# The persistence of neutralising antibodies up to 11 months after SARS-CoV-2 infection in the southern region of New Zealand

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which causes coronavirus disease 2019 (COVID-19), was first detected in New Zealand in February 2020. Following this initial introduction, and a community outbreak of 1,154 confirmed cases, New Zealand successfully eliminated the virus in the community.[1] With the exception of several isolated border incursions and short lockdowns in Auckland, the country remained largely COVID-free until the outbreak of the delta SARS-CoV-2 variant, which began in August 2021.

The emergence of novel viral variants of concern (VoC), such as delta (B.1.617.2) and most recently omicron (B.1.1.529), combined with reports of the gradual waning of antibodies over extended timeframes,[2] highlights a need for ongoing studies tracking immune responses following natural infections and vaccination, particularly since the initial waves of infections, and the currently licenced vaccines, are based on the original SARS-CoV-2 strain rather than VoC that have dominated global infections subsequently. Here we present a follow-up serological assessment of PCR-confirmed cases nearly oneyear post-infection, including levels of neutralising antibodies to alpha, beta, delta and omicron VoC.

During the first wave of infection in New Zealand, a cohort of PCR-confirmed COVID-19 cases was recruited in the Southern District Health Board (SDHB) region.[3] We have previously reported on antibody dynamics in this cohort, alongside participants from other cohorts, up to eight-months post-infection.[4] Antibody (IgG) responses to the viral spike protein and neutralising antibodies were relatively stable over this eight-month period compared with antibodies to the nucleocapsid protein. This persistence of spike-specific antibodies compared with the rapid decay of nucleocapsid antibodies has since been widely demonstrated, with the latter now being utilised as a marker of recent infection.[5]

The original SDHB cohort comprised n=78 PCR confirmed cases infected between 11 March and 5 April 2020, with up to three serum samples collected post-symptom onset (Figure 1 (clear circles) and Table 1). Of these, 30 participants donated further samples at later time points, represented as red circles in Figure 1. As there were no successive community outbreaks in SDHB during the study timeframe, nor had any participants received a COVID-19 vaccine, the immune responses observed likely represent a single exposure event tracked over the time course. Median days post symptom onset for this additional timepoint was 302 days (Table 1). Samples were assayed for antibodies to both nucleocapsid (Abbott Architect SARS-CoV-2 IgG assay, Figure 2a) and spike proteins (Abbott Alinity SARS-CoV-2 IgG II Quant assay, Figure 2b). We have previously reported 99.7% specificity for the nucleocapsid assay using 300 prepandemic anti-natal samples.[3] The same procedure was followed for the recently released Spike Alinty IgG assay for this study, for a calculated specificity of 100% (0/100 of anti-natal samples with sera available were above the 50 AU/mL cut-off).

Neutralising antibodies were measured using a surrogate viral neutralisation test (sVNT), based on the receptor binding domain of the spike protein (cPass™ SARS-CoV-2 Neutralization Antibody Detection Kit, GenScript). This domain contains >90% of neutralising antibody epitopes—that is, regions that block the entry of the virus into host cells via the hACE-2 receptor.[6] Specificity was previously determined to be 100% using the 300 anti-natal samples and an additional 113 pre-pandemic samples.[7]

Recent analyses suggest that the level of neutralising antibodies is an important component

**Figure 1:** Cohort summary. Individual participants are ordered by days post onset of symptoms, with temporal samples from the same individuals connected by grey lines. Samples were obtained at one to four timepoints over the study period. Samples included in this study are indicated by red circles with ealier timepoints indicated by unfilled circles.

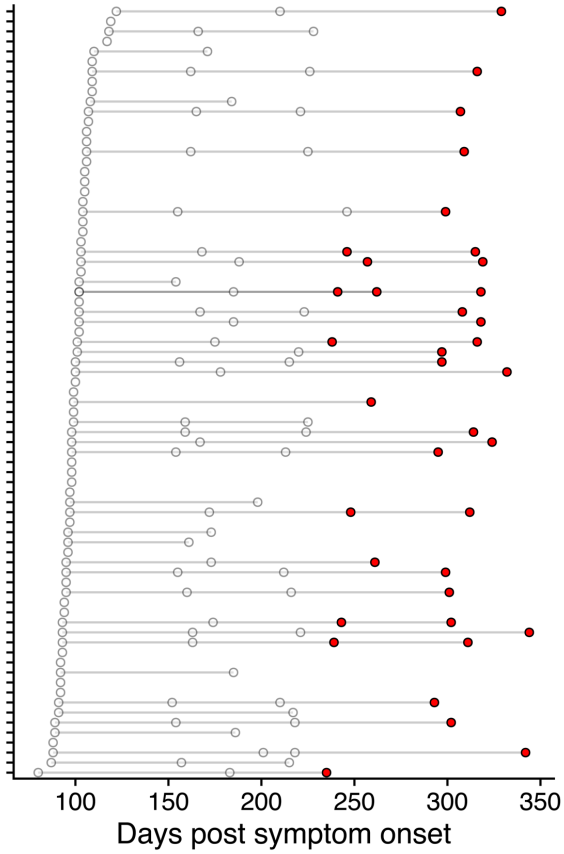



**Table 1:** Cohort demographics. All participants in this study had mild to moderate symptoms and none were admitied to hospital.

