

Immune responses and predictors of SARS-CoV-2 infection following a fourth dose of mRNA or protein-based COVID-19 vaccines

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Immune responses and predictors of SARS-CoV-2 infection following a fourth dose of mRNA or protein-based COVID-19 vaccines

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Abstract

Evidence on immune responses following SARS-CoV-2 infection in individuals boosted with mRNA or protein vaccines are limited. We examined the immune responses following SARS-CoV-2 infection and evaluated immunological markers associated with infection among adults who received a fourth dose of either a bivalent mRNA COVID-19 vaccine (Moderna), an ancestral protein-based vaccine (Novavax), or no fourth dose. Of 496 participants, 153 (30.8%; Moderna n=57, Novavax n=52, control n=44) self-reported at least one infection over 12 months. Serum samples collected at 28 days post-infection showed similar concentrations of binding IgG and surrogate virus neutralising antibodies against SARS-CoV-2 Ancestral and Omicron variants (BA.1, BA.4/5 and JN.1) across vaccine arms and control group. At 6 months post-fourth dose, higher antibody levels were associated with 3-42% lower odds of infection between 7–12 months. The time to infection from fourth-dose vaccination for 10% of participants was longer in the Moderna group compared with the Novavax (191 days [95%CI:138–225] vs 137 days [95%CI:63–199]). Immune response following SARS-CoV-2 infection was similar regardless of prior vaccine platform and a fourth dose mRNA vaccine may delay infection relative to protein-based vaccination. Antibody thresholds may be a useful marker for informing the timing of future booster doses.

Keywords: COVID-19; SARS-CoV-2; vaccination; booster; fourth dose; antibody; immunity; infection

Introduction

COVID-19 vaccines have been highly effective in reducing SARS-CoV-2-related morbidity and mortality. However, protection against infection declines over time due to waning immunity and the emergence of new SARS-CoV-2 variants. Regular COVID-19 vaccine booster doses are recommended by the World Health Organization (WHO) and regulatory agencies for certain populations, including older adults and people with comorbidities or who are immunocompromised^{1,2}. Original COVID-19 vaccines targeted the SARS-CoV-2 Ancestral/Wildtype (WT) strain, whereas next-generation vaccines have been developed to offer protection against emerging variants, including the bivalent mRNA vaccines (WT + Omicron BA.1 and WT + Omicron BA.4/5) and monovalent mRNA and protein vaccines (XBB.1, JN.1, KP.2/3, LP.8.1)³. However, there has been a substantial decline in booster vaccine coverage, with a global uptake of 13% in older adults and 6% in healthcare workers in 2022-23, mostly in high-income countries⁴. In the United States, routine COVID-19 vaccination is no longer recommended for healthy children and adults under 65 years of age. Evidence from randomised controlled trials (RCT) is now required to inform whether these recommendations should be reintroduced⁵.

While studies have shown that mRNA vaccines are more immunogenic than protein vaccines^{6,7}, it remains poorly understood how vaccine platform influences the immune responses to subsequent infection. Identifying correlates of protection (CoP) for SARS-CoV-2 infection is important for vaccine development and evaluation. In a cohort of Spanish healthcare workers who received a third mRNA (mRNA-1273) dose, higher anti-spike IgG and IgA levels to the WT, Delta or Omicron variants, prior infection, and shorter time since last infection were associated with protection against infection at 12 months post-booster.⁸ Several studies have suggested that neutralising antibodies could be a CoP⁹⁻¹⁴, but others dispute this due to a weakened neutralising antibody response following a booster dose in people with prior

infection¹⁵. Hybrid immunity, developed through both SARS-CoV-2 infection and vaccination, provides enhanced protection compared with infection or vaccination alone. However, there are limited long-term hybrid immunity data post a fourth COVID-19 vaccine dose.

Understanding both the durability of protection from booster vaccination and its underlying correlates is therefore essential to inform future COVID-19 vaccine recommendations. We conducted a RCT in adults in Australia to assess the immunogenicity of a fourth dose of either a bivalent mRNA vaccine (mRNA-1273.214/mRNA-1273.222; hereafter Moderna) or a protein vaccine (NVX-CoV-2373; hereafter Novavax)^{3,16}. We found higher antibody responses generated by Moderna compared with Novavax vaccine, but both vaccines appeared to provide comparable protection against SARS-CoV-2 Omicron variants over 12-months¹⁶. This post-hoc, exploratory analysis aimed to (i) assess the immune responses at 28 days after documented SARS-CoV-2 infection by rapid antigen test (RAT), (ii) compare time to first SARS-CoV-2 infection between vaccine arms over 12 months follow-up and (iii) identify immunological markers of protection against SARS-CoV-2 infection.

Results

Participant demographics

An overview of the study design and laboratory procedures are outlined in Figure 1A. A total of 1257 participants were screened between 28 February to 4 October 2023, with 496 enrolled (Figure 1B). Participants were allocated to the Moderna (n=177) and Novavax (n=176) arms. A self-selected control group with no fourth dose was also recruited (n=143). A total of 153 (30.8%; Moderna n=57, Novavax n=52, control n=44) participants had documented at least one infection, with 60 and 93 events occurring between 0-6 months and 7-12 months post-enrolment, respectively.

Among participants with a documented SARS-CoV-2 infection, the median age was similar between Moderna arm (33.8 years, IQR: 25.7 – 51.3) and Novavax arm (32.2 years, IQR: 26.6 – 39.0), while the control arm was older (44.3 years, IQR: 30.77 – 51.3). The proportion of participants aged <50 years was highest in the Novavax arm (92.3%), followed by Moderna (71.9%) and control (63.6%). The proportion of females was highest in the Novavax arm (82.7%), followed by control (81.8%) and Moderna (68.4%). The distribution of comorbidities was similar between arms and the full trial cohort (Table 1). Compared to the full trial cohort, participants with SARS-CoV-2 infection had a higher median age and higher proportion of females, as well as higher proportion of obesity in the Novavax and control group (Table 1). Approximately 25 – 40% of participants with an infection in each group provided a NP swab sample and 61 – 68% provided a blood sample. The baseline characteristics of participants who provided post-infection sample are similar to the overall cohort who reported a SARS-CoV-2 infection (Supplementary Table 1).

SARS-CoV-2 variant distribution

During the study period (March to November 2023), circulating variants in Australia were predominantly Omicron and related lineages, including JN.1, KP.3 and KP.3.1.1 (December 2023 to September 2024) (Figure 1C). A total of 47 swabs were collected from 153 participants with an infection (26.1%). Sequenced variants included XBB (March to August 2023; 27.5%), EG and HK (October to November 2023; 15% and 7.5%), JN.1 (November 2023 to March 2024; 37.5%), KW.1 (April 2024; 5.0%), KP (May 2024; 2.5%), and two unassigned lineages (May and November 2023; 5.0%) (Supplementary Figure 1). Sequenced variants were similar across arms (Supplementary Figure 1).

Immune responses following SARS-CoV-2 infection in participants who received mRNA or protein vaccine and control participants

Between 0-6 months, pre-infection levels (day 0 for control group and day 28 post-fourth dose for vaccine groups) of IgG against WT and Omicron BA.1, BA.4/5 and JN.1 and surrogate virus neutralising antibody titres (sVNT) against WT and Omicron BA.1 and BA.4/5 were highest for Moderna, then Novavax and control (Figure 2, Supplementary Table 2 and Supplementary Table 3). From pre- to post-infection, IgG and sVNT levels for all variants mostly decreased or remained similar for Moderna (GMR range: 0.40 – 1.12), increased for Novavax (0.86 – 3.78), and increased for control (1.11 – 8.77) (Figure 2 and Supplementary Table 3). Post-infection IgG and sVNT responses for all variants tested were similar across arms (Supplementary Figure 2). IFN γ levels by were similar at the pre- and post-timepoints and across arms (Figure 2 and Supplementary Table 2).

