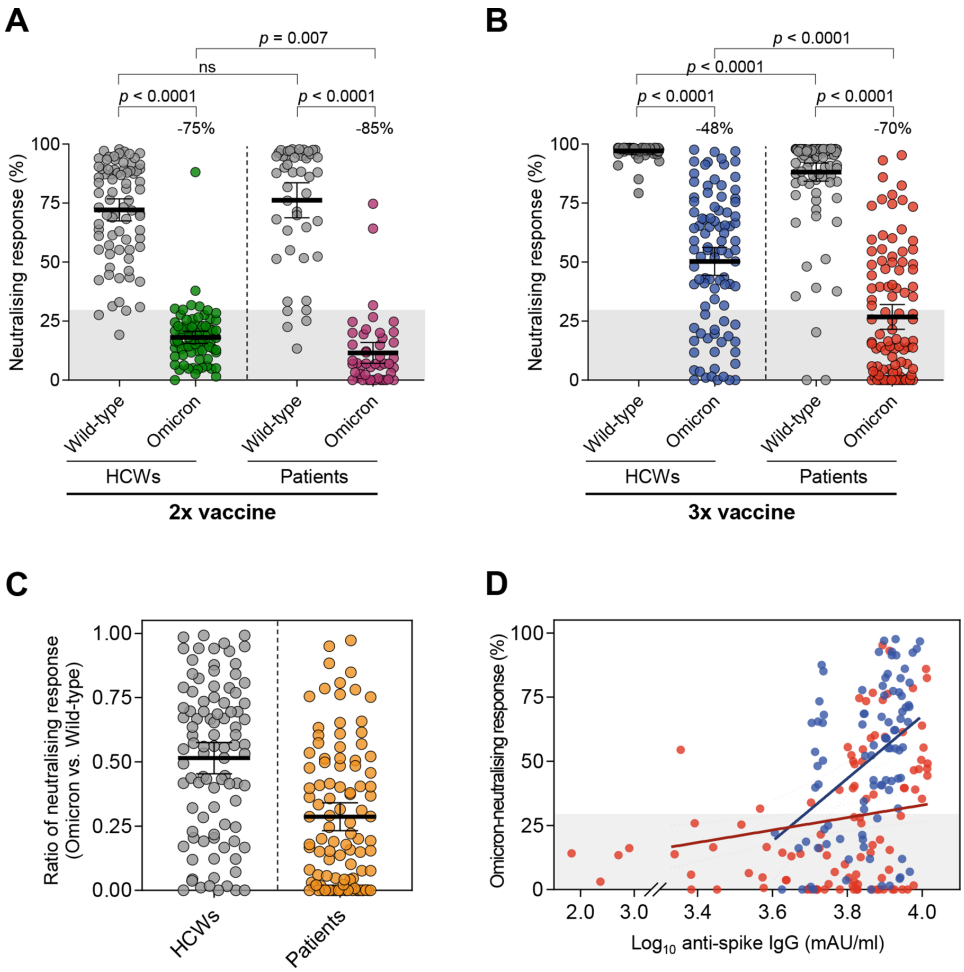

Figure 2 Cross-reactivity of neutralising antibody responses induced by COVID-19 vaccination. (A) Neutralisation responses against the wild-type SARS-CoV-2 and the Omicron variant were analysed for healthcare workers (HCWs) and patients with autoimmune rheumatic diseases vaccinated with primary series. (B) Neutralisation responses in HCWs and patients with autoimmune rheumatic diseases after the third dose of an mRNA vaccination. (C) The relative neutralisation capacity against the omicron variant compared with that against the wild-type SARS-CoV-2. (D) Results for neutralisation responses against the Omicron variant from study participants in (B) that received third vaccine doses were used for linear regression analysis of log-transformed ancestral anti-spike IgG titres in HCWs (blue) and patients with autoimmune rheumatic diseases (red). Dark horizontal lines for each group denote sample means, and the error bars and dotted lines indicate 95% CIs. NS, not significant.

antibody responses after immunisation with the third dose (figure 3H).