| | **Total** | **This study** |
|---|---|---|
| Participants, *n* (samples, *n*) | 78 (172) | 30 (37) |
| Sex, *n* (M/F) | 31/47 | 9/21 |
| **Age (year)** | | |
| Median | 51.5 | 52 |
| Range | 17-81 | 27-81 |
| **Days post symptom onset (days)** | | |
| Median | 158 | 302 |
| Range | 80–344 | 235–344 |

of a correlate of protection.[8] There is now intense effort on understanding how sequence changes within the receptor binding domain in VoC might impact on neutralising antibody activity, and protection from re-infection.[6] In this study neutralising antibodies were assessed to alpha (B.1.1.7), beta (B.1.351), delta (B.1.617.2) and omicron (B.1.1.529) VoC using an adapted sVNT assay that incorporates receptor binding domains corresponding to the sequence for each of these variants.[9]

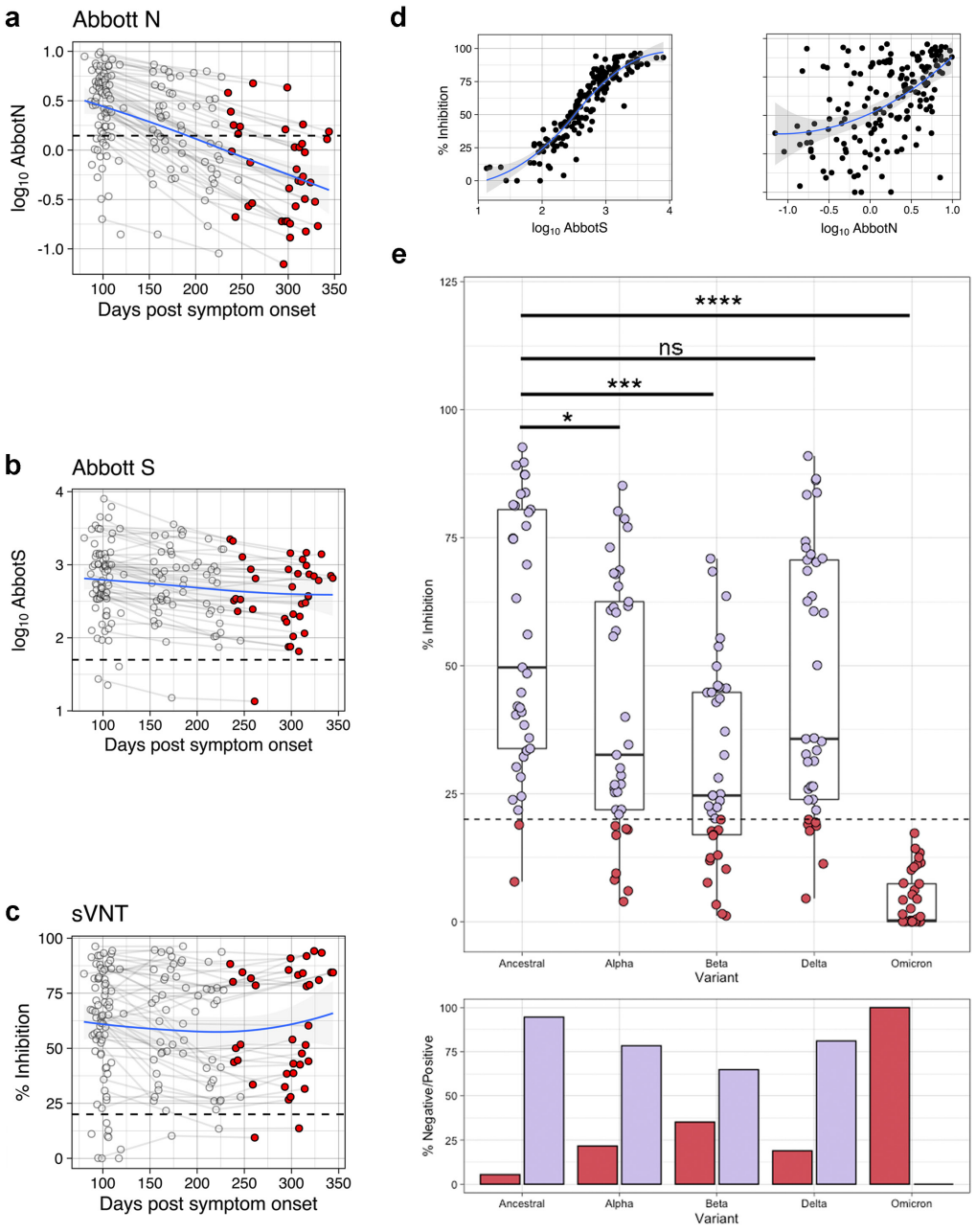
With the inclusion of later time-points, the relative stability of spike antibodies (Figure 2b), compared with nucleocapsid antibodies (Figure 2a), has been confirmed. Indeed, nucleocapsid antibodies continue to decline rapidly between 8 and 11 months, with 27/37 (72.97%) below the cut-off at this later time-point. This contrasts with spike antibodies where 36/37 (97.30%) remained positive at the later timepoint. Similarly, neutralising antibodies to the original SARS-CoV-2 virus showed no decline at this later timepoint (Figure 2c), with 35/37 (94.59%) remaining positive. This trend was also reflected in the strong correlation between spike and neutralising antibodies, which was not observed with nucleocapsid antibodies (Figure 2d).