Between 7-12 months, pre-infection levels (6 months post-enrolment for all arms) of IgG and sVNT for all variants tested were similar between both vaccine arms, while the control arm have lower responses overall (Figure 2, Supplementary Table 2 and Supplementary Table 3). From pre- to post-infection, IgG and sVNT levels increased for all arms (GMR range:

Moderna 0.63 – 1.57, Novavax 0.98 – 4.45, control 1.39 – 4.92) (Figure 2 and Supplementary Table 3). Post-infection IgG and sVNT responses for all variants tested were similar across arms (Supplementary Figure 2). IFN γ levels were similar at the pre- and post-timepoints and across arms (Figure 2 and Supplementary Table 2).

Within each study arm, post-infection levels of IgG, sVNT, and IFN γ did not differ according to whether infection occurred during the first or second 6-month follow-up period (Supplementary Figure 2).

Immune predictors of SARS-CoV-2 infection

We next wanted to compare the SARS-CoV-2 specific IgG, sVNT and IFN γ levels at the preceding study visit (day 0 for control arm or day 28 for vaccine arms if infection occurs within the first 6 months, and the 6-month timepoint for infection occurring 7–12 months for both control and vaccine arms) between participants who did or did not subsequently have a documented SARS-CoV-2 infection over the 12-month follow-up period. Pre-infection levels of IgG, sVNT and IFN γ were generally similar among participants who did not experience SARS-CoV-2 infection compared with those who did during either the first 6 months or the 7–12 months follow-up (Figure 3, Supplementary Figure 3 and Supplementary Table 4). Conversely, participants who experience a SARS-CoV-2 infection within the first 6 months or 7–12 months generally had higher IgG, sVNT and IFN γ levels at the 6- and 12-month study visits (post-enrolment), respectively compared to those who were uninfected (Supplementary Figure 4).

To assess the relationship between immunological markers (IgG, sVNT and IFN γ) and infection during the first 6 months post-vaccination and the subsequent 6-month period, we performed multivariable logistic regression, adjusted for age and study arm. Between 0-6 months, pre-infection levels of IgG, sVNT and IFN γ (day 28 post-fourth dose for vaccine

groups) were not associated with the odds of SARS-CoV-2 infection (Table 2). However, between 7–12 months, an increase of 100 units/mL of IgG against WT, BA.1, BA.4/5 and JN.1 were associated with 3%, 20%, 14% and 23% decreased odds of infection, respectively. Similarly, for sVNT, an increase of 50,000 U/mL against BA.1 and BA.4/5 was associated with 42% and 21% decreased odds of infection, respectively. In contrast, IFN γ levels were not associated with the odds of SARS-CoV-2 infection.

Survival time to infection

The time to infection from fourth-dose vaccination for 10% of participants was longer in the Moderna group compared with the Novavax group (191 days [95% CI: 138 – 225] vs 137 days [95% CI: 63 – 199]) (Figure 4). The 20th percentile of survival time was similar in the Moderna (247; 95% CI: 233 – 301) and Novavax (240; 95% CI: 198 – 310) groups. Additional exploratory analyses involving the self-selected control group (non-randomised and older) found time to infection for 10% of participants was longer in the Moderna group compared with the control group, while this was similar for Novavax and control groups (Supplementary Figure 5). When stratifying participants by IgG, sVNT and IFN γ levels at day 0 (control) or day 28 (vaccine arms), no consistent patterns were identified with regards to time to infection (Supplementary Figure 6 and Supplementary Table 5).

Discussion

This study found that the immune response among study participants who had a documented SARS-CoV-2 infection were similar regardless of whether they received a fourth dose of a bivalent mRNA vaccine (Moderna), an ancestral protein-based vaccine (Novavax), or no fourth dose. Higher antibody responses at 6 months following fourth-dose vaccination were associated with a lower risk of SARS-CoV-2 infection, and that the time to infection from fourth-dose vaccination/enrolment for 10% of participants was longer in the Moderna group compared with the Novavax group or control group. These findings extend our understanding of the immune response to SARS-CoV-2 infection following fourth dose vaccination and have implications for defining CoP and timing of booster vaccination in adults.

During the study period, circulating SARS-CoV-2 variants in Australia were Omicron lineages. Our trial participants were infected with similar lineages, and these did not differ between arms, suggesting that vaccine-induced immune pressure did not affect variant susceptibility in this trial. The increased transmissibility but reduced severity of Omicron compared to earlier variants has been widely described^{17,18}. Despite this, COVID-19 booster vaccines continue to provide protection against SARS-CoV-2 infection in healthy adults^{19,20}, including findings from our trial that showed greater than 50% vaccine efficacy against SARS-CoV-2 infection for both vaccine groups in the first 6 months¹⁶. Protection is even greater for severe disease, with a meta-regression estimating vaccine effectiveness of 70% (three mRNA doses) and 68% (four mRNA doses) at 20 weeks post-vaccination²¹.

A novel finding was that humoral and IFN γ response following SARS-CoV-2 infection were similar regardless of whether participants received a fourth dose of a bivalent Moderna mRNA vaccine, an ancestral Novavax protein-based vaccine, or no fourth dose, despite differences in their pre-infection immune levels. The limited post-infection boosting effect (ceiling effect)²², particularly during the first 6 months after the fourth mRNA vaccine dose, is

likely attributable to the high pre-infection antibody levels and the short interval since the most recent antigenic exposure through vaccination. Although post-infection immune responses were comparable across groups, we did not assess symptom severity or time to recovery, which may have differed based on trial arm, although all documented infections were mild in this study.

We found that humoral, but not cellular, immune levels were associated with documented (symptomatic) SARS-CoV-2 infection following a fourth dose. A meta-analysis by Cromer and colleagues found that neutralising antibody activity is a CoP for symptomatic infection¹¹. In our study, pre-infection levels of binding IgG and sVNT (surrogate neutralisation) were not predictive of infection in the first 6 months, but higher levels of IgG (WT, BA.1, BA.4/5, JN.1) and sVNT (BA.1, BA.4/5) were associated with reduced odds of infection between 7-12 months. Interestingly, unlike BA.1 and BA.4/5, sVNT levels for WT were not predictive of infection, contrary to Cromer et al.'s finding that neutralising activity against WT (ancestral) was highly predictive of protection against infection with subsequent variants of concern¹¹. A possible explanation for this could be that our data was based on newer variants such as JN.1 and response to a fourth dose vaccine, compared to the data from Cromer et al. which was largely based on pre-Omicron variants. Moreover, our study reported sVNT response, which is different to live virus neutralisation, although it has been shown to correlate well²³. IFN γ levels were similar across timepoints and arms and were not predictive of infection. As cellular responses are recognised as a proxy for severe disease²⁴, it is likely that the similar cellular responses we observed was due to no cases of severe infection reported in our study. Additionally, we used a single IFN γ assay and did not have data on key immune cell subsets, such as memory B cells and CD4⁺/CD8⁺ T cells. We are currently conducting more comprehensive cellular analyses.

We previously demonstrated vaccine efficacy against SARS-CoV-2 infection at 6 months was 61% (95% CI: 38 – 76) for Moderna and 50% (23 – 68) for Novavax when we included documented and undocumented infections (participants who experienced a ≥ 1.2 fold-change of Spike-specific SARS-CoV-2 WT IgG between study visits).¹⁶ By 12 months, vaccine efficacy declined to 12% (95% CI: –18.7 to 34.4) and 17% (95% CI: –12.5 to 39.2), respectively, consistent with other studies showing that protection against symptomatic infection is relatively short-lived²⁵⁻²⁷. Here, we found that Moderna appeared to provide slightly longer-lasting protection against infection than Novavax or control group, although this advantage diminished by 6 months, consistent with our previously reported vaccine efficacy findings. However, these findings should be interpreted with caution as this exploratory analysis was underpowered and included only participants who self-reported symptoms and tested, making the results susceptible to reporting bias and potential underascertainment of infections. Undocumented cases could not be used for this analysis because the exact day of infection was not available.