Vaccine breakthrough infections caused by SARS-CoV-2 Omicron

Of the 102 patients with ARDs who received the third dose, 99 responded to our interview survey (97.1% response rate) at the end of the follow-up. Throughout the observation period, 19.2% (19/99) of patients with ARDs and 33.0% (31/94) of HCWs developed breakthrough infections (online supplemental figure S2; log-rank test, p=0.710). Of note, the median time between the third dose vaccination and the date of confirmed breakthrough infection in patients with ARDs was significantly shorter compared with that in HCWs (93.0 days (IQR, 82.0–98.0) vs 122 days (IQR, 111–131); p<0.0001). Based on our findings, we postulated that limited neutralisation of the Omicron variant in sera have been implicated in the relatively short-lived protection from breakthrough infections in patients with ARDs.

Strikingly, 14 of the 19 breakthrough cases (73.7%) did not reach the threshold of Omicron-neutralisation capacity before SARS-CoV-2 infection (online supplemental table S1). Two vaccinated patients were hospitalised for COVID-19, and both had nil neutralising responses against the Omicron variant, despite high neutralising responses against the wild-type virus (96.9% and 94.3%, respectively). In our study cohort, patients with ARDs were stratified by the length of the observation time (the interval from the date of the immunogenicity assessment to the date of confirmed breakthrough infection or the end of the follow-up period) to better account for the difference in waning antibody responses over time (online supplemental figure S3). We found significantly lower Omicron-neutralising responses in sera from breakthrough-cases relative to those from non-cases (p=0.018), particularly within a 6-week interval from the immunogenicity assessments (figure 4A). These results suggest that levels of vaccine-induced cross-neutralising antibodies represented potential correlates of protection from breakthrough infections in patients with ARDs.

Next, we estimated the effect of the time elapsed from vaccination to neutralising responses against the wild-type virus and the Omicron variant during the initial 120 days after the third dose (figure 4B). As expected, sera from patients with ARDs efficiently neutralised the wild-type virus, showing a non-demonstrable decay in neutralising responses. In contrast, the same sera