To assess the neutralisation capacity of sera against VoC generated by a single natural exposure approximately 300 days prior, the sVNT with alpha, beta, delta and omicron was performed following standard protocols.[4,9] There was a dramatic and highly significant reduction in neutralisation capacity to the omicron variant compared with the ancestral strain (median inhibition 0.23% versus 49.7%, $p<0.0001$). There was also a notable reduction to the beta variant (median inhibition 24.7% versus 49.7%, $p<0.0001$), and a significant, but less marked, reduction to the alpha variant (32.6%, $p<0.05$). In contrast, there was a non-significant reduction to the delta variant (35.7%, $p=0.117$) (Figure 2e). This trend was mirrored in the proportion of samples below the assay cut-off (<20% inhibition), with omicron showing the highest proportion of negative samples (37/37, 100%) compared with beta (13/37, 35.1%), alpha (8/37, 21.6%) and delta (7/37, 18.9%) (Figure 2e).

These data are in keeping with observations internationally where the beta and omicron variants are thought to evade humoral immunity compared to the alpha and delta variants.[2,10,11] For beta, this is partly driven by the E484K mutation in the beta receptor binding domain that interferes with antibodies generated in response to ancestral strain sequences.[12] The omicron variant, first identified in November 2021, harbours a staggering 15 mutations in the receptor binding domain including at the 484 position.[10] Recent data have shown omicron crossneutralisation from a previous, non-omicron infection is extremely limited by six-months post-infection,[11] consistent with the lack of omicron cross-neutralisation observed in our study up to 11-months post-infection. This, combined with the increased transmissibility associated with omicron, highlights the need for vaccination of previously infected individuals as omicron surges globally. Although two doses of the Comirnaty (Pfizer/BioNTech) vaccine, which is based on the original spike protein sequence and currently being administered in New Zealand, produces antibodies that effectively neutralise the delta variant, a third booster dose is needed to restore high levels of neutralisation against omicron.[10]

In conclusion, this study provides novel insight from a unique setting in the Southern region of New Zealand where the probability of SARS-CoV-2 re-exposure has been extremely unlikely. Although a single exposure generated a neutralising antibody response that persists for at least 11 months for the original and delta variant, this was not the case for omicron. Given the risks of serious disease associated with SARS-CoV-2 infection, and the ongoing omicron surge, vaccination remains strongly recommended.

**Figure 2**: Antibody responses following SARS-CoV-2 infection over time. Antibody responses targeting Nucleocapsid (N) protein (a), spike (S) protein (b) as well as neutralising antibodies (c) over time. New samples are indicated by red circles with previously reported samples inidcated by unfilled circles (n=172). (d) Correlation between S protein antibodies and N protein antibodies versus neutralising antibodies. LOESS regression line shown in blue and standard error of regression is shaded in grey (n=172), with the residual standard error being 0.208 and 0.422 for S protein and N protein antibodies, respectively. When Spearman linear regression is applied the $r^2$ are 0.87 ($p<0.001$) for S protein and 0.28 ($p<0.001$) for N protein antibodies versus neutralising antibodies. (e) Neutralising antibody responses to variants of concern including only the most recent samples (represented by red circles in a-c) (top). Kruskal-Wallis test showed a significant difference ($p<0.001$) with follow up Wilcoxon test Holm adjusted p-values indicated by stars, *$p<0.05$, ***$p<0.001$, ****$p<0.0001$ (n=37). Percentage of samples above (purple) and below (pink) the assay cut-off (bottom). Dashed horizontal lines represent respective test cut-offs throughout.



**COMPETING INTERESTS**
Nil.

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