The main strength of our study was the use of a large, well-characterised cohort of participants enrolled in a RCT of a fourth dose of COVID-19 vaccine, with longitudinal follow-up for SARS-CoV-2 infection and immune responses over 12 months. The main limitation was swabs and blood samples were collected from a subset of participants who had a documented infection, which may limit the generalisability of our findings. However, the baseline characteristics of this subset were similar to all participants who had a documented infection, and there were minimal differences in the SARS-CoV-2 variants identified across study arms, suggesting that this is unlikely to have substantially influenced the results. Another limitation is that some of the regression analyses may have been underpowered, particularly the 7–12-month subgroup analyses, and therefore limited statistical power to detect associations. Lastly, we were unable to assess the impact of the change in Moderna vaccine formulation from BA.1

to BA.4/5, and our results may not be generalisable to newer variants or variant-based vaccine responses.

In summary, we did not observe any difference in the response to SARS-CoV-2 infection by vaccine platform. Higher pre-infection antibody responses, but not cellular responses, were associated with reduced risk of subsequent infection. These findings contribute to our understanding of the immune CoP following COVID-19 booster vaccination. Longer-term follow-up will be important to determine the durability of these responses and the impact of repeated infection and vaccination on hybrid immunity.

Methods

Study design and participants

This study is a post-hoc, exploratory analysis of a participant-blind, parallel-group RCT originally designed to determine the immunogenicity, reactogenicity, and safety of a fourth dose of COVID-19 vaccine in Melbourne, Australia (trial registration number: NCT05543356)³.

We recruited adults aged ≥ 18 years who received their third COVID-19 vaccine dose at least 6 months prior and had no self-reported SARS-CoV-2 infection by polymerase chain reaction (PCR) or RAT) in the last three months. Enrolment in the control arm occurred through self-selection and participants were eligible if they were aged < 60 years (as per Australian Technical Advisory Group on Immunisation [ATAGI] recommendations for booster doses). Exclusion criteria included: current receipt of immunosuppressants or anti-cancer chemotherapy, receipt of immunoglobulin or other blood products in the last three months, known history of severe allergic reaction to any COVID-19 vaccines or medical exemption to receive further COVID-19 vaccines, and unable to read or understand English.

Study procedures

Participants were randomly assigned to receive either the bivalent Moderna (mRNA-1273.214; 25 μ g WT SARS-CoV-2 + 25 μ g Omicron variant [B.1.1.529]) or Novavax (NVX-COV2373; 5 μ g SARS-CoV-2 spike protein adjuvanted with 50 μ g Matrix-M) vaccine in a 1:1 allocation ratio and stratified by age (< 50 and ≥ 50 years). An independent statistician provided a secure, password-protected web-based randomisation schedule using random blocks of permuted length. In line with advice from ATAGI, the Moderna BA.1 bivalent vaccine was replaced by the BA.4/5 bivalent vaccine (mRNA-1273.222; 25 μ g WT SARS-CoV-2 + 25 μ g Omicron variant [BA.4/5]) on 26 April 2023. A non-blinded and non-randomised control arm

(no fourth COVID-19 vaccine dose) was enrolled concurrently. A subset of participants in each arm (~50%) was recruited into the cell-mediated immunity (CMI) analysis and monitored to ensure even representation of age strata (<50 and ≥50 years). A non-blinded study nurse administered the vaccine. Laboratory staff were blinded for all laboratory assays and analyses. Blood samples (5 mL) were taken at baseline (day 0), and at 28 days, 6 months and 12 months post-vaccination in the vaccine arms, and at baseline (day 0), 6 months and 12 months post-enrolment in the control arm. Additional blood samples (25 mL) were collected from participants in the CMI subset at each timepoint. All participants were given RAT kits and asked to test if they developed any symptoms of SARS-CoV-2 infection throughout the study period. Documented infection was defined as an infection identified by RAT ≥14 days post-vaccination and/or enrolment. For participants with infection, a nasopharyngeal (NP) swab was self-collected by the participants within five days and an additional blood sample (10 mL) at 28 days post-symptom onset was collected by the study team.

Laboratory procedures

Blood samples were processed for serum and stored at -80 °C until use. Binding IgG antibodies (against WT, Omicron BA.1, Omicron BA.4/5, JN.1) and functional antibodies (against WT, Omicron BA.1, Omicron BA.4/5) were measured on all samples. Binding IgG antibodies against WT and Omicron BA.1 were measured using the commercial EUROIMMUN S1 IgG ELISA kit (EUROIMMUN Medizinische Laboradiagnostika AG, Lübeck, Germany) and were reported as relative units/mL (RU/mL). WT IgG results were converted to binding antibody units (BAU/mL) as per the manufacturer's instructions (multiplication factor = 3.2), with WT IgG >35.2 BAU/mL and Omicron BA.1 IgG >11 RU/mL considered positive. Binding IgG antibodies against Omicron BA.4/5 and JN.1 were measured using an in-house ELISA, as previously described,²⁸⁻³¹ and reported as ELISA units/mL

(EU/mL). Omicron BA.4/5 or JN.1 ≥ 1.5 EU/mL was considered positive. Functional antibodies were measured using a surrogate virus neutralising test (sVNT) (GenScript cPass SARS-CoV-2, New Jersey, USA) as per the manufacturer's instructions and reported in units/mL (U/mL). Additional whole blood samples from the CMI subset were stimulated with antigen coated blood collection tubes (Mitogen, Ag1 and Ag2) or unstimulated (nil) for 24 hours, followed by plasma isolation and stored at -80°C until use. Interferon-gamma (IFN γ) was measured using the QuantiFERON Human IFN γ SARS-CoV-2 kit (QIAGEN, Hilden, Germany) as per the manufacturer's instructions, background corrected for negative control (nil) and reported in international units/mL (IU/mL).

NP swabs were stored at -80 °C until use. Viral RNA was extracted from NP swabs using the QIAamp viral RNA kit (QIAGEN, Hilden, Germany) as per the manufacturer's instructions. SARS-CoV-2 viral load was measured using a real-time reverse transcription PCR panel targeting nucleocapsid protein genes (N1 and N2) developed by the U.S. Centers for Disease Control and Prevention (CDC),³² and a standard (Exact Diagnostics, Texas, USA) was used as the standard curve for each assay. Whole genome sequencing was performed as previously described.³³ Samples were classified into recognised SARS-CoV-2 lineages using Pangolin.³⁴

Statistical analysis

We used Stata/SE 19.5 for statistical analyses and to generate figures. Publicly available data from GISAID were used to plot the distribution of variants circulating in Australia during the study period.³⁵ Sequencing data from NP swabs were used to plot the distribution of variants in our RCT. For the remaining analyses, we used infection status and levels of IgG (WT, BA.1, BA.4/5, JN.1), sVNT (WT, BA.1, BA.4/5) and IFN γ (Ag1 and Ag2), hereafter denoted as immune levels, at each timepoint. Infection events were aggregated by study period (0-6 and

7-12 months). For participants who experienced ≥ 1 infection during the study period, only the first event was used in our analysis. The pre-infection timepoint was defined as the study visit immediately preceding the infection event and the post-infection timepoint was the day 28 post-infection visit. For the immune parameters analysis at 28 days post-infection, we used scatterplots with medians and interquartile ranges (IQR) to visualise the immune parameters as follows: (1) paired levels at the pre- and post-infection timepoints; (2) levels at the post-infection timepoint; (3) levels at the pre-infection timepoint; and (4) levels at 6 and 12 months post-enrolment. We calculated geometric mean ratios (GMR) to compare IgG, sVNT and IFN γ levels at (1) the post- vs pre-infection timepoints (paired); and (2) Moderna vs Novavax (unpaired).