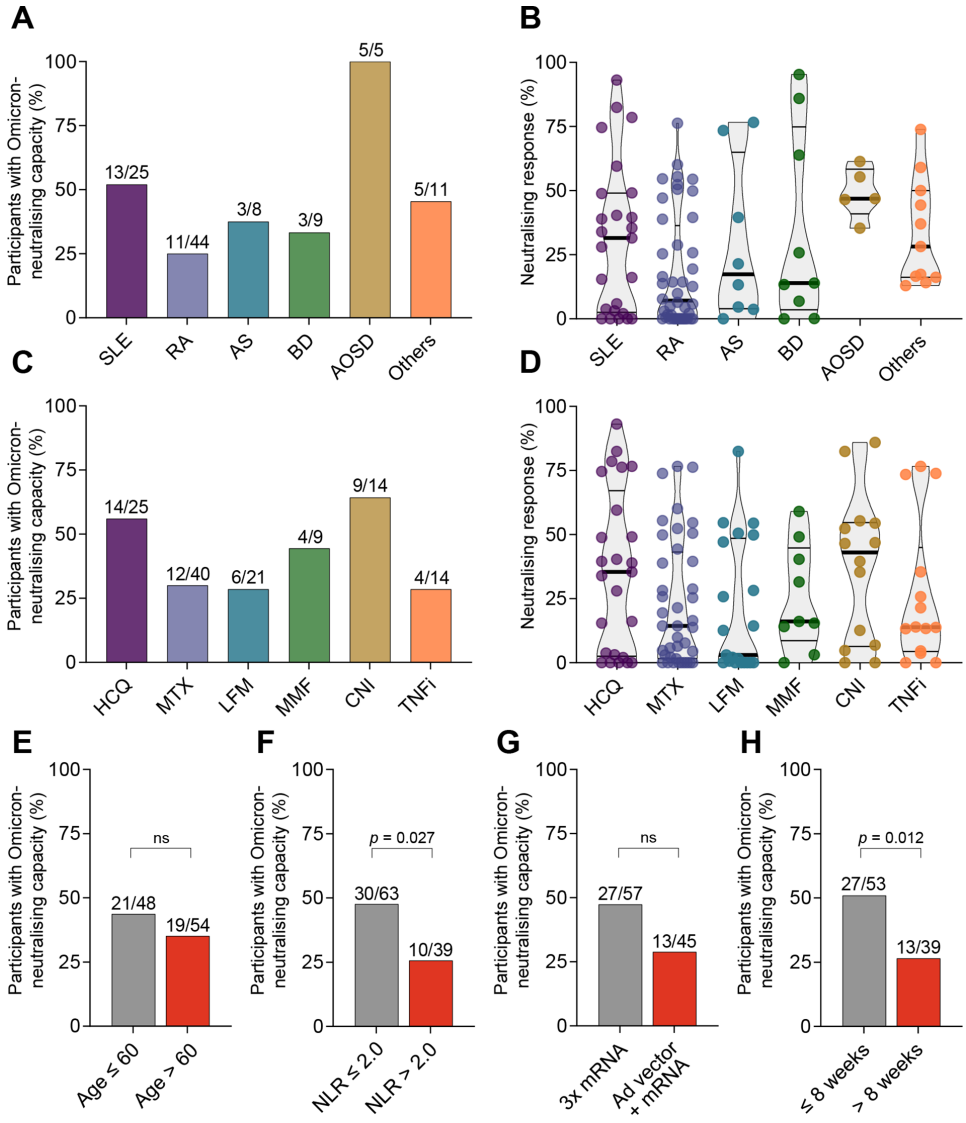

Figure 3 The functional neutralisation of the Omicron variant by immunised sera from patients with autoimmune rheumatic diseases. (A) percentages of sera from patients with autoimmune rheumatic diseases exhibiting Omicron-neutralising capacity defined by Omicron-specific neutralising responses ≥30% stratified by disease entity. (B) results for neutralisation responses against the Omicron variant from study participants in (A). (C) Percentages of sera from patients with autoimmune rheumatic diseases exhibiting Omicron-neutralising capacity stratified by immunomodulator use. (D) Results for neutralisation responses against the omicron variant from study participants in (C). (E–H) Percentages of sera from patients with autoimmune rheumatic diseases exhibiting Omicron-neutralising capacity stratified by age, neutrophil-to-lymphocyte ratio (NLR), vaccine type and time elapsed since the third dose. The numbers above the bar graph represent the number of participants in each group. The dark horizontal lines for each group denote sample medians, and the error bars indicate interquartile ranges. AOSD, adult-onset Still's disease; AS, ankylosing spondylitis; BD, Behçet's disease; CNI, calcineurin inhibitor; HCQ, hydroxychloroquine; LFM, leflunomide; MMF, mycophenolate mofetil; MTX, methotrexate; ns, not significant; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; TNFi, tumour necrosis factor inhibitor.

neutralised the Omicron variant to a lesser extent, demonstrating a significant decline in cross-neutralising responses over time, with a predicted decay rate of −0.351% /day (95% CI −0.559 to −0.144, p=0.001), suggesting the potential for a substantial loss of the protection from breakthrough infection.

SARS-CoV-2-specific cellular immunity

A robust T cell responses likely play a role in prevention and resolution of severe SARS-CoV-2 infection. Hence, we examined SARS-CoV-2–specific T cell reactivity in patients with ARDs, at a median of 6.4 weeks (IQR 4.7–8.7) after receiving the third dose of an mRNA vaccine. Released IFN-γ levels in response to spike-based antigens declined slightly from a median of 324 mIU/mL (IQR 118–555) in HCWs to 203 mIU/mL(IQR, 37.5–470) in patients with ARDs, but the difference was not significant (p=0.262; figure 5A). A total of 53.5% of the participants had positive IGRA responses, and T cell reactivity in vaccinated individuals displayed similar patterns between the two cohorts (figure 5B), even if we could perform IGRAs only for some of the samples due to logistical issues at the time of study implementation (online supplemental table S1).

