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Antibody correlates of protection from SARS-CoV-2 reinfection prior to vaccination: A nested case-control within the SIREN study

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SUMMARY

Objectives: To investigate serological differences between SARS-CoV-2 reinfection cases and contemporary controls, to identify antibody correlates of protection against reinfection.
Methods: We performed a case-control study, comparing reinfection cases with singly infected individuals pre-vaccination, matched by gender, age, region and timing of first infection. Serum samples were tested for anti-SARS-CoV-2 spike (anti-S), anti-SARS-CoV-2 nucleocapsid (anti-N), live virus microneutralisation (LV-N) and pseudovirus microneutralisation (PV-N). Results were analysed using fixed effect linear regression and fitted into conditional logistic regression models.
Results: We identified 23 cases and 92 controls. First infections occurred before November 2020; reinfections occurred before February 2021, pre-vaccination. Anti-S levels, LV-N and PV-N titres were significantly lower among cases; no difference was found for anti-N levels. Increasing anti-S levels were associated with reduced risk of reinfection (OR 0·63, CI 0·47-0·85), but no association for anti-N levels (OR 0·88, CI 0·73-1·05). Titres >40 were correlated with protection against reinfection for LV-N Wuhan (OR 0·02, CI 0·001–0·31) and LV-N Alpha (OR 0·07, CI 0·009–0·62). For PV-N, titres >100 were associated with protection against Wuhan (OR 0·14, CI 0·03–0·64) and Alpha (0·06, CI 0·008–0·40).

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Conclusions: Before vaccination, protection against SARS-CoV-2 reinfection was directly correlated with anti-S levels, PV-N and LV-N titres, but not with anti-N levels. Detectable LV-N titres were sufficient for protection, whilst PV-N titres >100 were required for a protective effect.
Trial registration number: ISRCTN11041050

Introduction

The durability of infection-acquired immunity and the nature of SARS-CoV-2 reinfection remains a critical and continued knowledge gap. Prior to Omicron variant emergence, infection-acquired protection for healthcare workers (HCW) was over 80% a year or more after primary infection, and higher still in those subsequently vaccinated.[1–4] For Omicron, a 16-fold increased reinfection risk was reported compared to the Delta dominance period,[5,6] reflecting the antigenic distances between variants and highlighting the impact of a partial immune-escape variant. Understanding reinfections that occurred early in the pandemic, prior to antigenically distinct variants and vaccine deployment, is essential to inform ongoing clinical management, vaccine boosters and continued vaccine development.

Detectable anti-SARS-CoV-2 spike binding antibodies are associated with a substantial reduction of reinfection risk.[2,7–9] However, whether and how binding antibody levels translate into functional protection against further infections with different SARS-CoV-2 variants is not yet elucidated. The absence of a suitable antibody response after first infection, influenced by epidemiological factors such as severity of infection and immunosuppression, and decreasing neutralising antibody (nAb) titres over time were associated with SARS-CoV-2 reinfection.[10–13] nAb titres to specific variants may be more relevant for sterilising immunity than total IgG or binding antibody levels, thus a more accurate correlate of protection against infection.[14,15]

When comparing individuals who experienced reinfection and those after recover from primary infection (convalescent), no difference in antibody levels within weeks after reinfection were found.[9] Nonetheless, there is increasing evidence that antibody levels at the time of exposure are especially relevant to prevent an infection episode,[11,12] forming the basis of treatment and prophylaxis with monoclonal therapies.[16–18]

The SIREN (SARS-CoV-2 Immunity & REinfection EvaluatioN) study - a large prospective cohort of UK HCW - was designed to enable the timely detection and characterisation of reinfection cases.[2,19] In this analysis, we aimed to investigate differences in serological response to primary infection between reinfection cases and singly infected controls prior to vaccination, to inform how antibody levels and neutralisation titres correlate with protection.

Methods

Study population and design

We conducted a case-control study, comparing reinfection cases and matched controls nested within the SIREN study, who underwent regular SARS-CoV-2 antibody and PCR testing. The study protocol was approved by the Berkshire Research Ethics Committee on May 22, 2020, and is described elsewhere.[19]

Case selection

A potential reinfection case was defined as a participant with two positive PCR results at least 90 days apart or a participant with a new positive PCR test at least 4 weeks after their first antibody-positive result, prior to vaccination. Participants with recurrent positive PCR results less than 90 days apart were excluded irrespective of their antibody status. All potential reinfections detected by 30th June 2021 were allocated as possible, probable, confirmed or excluded, based on genomic and sequential serological data, according to our case definitions (Supplementary material).