To assess the relationship between immunological markers (IgG, sVNT and IFN γ) and infection during the first 6 months post-vaccination and the subsequent 6-month period, we performed multivariable logistic regression, adjusted for age and study arm, and reported results as odds ratios (OR) and 95% confidence intervals (CI); for this analysis, participants who experienced a ≥ 1.2 fold-change of Spike-specific SARS-CoV-2 WT IgG between study visits were excluded to minimise the inclusion of participants with potential undocumented SARS-CoV-2 infection, as previously defined¹⁶. Exposure variables (pre-infection immune levels) were scaled to 100 units/mL (IgG), 50,000 U/mL (sVNT) and 1 IU/mL (IFN γ).

For time to first infection from fourth dose vaccination, we plotted Kaplan-Meier curves of infection and calculated survival times to infection at the 10th, 20th and 30th percentile (i.e. 10, 20 and 30 percent survival probability), by arm and levels of immune parameters at the pre-infection timepoint. Immune levels were classified as low (< 25th percentile), medium (25th-75th percentile) and high (>75th percentile). Further details regarding statistical analyses are provided in the corresponding figure and table legends.

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359 The funder had no role in the study design, data collection, data analysis, data interpretation,
360 writing of the report, or decision to publish.

361

362 *Ethics and inclusion statement*

363 Ethics approval was obtained by The Royal Children's Hospital Melbourne Human Research
364 Ethics Committee (HREC 91108). All participants provided written informed consent to enrol
365 in the trial.

366

367 **Author contributions**

368 Conceptualization: NM, ZQT, LAHD, CDN, KM, CVM and PVL; Data curation: EFGN and
369 KAM; Formal analysis: DSO, NM, ZQT, LAHD, CDN and PVL; Funding acquisition: KM,
370 CVM and PVL; Investigation: DSO, NM, ZQT, LAHD, SL, LQ, YYN, RC, JDH, SD and RAH;
371 Methodology: NM, ZQT and LAHD; Project administration: DSO, NM, KB, AR, FJ and EW;
372 Supervision: NM, ZQT, LAHD, SM, KS, KM, CVM and PVL; Validation: NM, ZQT and
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374 authors.

375

376 **Declaration of competing interests**

377 The authors have no conflicts of interest to disclose.

378

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Data availability

The de-identified participant data that support the findings of this study are available from the corresponding author upon reasonable request, subject to institutional ethics approval and data sharing agreements.

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Figure legends

Figure 1. (A) Study overview, (B) participant flow chart and (C) SARS-CoV-2 variants circulating in Australia during the study period. Study overview was created with BioRender.com. Data of SARS-CoV-2 variants circulating in Australia were obtained from GISAID (<https://gisaid.org/hcov-19-variants-dashboard/>) (cited 11 Dec 2025).

Figure 2. Paired SARS-CoV-2 IgG, sVNT and IFN γ responses at the pre-infection and day 28 post-infection visits for participants with SARS-CoV-2 infection, by vaccine arm and timepoint. This analysis only includes the first SARS-CoV-2 infection event for each participant and excludes SARS-CoV-2 infections that occurred within 14 days of enrolment or after the 12-month visit. The preceding study visit for 0-6 months is day 0 (control arm) or day 28 (vaccine arms), and for 7-12 months is 6 months (all arms). Graphs for IFN γ are in the linear scale due to small values. A zero value for sVNT BA.1 0-6 months was not plotted to maintain visualisation on the logarithmic scale. Solid vertical lines are medians and interquartile ranges (IQR). Coloured lines connect the medians of the pre- and post-infection visits. 0-6 months: Moderna (N=13), Novavax (N=17), Control (N=18); 7-12 months: Moderna (N=22), Novavax (N=15), Control (N=12).

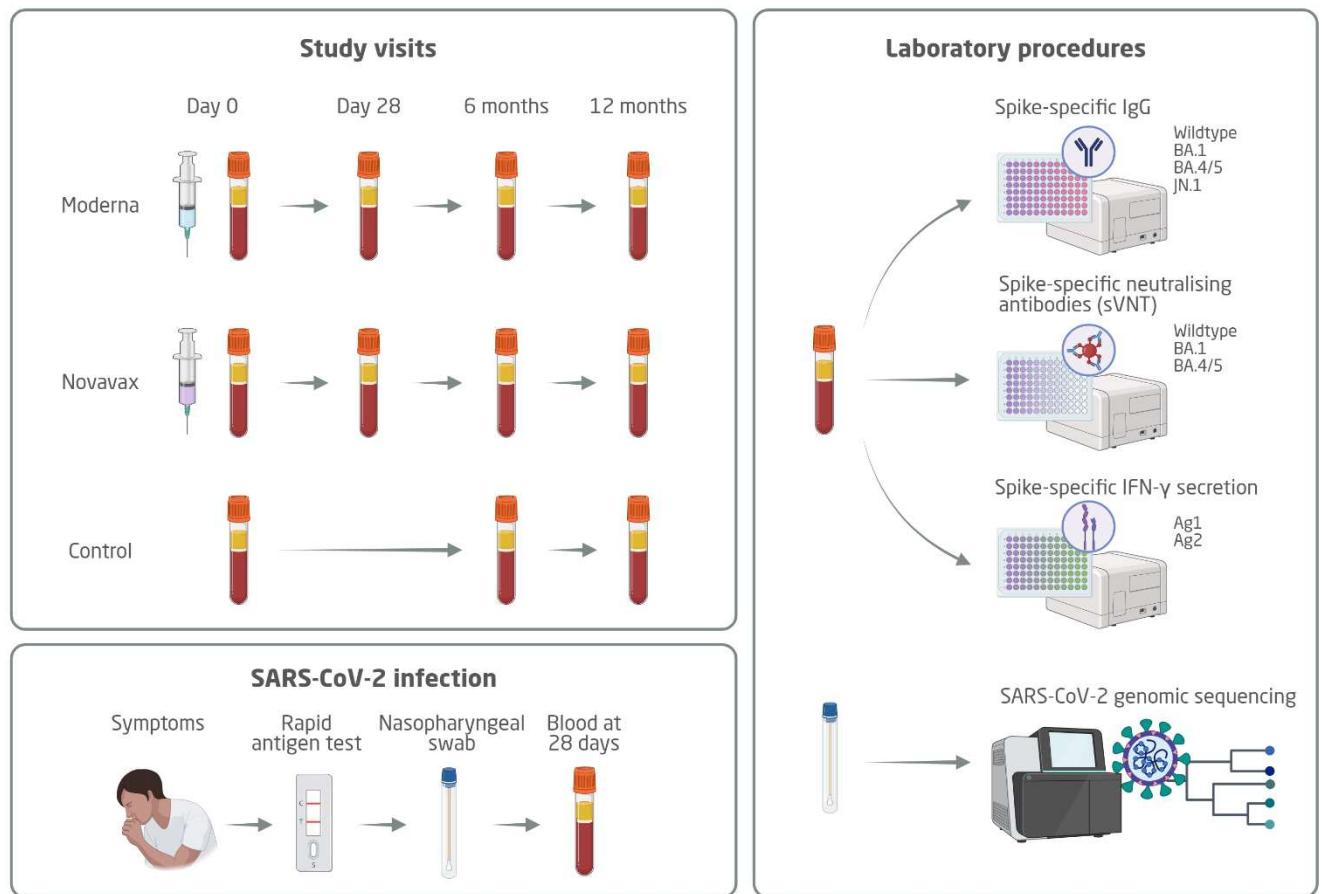
Figure 3. SARS-CoV-2 IgG, sVNT and IFN γ responses at the preceding study visit for participants with and without SARS-CoV-2 infection, by vaccine arm and timepoint. This analysis only includes the first SARS-CoV-2 infection event for each participant and excludes SARS-CoV-2 infections that occurred within 14 days of enrolment or after the 12-month visit. This analysis excludes participants who experienced a ≥ 1.2 fold-change of Spike-specific SARS-CoV-2 WT IgG between study visits. The preceding study visit for 0-6 months is day 0 (control arm) or day 28 (vaccine arms), and for 7-12 months is 6 months (all arms). Graphs for