DISCUSSION

The immunogenicity of the COVID-19 vaccine in patients with ARDs is of concern.[37 38] However, most published data regarding immunocompromised patients do not consider VOCs, and thus offer limited real-world application. Although a few studies have reported neutralisation responses against alpha, beta and delta

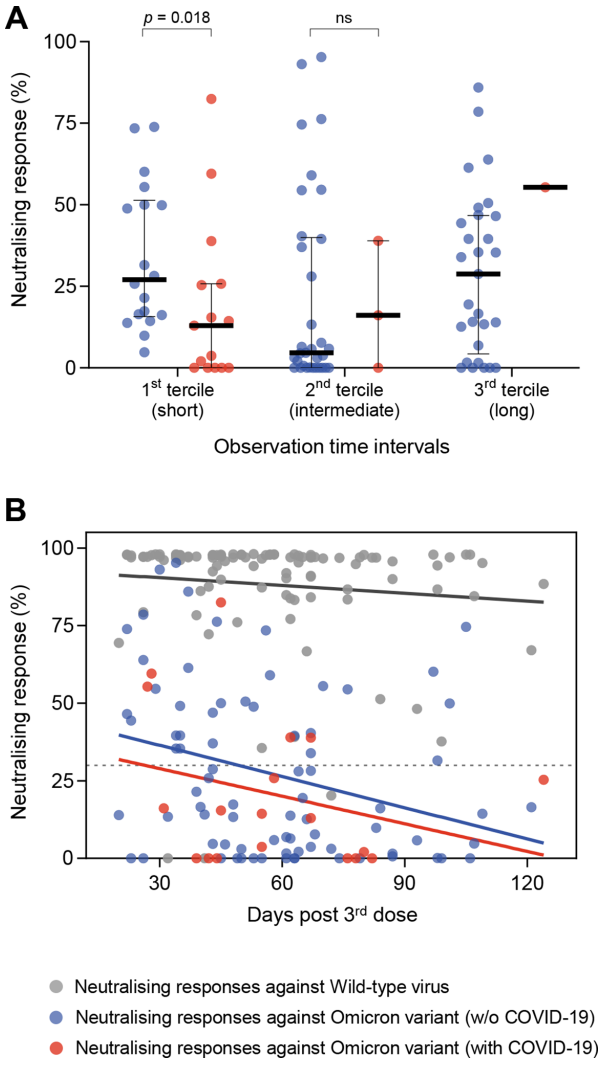

Figure 4 COVID-19 breakthrough infections in patients with autoimmune rheumatic diseases who received a third vaccine dose. (A) neutralisation responses in patients with autoimmune rheumatic diseases against the omicron variant are compared between those with (red) or without (blue) confirmed breakthrough infections in relation to the length of follow-up time. (B) Neutralisation responses against the wild-type SARS-CoV-2 (grey) and the omicron variant (blue and red) with regression lines are plotted over time elapsed since the receipt of the third dose. The dark horizontal lines for each group denote sample medians, and the error bars indicate interquartile ranges. NS, not significant.

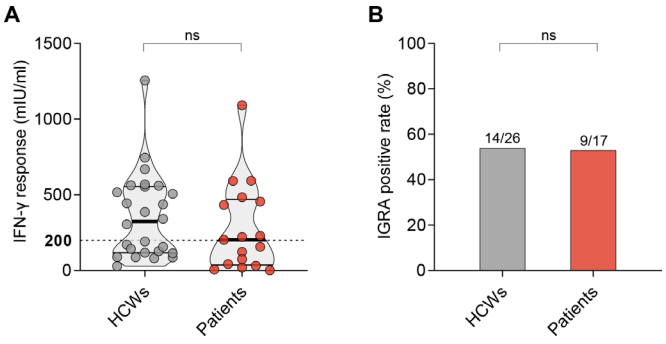

Figure 5 SARS-CoV-2-specific T cell responses after the third dose. Interferon gamma (IFN-γ) levels in plasma after whole blood stimulation with peptide pools spanning the SARS-CoV-2 spike protein. (B) Positivity rates of the interferon gamma release assay (IGRA). The IFN-γ response-positive cut-off was set at ≥200 mIU/mL. The dark horizontal lines for each group denote sample medians, and the error bars indicate interquartile ranges. The numbers above the bar graph represent the number of participants in each group. HCWs, healthcare workers; NS, not significant.