For this analysis, probable or confirmed reinfections were included, which occurred before individual vaccination and no later than February 2021. We excluded participants who had withdrawn and had their data deleted, or for whom there were no matched controls available.

Control selection

SIREN participants with history of SARS-CoV-2 infection (either SARS-CoV-2 antibody positive at UKHSA Porton testing or PCR positive in local testing) but no SARS-CoV-2 reinfection detected by 15th July 2021 were selected as controls. Additionally, controls must had a minimum of four serology samples available for testing before individual vaccination, over at least a three-month period. Controls identified as potential reinfections after analysis of sequential antibody results were excluded. Controls were matched to cases, initially in a 1:4 ratio, on the following criteria: gender (male/female), age (<25, 25-34, 35-44, 45-54, ≥55 years), geographic region (England: South, London, Midlands, North, Devolved Administrations) and estimated time of primary infection (March-June 2020, July-October 2020, November-February 2021 and March-June 2021), in which either the first PCR or first antibody positive test was used as a proxy. Where more than four controls per case were available, random selection was used. If less than four controls were available, all were included in the analysis.

Sample testing

All sera samples from cases before reinfection and at least two samples after reinfection were tested. For controls, we tested one sample prior to their vaccination, taken at a similar time to the corresponding pre-reinfection case sample. The following blinded sample testing was performed at three different laboratories: anti-SARS-CoV-2 spike RBD (anti-S) and anti-SARS-CoV-2 nucleocapsid (anti-N) antibody testing, live virus neutralisation (LV-N) and pseudovirus microneutralisation (PV-N) against variants circulating at time reinfections occurred (Wuhan and Alpha) were performed as previously described.[20–22] The detailed laboratory methodology is provided in the Supplementary material.

Data analysis

Anti-S results were expressed in binding antibody units/mL (BAU/mL) and anti-N results were expressed as a cutoff index (COI). nAb results were reported as IC50 titres, which provide estimated values for 50% of protection. Description of cases and controls included for each analysis can be found in the Supplementary material.

We compared antibody levels and nAb titres pre-reinfection for cases with control samples taken at a similar calendar time. Fixed-

effect linear regression was used to compare the geometric means of anti-S, anti-N and PV-N titres in cases before and after reinfection, as well as for cases and controls before reinfection. For LV-N assays (not quantitative below the detection threshold of 40), we compared the proportions of cases that were positive before and after reinfection, and cases and controls that were positive pre-reinfection, using McNemar's test.

Conditional logistic model

We modelled the probability of reinfection as a function of antibody levels and activities, using conditional logistic regression, compatible with the binary outcome (reinfected/not reinfected) and the matched design of the study. For anti-S and anti-N, we used the $\log_2$ as a continuous predictor. For LV-N and PV-N, we categorised titres into $\leq$ 40 (below positivity threshold), 41–100 and >100. We coded the resulting ordinal predictor,[23] suited to identifying contrasts between successive categories and, therefore, a potential critical threshold for protection. The cut-off of 100 was an estimation based on previously reported IC50 titres associated with less than 5% of in vivo replication-competent virus.[24,25] In case a significant protection was highlighted in the highest, open-ended category (>100), we used logistic regression with the nAB titres as continuous predictor to ascertain whether higher titres are associated with additional benefits.

In all conditional logistic models, we controlled on frequency of exposure to COVID-19 patients (FEC) - a potential confounder for probability of reinfection and antibody levels. Including FEC in the models decreased the ORs for antibody titres. Likelihood ratio test (LRT) confirmed that a model including FEC was favoured for all antibody assays, which was not seen for other characteristics (underlying medical condition, staff type, patient contact; models including ethnic group did not converge due to small numbers in most categories).

Correlation between assays

For correlation between assays, we used linear regression and Spearman's correlation. To investigate whether LV-N (PV-N) positivity could be inferred from anti-S and anti-N levels or PV-N (LV-N) positivity, we used a mixed effect logistic regression model, which included all available samples for each participant and mixed models. We fitted logistic regression with participant-specific random intercept and random slopes. We used Wald tests on estimated coefficients and LRTs for model selection. We reported a random-slope model over a random-intercept model when LRT showed a better fit. We allowed for correlated random effects when covariance was significantly different from zero (Wald test, 0.05 level) and favoured by LRT (0.05 level).