IFN γ are in the linear scale due to small values. A zero value for sVNT BA.1 0-6 months was not plotted to maintain visualisation on the logarithmic scale. Solid lines are medians and interquartile ranges (IQR). 0-6 months: IgG (N=436), sVNT (N=436), IFN γ (N=235); 7-12 months: IgG (N=427), sVNT (N=427), IFN γ (N=231).

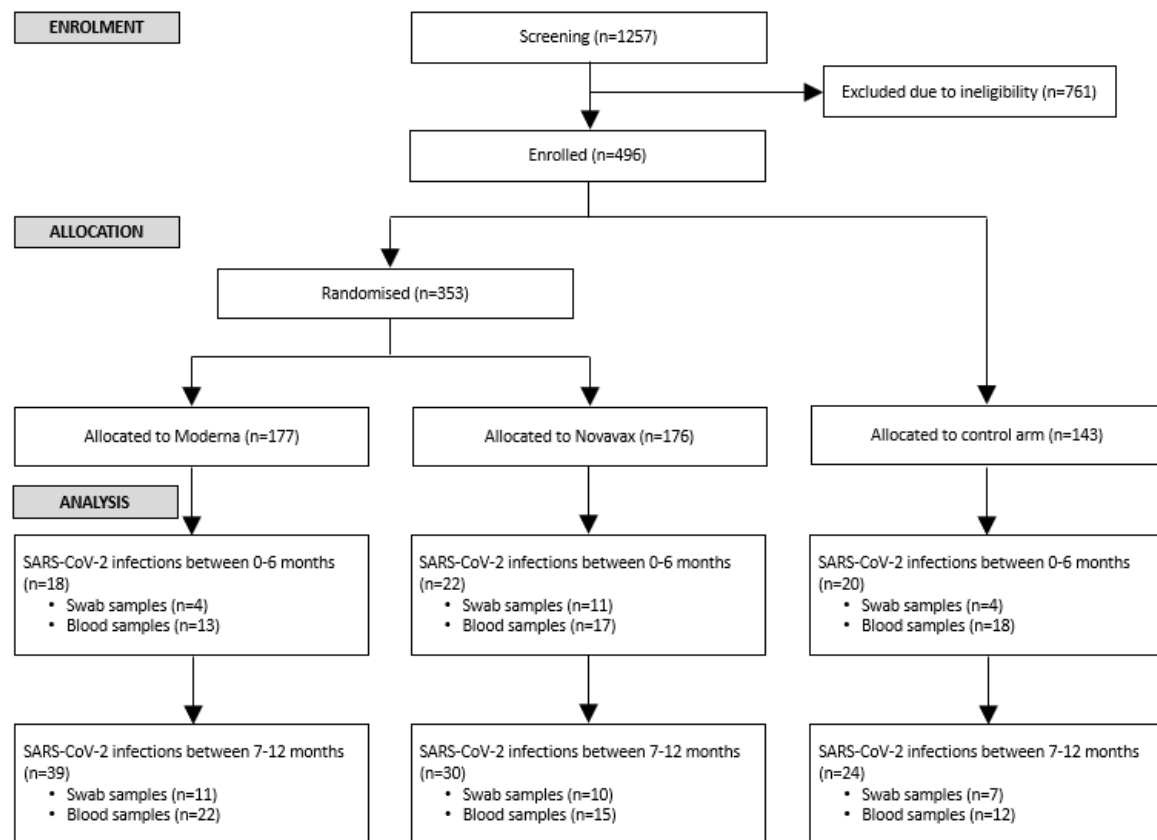
Figure 4. (A) Kaplan-Meier curves of SARS-CoV-2 infection and (B) survival time to infection, by vaccine arm. This analysis only includes the first SARS-CoV-2 infection event for each participant and excludes SARS-CoV-2 infections that occurred within 14 days of enrolment or after the 12-month visit. For participants whose first SARS-CoV-2 infection occurred within 14 days of enrolment but had a subsequent infection before the 12-month visit, the second infection was used in this analysis. The 10th, 20th and 30th percentiles of survival times (days [95% confidence intervals]) were calculated.

Figure 1.

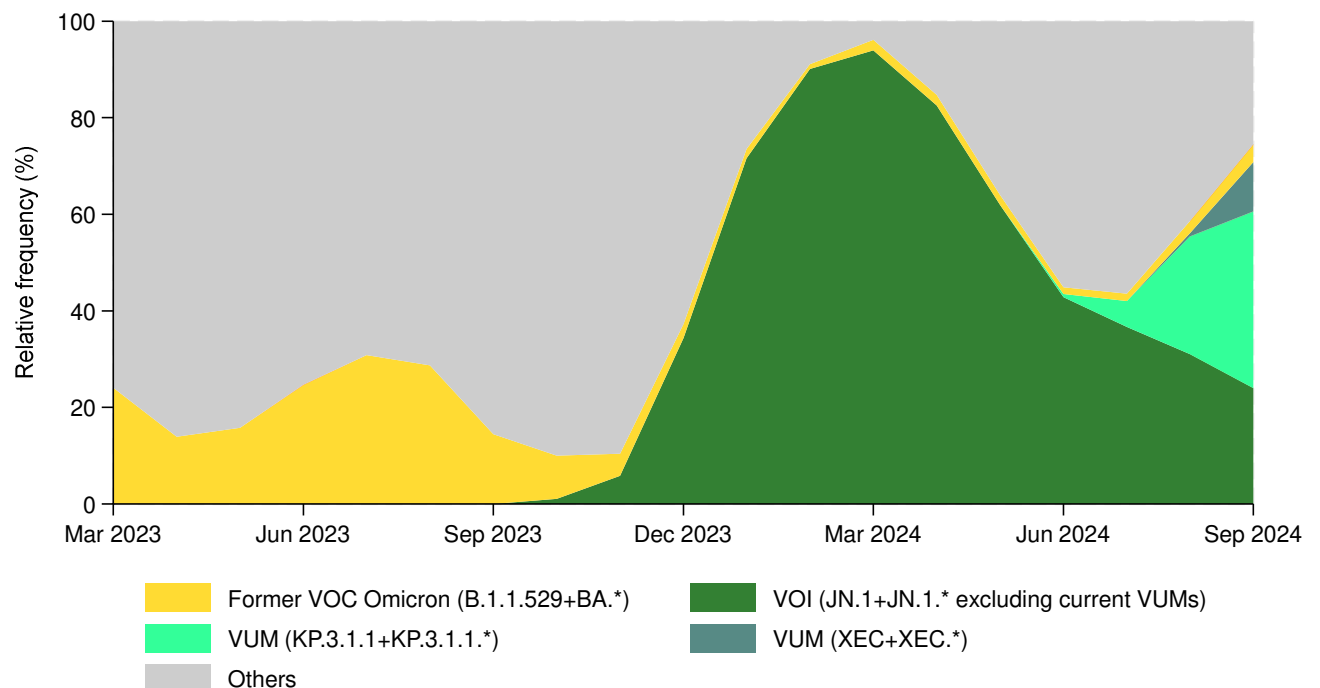
(A)