variants in solid organ transplant recipients[39 40] and a heterogeneous population of immunocompromised patients,[41] studies on patients with ARDs regarding the latest Omicron variant remain limited. Hence, we delineated the cross-reactivity of vaccine-induced humoral responses against the SARS-CoV-2 Omicron variant compared with that against the wild-type virus. Our findings suggested that neither primary series vaccinations nor booster doses are sufficient to induce Omicron-neutralising responses above the threshold in patients with ARDs, although responses were noticeably increased following the third dose of an mRNA vaccine. This impairment of cross-neutralisation responses across most of our patients contrasts starkly with a potent elicitation of the Omicron-neutralising responses after the third vaccination in healthy recipients. These differences could potentially be attributed to the nature of the patients undergoing immunomodulatory therapy, who typically exhibit profoundly blunted RBD-specific germinal centre B cell responses even after the third vaccination.[42 43]

High-throughput measurements of IgG antibodies that bind to the ancestral spike constitute a major part of immunogenicity assessments. Such analyses of an mRNA vaccine trial in the general population found that IgG titres correlated with the degree of vaccine efficacy, although this study precluded the assessment of SARS-CoV-2 VOCs.[44] Accordingly, considering that potent germinal centre B cell reactions are closely intertwined with efficient induction of neutralising antibodies, the poor correlation between anti-spike IgG and neutralising responses in patients with ARDs may be due to a relatively greater proportion of IgG recognising non-RBD spike epitopes and low-affinity IgG originating from extrafollicular B cells. Our results demonstrate that while booster doses may bring about an overall increase in total anti-spike IgG titres, such increases do not necessarily equate to improved neutralisation responses. Thus, quantifying the functional neutralisation capacity rather than the ancestral anti-spike IgG may be a more precise approach for determining the immunological benefit conferred by booster doses in patients with ARDs.

Protection against SARS-CoV-2 infection provided by third doses has now been well-demonstrated.[45] Such benefits are also conferred to immunosuppressed patients who exhibit greater risks of prolonged viral replication, potentially facilitating the emergence of new SARS-CoV-2 genetic mutations.[46 47] However, in our study, booster vaccination-induced Omicron-neutralising responses varied greatly between patients with ARDs, undoubtedly based on the properties of immunomodulators and patient demographics such as age and comorbidities. No clear trends were observed between the Omicron-neutralisation capacity and disease entities. While patients with ARDs have predictably diminished cross-neutralising responses to vaccination, the humoral reactivity of SLE patients solely on hydroxychloroquine therapy was less affected. Likewise, sera from patients treated with calcineurin inhibitors had an increased chance of exerting neutralisation responses, given that the inhibition of the nuclear factor of activated T cells does not necessarily hinder memory B cell expansion and differentiation into plasma cells, though the function of follicular helper T cells may be affected. Indeed, four of the five AOSD patients treated with calcineurin inhibitors

and the remaining patients who were treated with low-dose azathioprine demonstrated Omicron-neutralisation capacities. Furthermore, a strong association between NLR and Omicron-neutralising responses indicated a potentially skewed balance towards innate over adaptive immune responses.[48]

An initial report of breakthrough infections showed neutralising antibody levels in cases to be lower than that in uninfected controls.[49] We found similar results indicating limited protection from breakthrough infection in patients with poor cross-neutralising responses until 6 weeks following the immunological assessment. However, this may not be generalisable to settings with longer time intervals between the immunological assessment and the confirmation of breakthrough infection. The low breakthrough infection rate observed in patients with a prolonged follow-up period may be affected by the greater proportion of recently vaccinated individuals and the gradually decreasing trend in the incidence of COVID-19 during the post-peak phase of the pandemic (online supplemental figures S1 and S2).

Further, to account for variability in the duration of neutralising antibody-mediated protection from breakthrough infection, we calculated the rate of breakthrough infections according to the time elapsed since the third vaccination in both cohorts (online supplemental figure S2). Notably, our analysis indicated a tendency for a shorter duration of protection from the third dose in patients with ARDs than HCWs, although there was no statistically significant between-group difference in the overall incidence of breakthrough infections.

Taken together, as the magnitude of the Omicron-specific neutralising antibody responses induced by the third dose was markedly diminished and was suggested to decay quickly relative to the wild-type-specific neutralising antibody responses in patients with ARDs, this population is anticipated to be at an increased risk of developing breakthrough infections. Since the fourth dose is beginning to be administered, it remains to be determined whether such additional doses will provide improved neutralising responses in patients with exceptionally weak cross-neutralising responses. At the same time, more research into the potential benefits afforded by alternative Omicron-specific boosters may be necessary to effectively protect such immunologically vulnerable individuals.