Results

A total of 23 reinfection cases and 92 controls were initially identified and included for demographic analysis (Table 1). Seventy eight percent of cases and 86% of controls were white and 22% of cases and 27% of controls had reported underlying medical conditions. Workplace exposure to SARS-CoV-2 was higher in cases than controls, with more cases employed in clinical roles (78% vs. 66%) and reporting being exposed at least weekly to SARS-CoV-2 at work (61% vs. 47%).

Among cases, first infections occurred between April and September 2020 and reinfections occurred between October 2020 and February 2021. The median time to reinfection was 160 days (IQR 99-204). Primary infections were mild or asymptomatic in both cases and controls, with just two cases (9%) and 23 controls (25%) reporting COVID-19 symptoms, according to the UK case definition in use at the time (fever, persistent cough, anosmia, ageusia); no cases and three (3%) controls reported a hospital attendance during their primary infection, but none were admitted. During the reinfection episode, 16 (70%) of cases reported symptoms, of which 9 (39%) had COVID-19 symptoms.

For cases, we analysed trajectories of antibody levels and neutralization titres before and after reinfection (Fig. 1). Prior to reinfection, all cases were positive for anti-S, whereas two cases (9%) had anti-N levels below the positivity threshold. Regarding nAb titres, 85% of cases had LV-N titres against Alpha below the quantitative range (LV-N Wuhan [65%]; PV-N Alpha [60%]; PV-N Wuhan [35%]). Comparing geometric means before and after reinfection, we observed a significant boosting after reinfection in anti-S and anti-N levels, as well as in LV-N and PV-N titres (Fig. 2).

Comparing antibodies between cases and controls

We compared antibody levels and neutralisation titres from cases and controls before reinfection (Figs. 3 and 4). Anti-S levels were significantly higher in controls ($p = 0{\cdot}001$) than in cases before reinfection, while no significant difference was observed for anti-N ($p = 0{\cdot}29$). For PV-N Wuhan and PV-N Alpha titres, geometric means were significantly higher in controls than in cases ($p = 0{\cdot}01$ and $p = 0{\cdot}004$, respectively). For LV-N, a higher proportion of controls had detectable titres than cases: 88% vs 35% for LV-N Wuhan, 54% vs 15% for LV-N Alpha.

In the conditional logistic regression model, doubling in anti-S levels was associated with a significant reduction in odds of reinfection of 37% (OR 0·63, CI 0·47-0·85, for doubling levels); such association has not been found for anti-N levels (OR 0·88, CI 0·73-1·05, for doubling of levels).

For LV-N Wuhan, titres between 41-100 were associated with a significant reduction in the odds of reinfection, when comparing with values $\leq$ 40 (p=0·002) and no additional benefits observed for titres >100 ($p = 0{\cdot}82$) (Table 2). Similar findings were observed for LV-N Alpha: titres between 41-100 were associated with a significant reduction in the odds of reinfection with respect to titres $\leq$ 40 ($p = 0{\cdot}006$), and no additional benefits for titres >100 ($p = 0{\cdot}47$). The lower limit of the assay's quantitative range (40) was therefore the threshold associated with protection for LV-N Wuhan (OR 0·02, CI 0·00-0·31) and LV-N Alpha (OR 0·07, CI 0·01-0·62).

For PV-N Wuhan, titres between 41-100 were not associated with protection ($p = 0{\cdot}12$), whereas there was evidence of protection for titres above 100, both with respect to titres $\leq$ 40 ($p = 0{\cdot}03$) and $\leq$ 100 (OR 0·14, CI 0·03-0·64) (Table 2), respectively. Findings for PV-N Alpha were similar: no evidence of protection for titres between 41-100 ($p = 0{\cdot}48$), but titres >100 were associated with protection, when comparing with titres $\leq$ 40 ($p = 0{\cdot}005$) and $\leq$ 100 (OR 0·06, CI 0·01-0·40). For PV-N Wuhan titres >100 (continuous variable), we found no additional protection associated with titres above that range ($p = 0{\cdot}98$, for doubling of titres). For PV-Alpha, titres >100 did not show any additional protection when increasing titres ($p = 0{\cdot}85$, for doubling of titres).