(B)

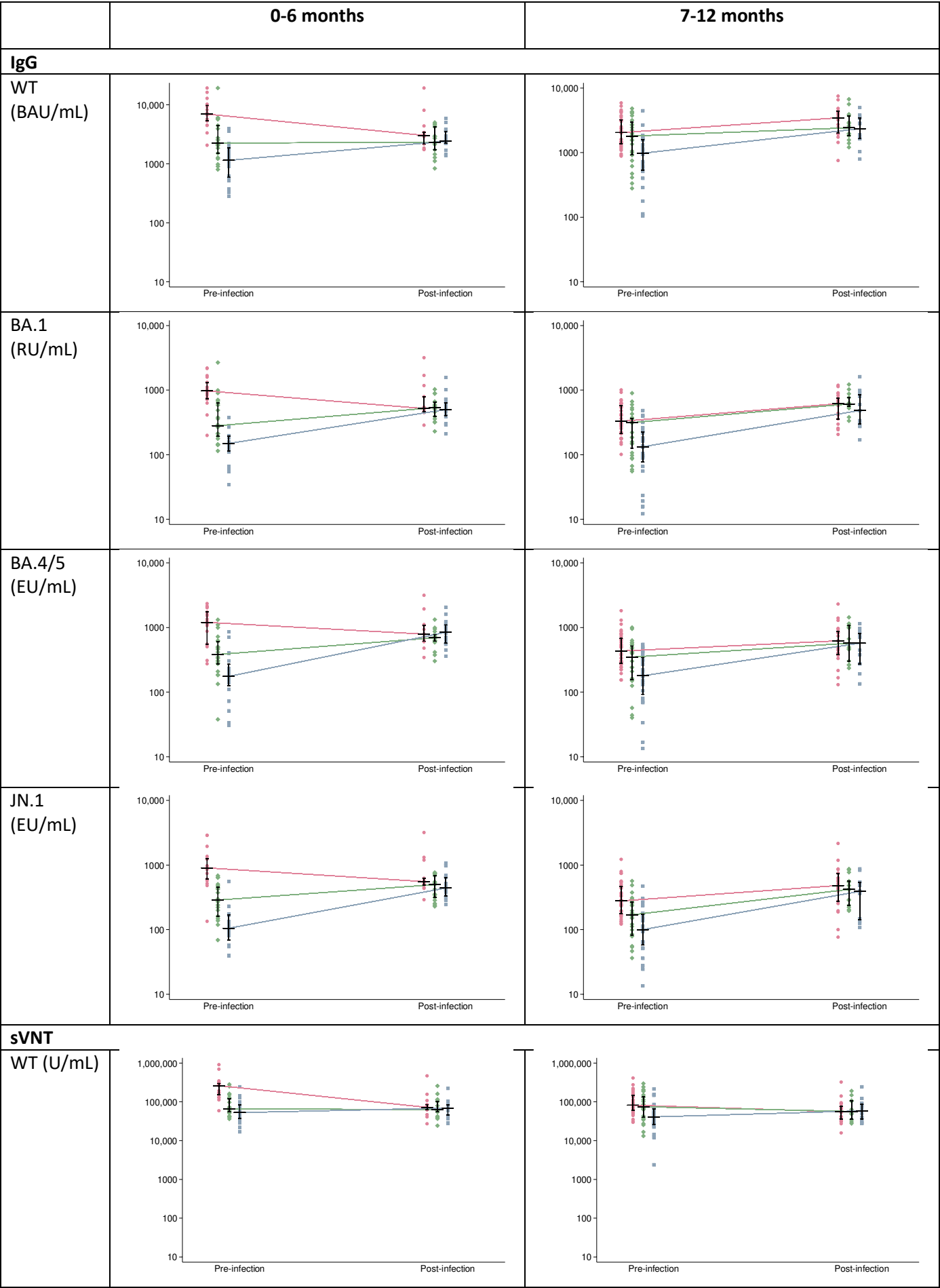


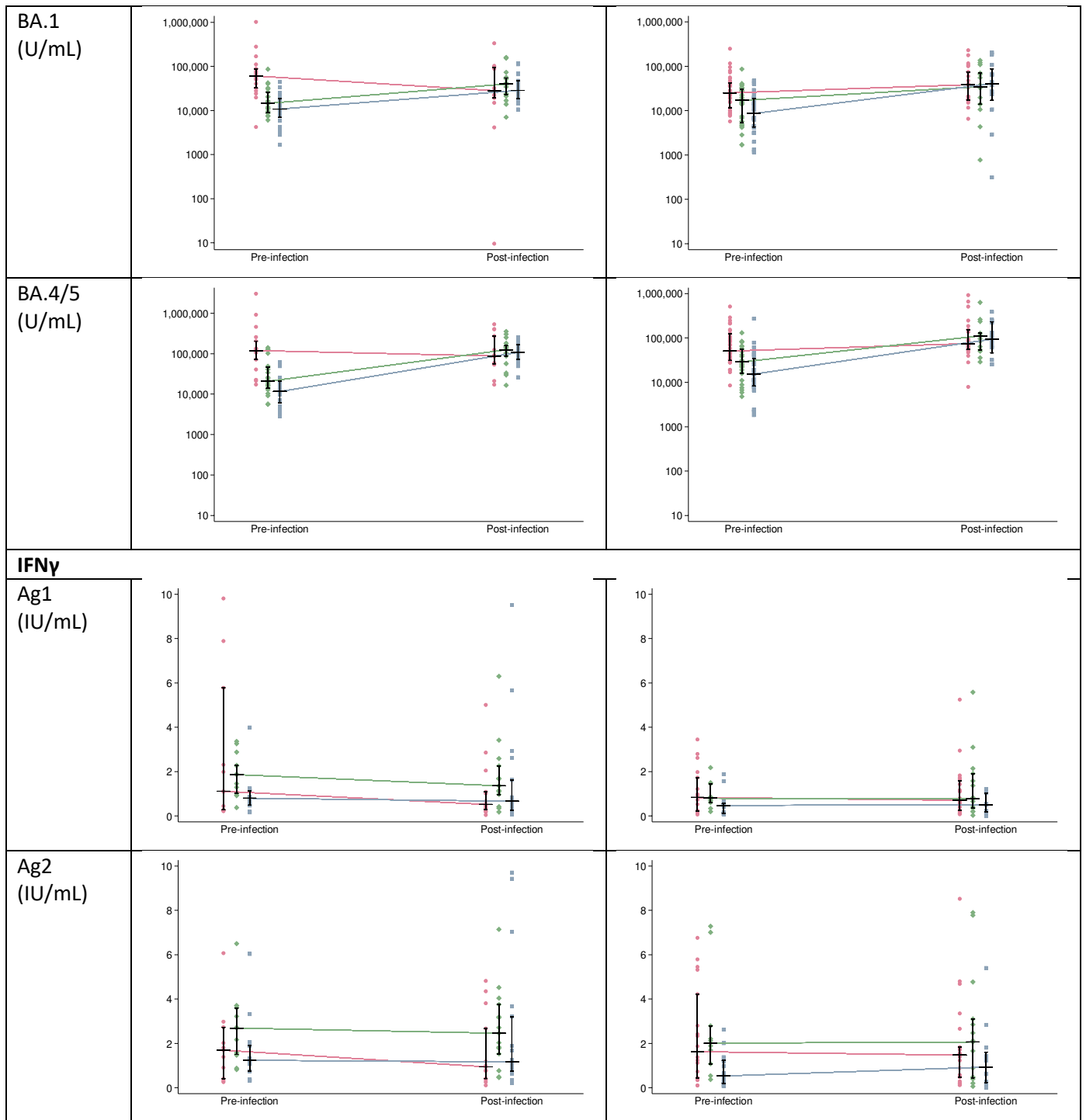
(C)



BMI: body mass index; VOC: variant of concern; VUM: variant under monitoring.