This study had several limitations. First, neutralising antibody responses were assessed at once after the third dose vaccination. Thus, longitudinal antibody responses to the SARS-CoV-2 Omicron variant and whether and how the waning of immunity might affect breakthrough infection risks remain to be determined. Second, the enrolled patients were generally older than the recruited HCWs, and age-associated immunosenescence might have contributed to the deterioration in cross-neutralisation capacity. Third, our patient cohort was recruited from the outpatient clinic in a single academic hospital that comprises several distinct clinicopathological entities, making robust statistical analysis challenging. Fourth, SARS-CoV-2 Omicron-specific T cell responses were not examined; however, T cell responses are largely preserved against the Omicron variant.[50] Lastly, vaccine breakthrough cases in the patient cohort were identified by in-depth interviews. Despite a high response rate (97.1%) and our endeavours to obtain accurate information, the possibility of unidentified or unreported cases of SARS-CoV-2 infection during the observation period could not be ruled out.

In conclusion, the third dose of an mRNA vaccine could improve the cross-neutralisation of the SARS-CoV-2 Omicron variant in patients with ARDs, although more than half of the patients failed to generate Omicron-neutralising antibodies. Our study sheds light on the relative deficiency of the Omicron-specific neutralising responses in patients with ARDs and their anticipated vulnerability to breakthrough infection. As new SARS-CoV-2 variants are expected to circulate, further research on effective vaccination strategies for patients with immune dysfunction is urgently required.

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Patient consent for publication Consent obtained directly from patient(s)

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REFERENCES

1 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.
2 Conway R, Grimshaw AA, Konig MF, *et al*. SARS-CoV-2 infection and COVID-19 outcomes in rheumatic diseases: a systematic literature review and meta-analysis. *Arthritis Rheumatol* 2022;74:766-775.
3 Bok K, Sitar S, Graham BS, *et al*. Accelerated COVID-19 vaccine development: milestones, lessons, and prospects. *Immunity* 2021;54:1636–51.