Figure 2.

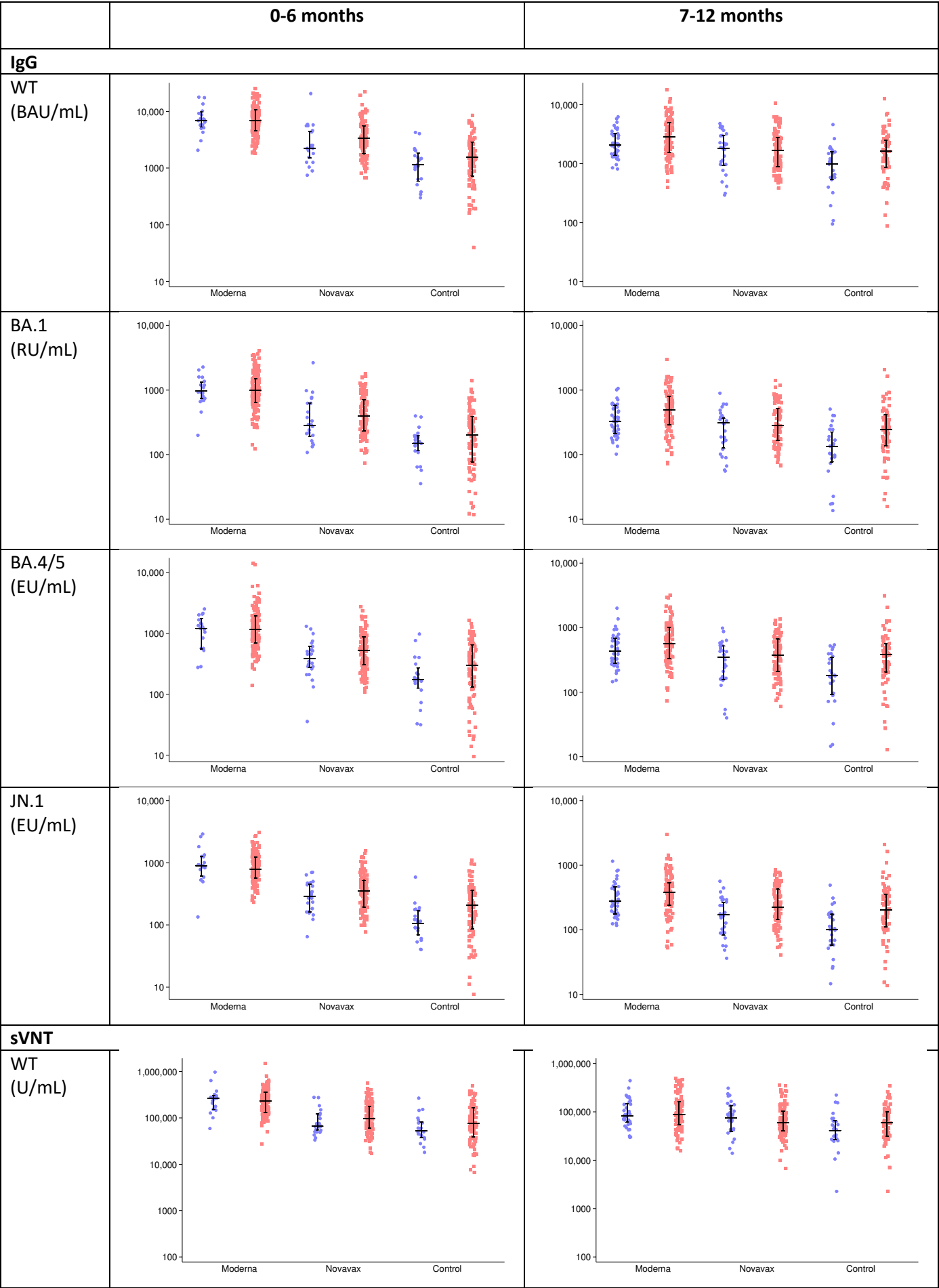


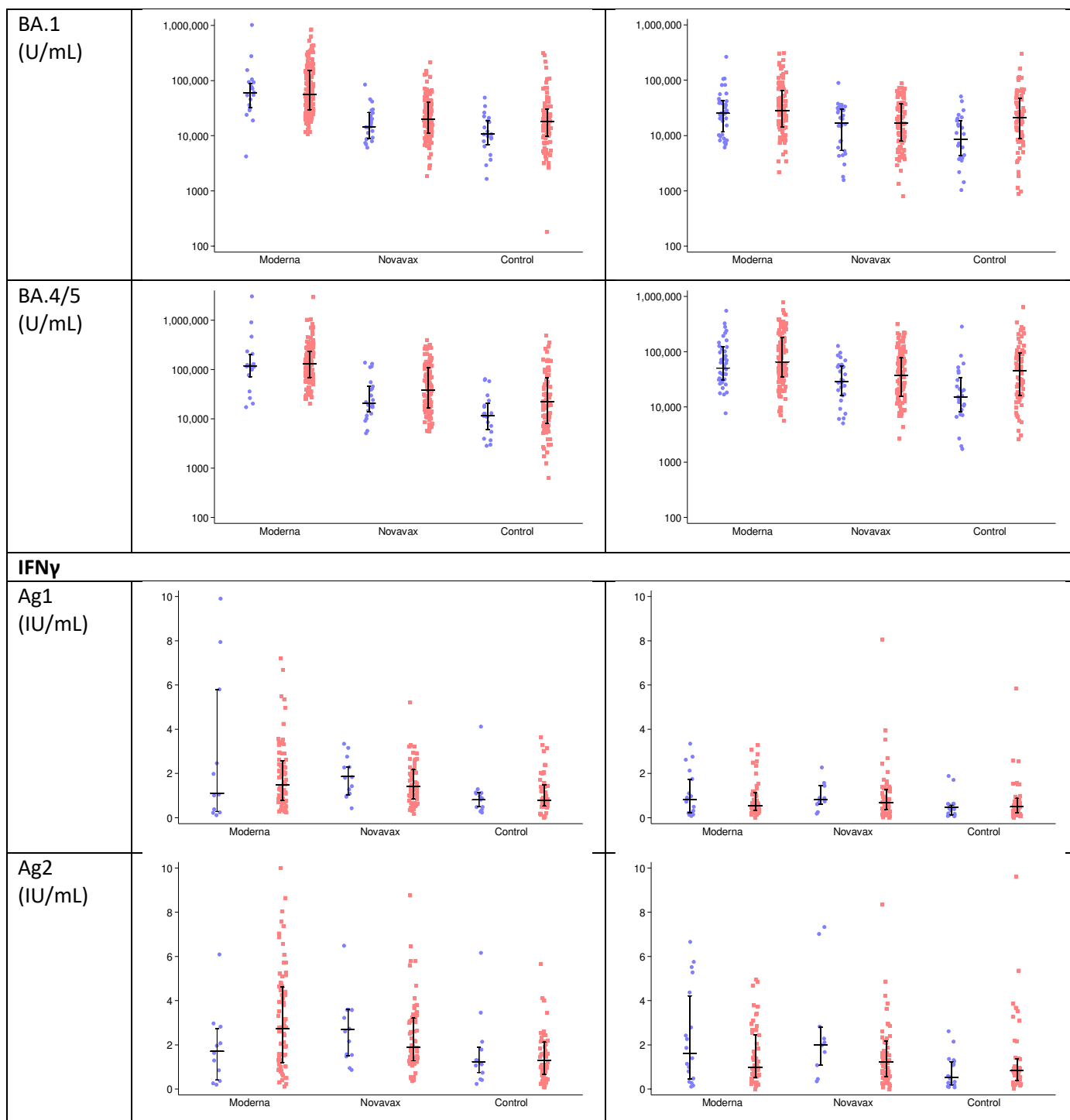


● Moderna
 ◆ Novavax
 ■ Control

Ag: antigen; BAU: binding antibody units; EU: ELISA units; IFN γ : interferon-gamma; IU: international units; RU: relative units; sVNT: surrogate virus neutralisation test; U: units; WT: wildtype.