4 Sun J, Zheng Q, Madhira V, *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.
5 Khoury DS, Cromer D, Reynaldi A, *et al*. Neutralizing antibody levels are highly predictive of immune protection from symptomatic SARS-CoV-2 infection. *Nat Med* 2021;27:1205–11.
6 Bar-On YM, Goldberg Y, Mandel M, *et al*. Protection of BNT162b2 vaccine booster against Covid-19 in Israel. *N Engl J Med* 2021;385:1393–400.
7 Bar-On YM, Goldberg Y, Mandel M, *et al*. Protection against Covid-19 by BNT162b2 booster across age groups. *N Engl J Med Overseas Ed* 2021;385:2421–30.
8 Munro APS, Janani L, Cornelius V, *et al*. Safety and immunogenicity of seven COVID-19 vaccines as a third dose (booster) following two doses of ChAdOx1 nCov-19 or BNT162b2 in the UK (COV-BOOST): a blinded, multicentre, randomised, controlled, phase 2 trial. *The Lancet* 2021;398:2258–76.
9 Atmar RL, Lyke KE, Deming ME, *et al*. Homologous and heterologous Covid-19 booster vaccinations. *N Engl J Med Overseas Ed* 2022;386:1046–57.
10 Landewé RBM, Kroon FPB, Alunno A, *et al*. EULAR recommendations for the management and vaccination of people with rheumatic and musculoskeletal diseases in the context of SARS-CoV-2: the November 2021 update. *Ann Rheum Dis* 2022. doi:10.1136/annrheumdis-2021-222006. [Epub ahead of print: 23 Feb 2022].
11 Lambrou AS, Shirk P, Steele MK, *et al*. Genomic surveillance for SARS-CoV-2 variants: predominance of the delta (B.1.617.2) and omicron (B.1.1.529) variants — United States, June 2021–January 2022. *MMWR Morb Mortal Wkly Rep* 2022;71:206–11.
12 Planas D, Saunders N, Maes P, *et al*. Considerable escape of SARS-CoV-2 omicron to antibody neutralization. *Nature* 2022;602:671–5.
13 Liu L, Iketani S, Guo Y, *et al*. Striking antibody evasion manifested by the omicron variant of SARS-CoV-2. *Nature* 2022;602:676–81.
14 Carreño JM, Alshammary H, Tcheou J, *et al*. Activity of convalescent and vaccine serum against SARS-CoV-2 omicron. *Nature* 2022;602:682–8.
15 Dejnirattisai W, Huo J, Zhou D, *et al*. SARS-CoV-2 Omicron-B.1.1.529 leads to widespread escape from neutralizing antibody responses. *Cell* 2022;185:e15:467–84.
16 Hoffmann M, Krüger N, Schulz S, *et al*. The omicron variant is highly resistant against antibody-mediated neutralization: implications for control of the COVID-19 pandemic. *Cell* 2022;185:e11:447–56.
17 Tarke A, Coelho CH, Zhang Z, *et al*. SARS-CoV-2 vaccination induces immunological T cell memory able to cross-recognize variants from alpha to omicron. *Cell* 2022;185:e11:847–59.
18 Keeton R, Tincho MB, Ngomti A, *et al*. T cell responses to SARS-CoV-2 spike cross-recognize omicron. *Nature* 2022;603:488–92.
19 Liu J, Chandrashekar A, Sellers D, *et al*. Vaccines elicit highly conserved cellular immunity to SARS-CoV-2 omicron. *Nature* 2022;603:493–6.
20 Gao Y, Cai C, Grifoni A, *et al*. Ancestral SARS-CoV-2-specific T cells cross-recognize the omicron variant. *Nat Med* 2022;28:472–6.
21 Accorsi EK, Britton A, Fleming-Dutra KE, *et al*. Association between 3 doses of mRNA COVID-19 vaccine and symptomatic infection caused by the SARS-CoV-2 omicron and delta variants. *JAMA* 2022;327:639–51.
22 Garcia-Beltran WF, St. Denis KJ, Hoelzemer A, *et al*. mRNA-Based COVID-19 vaccine boosters induce neutralizing immunity against SARS-CoV-2 omicron variant. *Cell* 2022;185:e4:457–66.
23 Wratil PR, Stern M, Priller A, *et al*. Three exposures to the spike protein of SARS-CoV-2 by either infection or vaccination elicit superior neutralizing immunity to all variants of concern. *Nat Med* 2022;28:496–503.
24 Gruell H, Vanshylla K, Tober-Lau P, *et al*. mRNA booster immunization elicits potent neutralizing serum activity against the SARS-CoV-2 omicron variant. *Nat Med* 2022;28:477–80.
25 Gudbjartsson DF, Norddahl GL, Melsted P, *et al*. Humoral immune response to SARS-CoV-2 in Iceland. *N Engl J Med* 2020;383:1724–34.
26 Simon D, Tascilar K, Fagni F, *et al*. Efficacy and safety of SARS-CoV-2 revaccination in non-responders with immune-mediated inflammatory disease. *Ann Rheum Dis* 2022;81:1023–7.
27 Bradley T, Grundberg E, Selvarangan R, *et al*. Antibody responses after a single dose of SARS-CoV-2 mRNA vaccine. *N Engl J Med Overseas Ed* 2021;384:1959–61.