Figure 3.



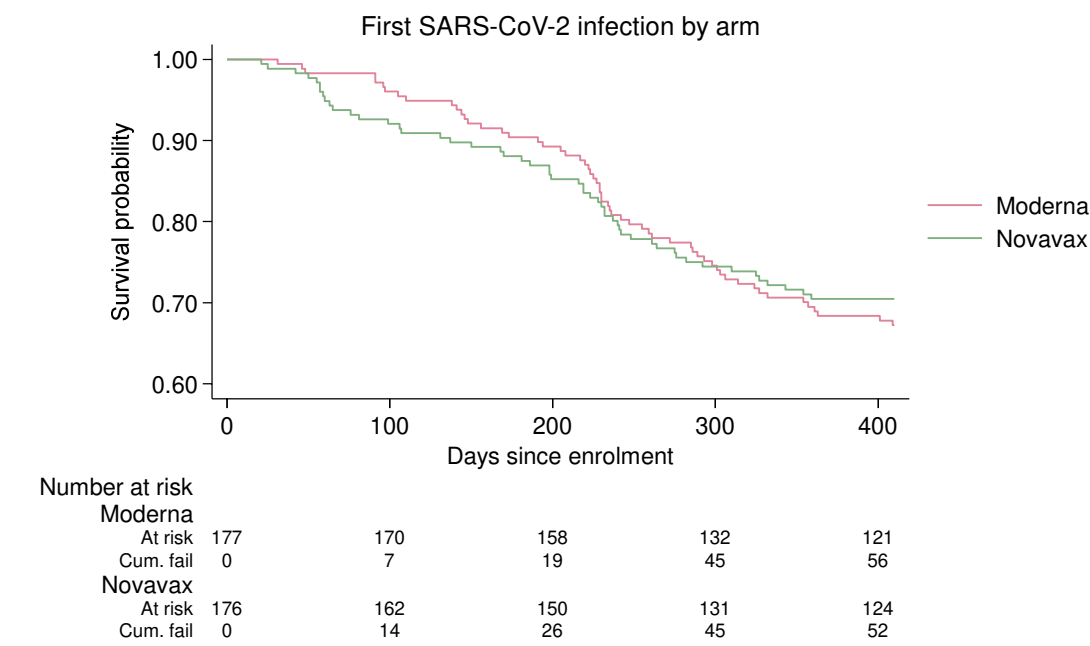


• SARS-CoV-2 infection ■ No infection

Ag: antigen; BAU: binding antibody units; EU: ELISA units; IFN γ : interferon-gamma; IU: international units; RU: relative units; sVNT: surrogate virus neutralisation test; U: units; WT: wildtype.

Figure 4.

(A)



(B)

Arm	10 th percentile	20 th percentile	30 th percentile
	191	247	357
Moderna	(138, 225)	(223, 301)	(285, -)
	137	240	-
Novavax	(63, 199)	(198, 310)	(264, -)

Table 1. Baseline characteristics of participants.

	All participants			Participants with a SARS-CoV-2 infection		
	Moderna (N=177)	Novavax (N=176)	Control* (N=143)	Moderna (N=57)	Novavax (N=52)	Control* (N=44)
Age (years), median (IQR)	30.4 (25.7, 42.4)	30.7 (25.2, 42.7)	39.2 (28.9, 50.8)	33.8 (25.7, 51.3)	32.2 (26.6, 39.0)	44.3 (30.7, 51.3)
<50, n (%)	150 (84.8)	149 (84.7)	100 (69.9)	41 (71.9)	48 (92.3)	28 (63.6)
≥50, n (%)	27 (15.3)	27 (15.3)	43 (30.1)	16 (28.1)	4 (7.7)	16 (36.4)
Female, n (%)	116 (65.5)	128 (72.7)	114 (79.7)	39 (68.4)	43 (82.7)	36 (81.8)
Comorbidities, n (%)						
Obesity (BMI ≥30 kg/m ²)	28 (15.8)	33 (18.8)	21 (14.7)	9 (15.8)	12 (23.1)	8 (18.2)
Hypertension	8 (4.5)	9 (5.1)	8 (5.6)	3 (5.3)	1 (1.9)	2 (4.6)
Diabetes mellitus	4 (2.3)	4 (2.3)	3 (2.1)	0	1 (1.9)	0
Cardiovascular disease	1 (0.6)	3 (1.7)	1 (0.7)	0	1 (1.9)	0

*IQR: interquartile range. *This was a non-randomised arm.*

Table 2. Multivariable logistic regression of IgG, sVNT and IFN γ pre-infection levels and SARS-CoV-2 infection, adjusted for age and study arm. The comparison group is participants with no SARS-CoV-2 infection, which excludes participants who experienced a ≥ 1.2 fold-change of Spike-specific SARS-CoV-2 WT IgG between study visits. This analysis only includes the first SARS-CoV-2 infection event for each participant and excludes SARS-CoV-2 infections that occurred within 14 days of enrolment (control arm), within 28 days of enrolment (vaccine arms) or after the 12-month visit (all arms). IgG, sVNT and IFN γ pre-infection levels for 0-6 months were measured at day 0 (control arm) or day 28 (vaccine arms), and for 7-12 months at 6 months (all arms). Immune levels were scaled by 100 units for IgG, 50,000 units for sVNT and 1 unit for IFN γ .

	0-6 months		7-12 months	
	OR (95% CI)	p	OR (95% CI)	p
IgG	N=428		N=369	
WT (BAU/mL)	1.00 (0.99, 1.00)	0.368	0.97 (0.96, 0.99)	0.001
BA.1, (RU/mL)	0.97 (0.91, 1.03)	0.348	0.80 (0.72, 0.90)	<0.001
BA.4/5, (EU/mL)	0.95 (0.90, 1.01)	0.083	0.86 (0.80, 0.94)	<0.001
JN.1, (EU/mL)	0.99 (0.92, 1.07)	0.829	0.77 (0.67, 0.88)	<0.001
sVNT (U/mL)	N=428		N=369	
WT	0.92 (0.81, 1.05)	0.218	0.91 (0.79, 1.06)	0.231
BA.1	0.97 (0.81, 1.15)	0.693	0.58 (0.38, 0.88)	0.011
BA.4/5	1.02 (0.97, 1.07)	0.519	0.79 (0.67, 0.94)	0.007
IFNγ (IU/mL)	N=232		N=198	
Ag1	1.21 (0.95, 1.54)	0.132	0.98 (0.68, 1.41)	0.905
Ag2	0.92 (0.73, 1.15)	0.455	1.18 (0.97, 1.44)	0.099

Ag: antigen; BAU: binding antibody units; EU: ELISA units; IFN γ : interferon-gamma; IU: international units; RU: relative units; sVNT: surrogate virus neutralisation test; U: units; WT: wildtype.

Supplementary Files

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