28 Sattler A, Schrezenmeier E, Weber UA, *et al*. Impaired humoral and cellular immunity after SARS-CoV-2 BNT162b2 (tozinameran) prime-boost vaccination in kidney transplant recipients. *J Clin Invest* 2021;131.
29 Stankov MV, Cossmann A, Bonifacius A, *et al*. Humoral and cellular immune responses against severe acute respiratory syndrome coronavirus 2 variants and human coronaviruses after single BNT162b2 vaccination. *Clin Infect Dis* 2021;73:2000–8.
30 Fraley E, LeMaster C, Khanal S, *et al*. The impact of prior infection and age on antibody persistence after severe acute respiratory syndrome coronavirus 2 messenger RNA vaccine. 2021;384.
31 Ujjainiya R, Tyagi A, Sardana V, *et al*. High failure rate of ChAdOx1-nCoV19 immunization against asymptomatic infection in healthcare workers during a delta variant surge. *Nat Commun* 2022;13:1726.
32 Taylor SC, Hurst B, Charlton CL, *et al*. A new SARS-CoV-2 dual-purpose serology test: highly accurate infection tracing and neutralizing antibody response detection. *J Clin Microbiol* 2021;59. doi:10.1128/JCM.02438-20. [Epub ahead of print: 19 03 2021].
33 Mariën J, Michiels J, Heyndrickx L, *et al*. Evaluation of a surrogate virus neutralization test for high-throughput serosurveillance of SARS-CoV-2. *J Virol Methods* 2021;297:114228.
34 Fernández-González M, Agulló V, Padilla S, *et al*. Clinical performance of a standardized SARS-CoV-2 interferon-γ release assay for simple detection of T-cell responses after infection or vaccination. *Clin Infect Dis* 2021. doi:10.1093/cid/ciab1021. [Epub ahead of print: 10 Dec 2021].
35 Markewitz R, Pauli D, Dargvainiene J, *et al*. The temporal course of T- and B-cell responses to vaccination with BNT162b2 and mRNA-1273. *Clin Microbiol Infect* 2022;28:701-709.
36 Tan CW, Chia WN, Qin X, *et al*. A SARS-CoV-2 surrogate virus neutralization test based on antibody-mediated blockage of ACE2-spike protein-protein interaction. *Nat Biotechnol* 2020;38:1073–8.
37 Lee ARYB, Wong SY, Chai LYA, *et al*. Efficacy of covid-19 vaccines in immunocompromised patients: systematic review and meta-analysis. *BMJ* 2022;376:e068632.
38 Jena A, Mishra S, Deepak P, *et al*. Response to SARS-CoV-2 vaccination in immune mediated inflammatory diseases: systematic review and meta-analysis. *Autoimmun Rev* 2022;21:102927.
39 Kumar D, Ferreira VH, Hall VG, *et al*. Neutralization of SARS-CoV-2 variants in transplant recipients after two and three doses of mRNA-1273 vaccine: secondary analysis of a randomized trial. *Ann Intern Med* 2022;175:226–33.
40 Benning L, Morath C, Bartenschlager M, *et al*. Neutralization of SARS-CoV-2 variants of concern in kidney transplant recipients after standard COVID-19 vaccination. *Clin J Am Soc Nephrol* 2022;17:98–106.
41 Obeid M, Suffiotti M, Pellaton C, *et al*. Humoral responses against variants of concern by COVID-19 mRNA vaccines in immunocompromised patients. *JAMA Oncol* 2022;8:e220446.
42 Charmetant X, Espi M, Benotmane I, *et al*. Infection or a third dose of mRNA vaccine elicits neutralizing antibody responses against SARS-CoV-2 in kidney transplant recipients. *Sci Transl Med* 2022;14:eabl6141.
43 Lederer K, Bettini E, Parvathaneni K, *et al*. Germinal center responses to SARS-CoV-2 mRNA vaccines in healthy and immunocompromised individuals. *Cell* 2022;185:e15:1008–24.
44 Gilbert PB, Montefiori DC, McDermott AB, *et al*. Immune correlates analysis of the mRNA-1273 COVID-19 vaccine efficacy clinical trial. *Science* 2022;375:43–50.
45 Andrews N, Stowe J, Kirsebom F. Covid-19 vaccine effectiveness against the omicron (B.1.1.529) variant. *N Engl J Med* 2022.
46 Shen C, Risk M, Schiopu E. Efficacy of COVID-19 vaccines in patients taking immunosuppressants. *Ann Rheum Dis* 2022;81:875–80.
47 Corey L, Beyrer C, Cohen MS, *et al*. SARS-CoV-2 variants in patients with immunosuppression. *N Engl J Med* 2021;385:562–6.
48 Kim S, Eliot M, Koestler DC, *et al*. Association of neutrophil-to-lymphocyte ratio with mortality and cardiovascular disease in the Jackson heart study and modification by the Duffy antigen variant. *JAMA Cardiol* 2018;3:455–62.
49 Bergwerk M, Gonen T, Lustig Y, *et al*. Covid-19 breakthrough infections in vaccinated health care workers. *N Engl J Med* 2021;385:1474–84.
50 Moss P. The T cell immune response against SARS-CoV-2. *Nat Immunol* 2022;23:186–93.