Correlation between assays

Correlations between anti-S levels and PV-N and LV-N titres are plotted in Fig. 5. We found a positive correlation between PV-N and anti-S (Fig. 5A) and LV-N and anti-S (Fig. 5B). For PV-N, whilst titres >100 were associated with protection from reinfection, its distribution appears continuous across its range. For LV-N, this threshold falls below the lower limit of the quantitative range.

Table 1
Description of the demographic profile and workplace exposure to SARS-CoV-2 of reinfection cases ($n = 23$) and controls ($n = 92$).

Characteristics	Cases n (%)	Controls n (%)
Age group		
18-39	9 (39·13)	36 (39·13)
40 - 49	0 (0)	1 (1·09)
40-59	13 (56·2)	51 (55·43)
60+	1 (4·35)	4 (4·35)
Gender		
Male	4 (17·39)	18 (19·57)
Female	18 (78·26)	74 (80·43)
Other	1 (4·35)	0 (0)
English Region		
East Midlands	2 (8·7)	8 (8·7)
East of England	3 (13·04)	13 (14·13)
London	6 (26·09)	24 (26·09)
Northwest	1 (4·35)	2 (2·17)
Southeast	4 (17·39)	9 (9·78)
Southwest	3 (13·04)	19 (20·65)
West Midlands	2 (8·7)	7 (7·61)
Yorkshire and the Humber	2 (8·7)	10 (10·87)
Ethnic group		
Asian	3 (13·04)	6 (6·52)
Black	0 (0)	4 (4·35)
Other	1 (4·35)	2 (2·17)
Prefer not to say	1 (4·35)	1 (1·09)
White	18 (78·26)	79 (85·87)
Underlying medical conditions		
Chronic non-respiratory	1 (4·35)	8 (8·7)
Chronic respiratory	4 (17·39)	15 (16·3)
Immunosuppression	0 (0)	2 (2·17)
None	18 (78·26)	67 (72·83)
Patient Facing Role		
Yes	21 (91·3)	78 (84·78)
No	2 (8·7)	14 (15·22)
Staff Type[ı]		
Clinical	18 (78·26)	61 (66·3)
Administrative	3 (13·04)	12 (13·04)
Other	1 (4·35)	19 (20·65)
Support	1 (4·35)	0 (0)
Frequency of Exposure to COVID-19 patients (FEC)		
Daily	7 (30·43)	27 (29·35)
Weekly	7 (30·43)	16 (17·39)
Monthly	4 (17·39)	4 (4·35)
Less than monthly	0 (0)	12 (13·04)
Never	5 (21·74)	33 (35·87)
Weekly exposure to COVID-19 patients		
At least once a week	14 (60·87)	43 (46·74)
Less than once a week	9 (39·13)	49 (53·26)
TOTAL	**23**	**92**

[ı] Clinical: Dental, Dietician, Healthcare Assistant, Healthcare Scientists, Medical, Midwife, Midwifery student, Nursing, Nursing student, Occupational Therapist, Paramedic, Pharmacist, Pharmacy technician, Physiotherapy, Psychologist, Radiographer, Speech & Language Therapy and Other Allied Health Professional. Administrative: Administrative & Clerical (e.g. receptionist, secretary, database manager) and Senior manager / Executive / Hospital Administration. Support: Estates & Ancillary (e.g. domestic cleaner, housekeeper, engineer), Porter and Security services. Other: Apprenticeships, Other Professional Scientific & Technical, Other student and Other.

Table 2
Associations between neutralising antibody titres and reinfections - conditional logistic regression model.

Neutralising antibody titres[ı]	Probability of reinfection Odds Ratio (95% Confidence Interval)			
	LV-N Wuhan	LV-N Alpha	PV-N Wuhan	PV-N Alpha
41-100	0.02 (0·00–0·26)	0·04 (0·00–0·40)	0·29 (0·06–1·36)	0·59 (0·13–2·57)
>100	0·81 (0·14–4·90)	3·06 (0·14–65·12)	0·14 (0·03–0·64)	0·06 (0·01–0·40)

The table is complementary to the findings on nAB titres and probability of reinfection. The ORs were obtained using conditional logistic regression with the scheme detailed in Data Analysis section of Methods. Each OR is relative to the previous category of nAb titres. The reference for the 41-100 interval is ≤ 40.

[ı] : above threshold of 40

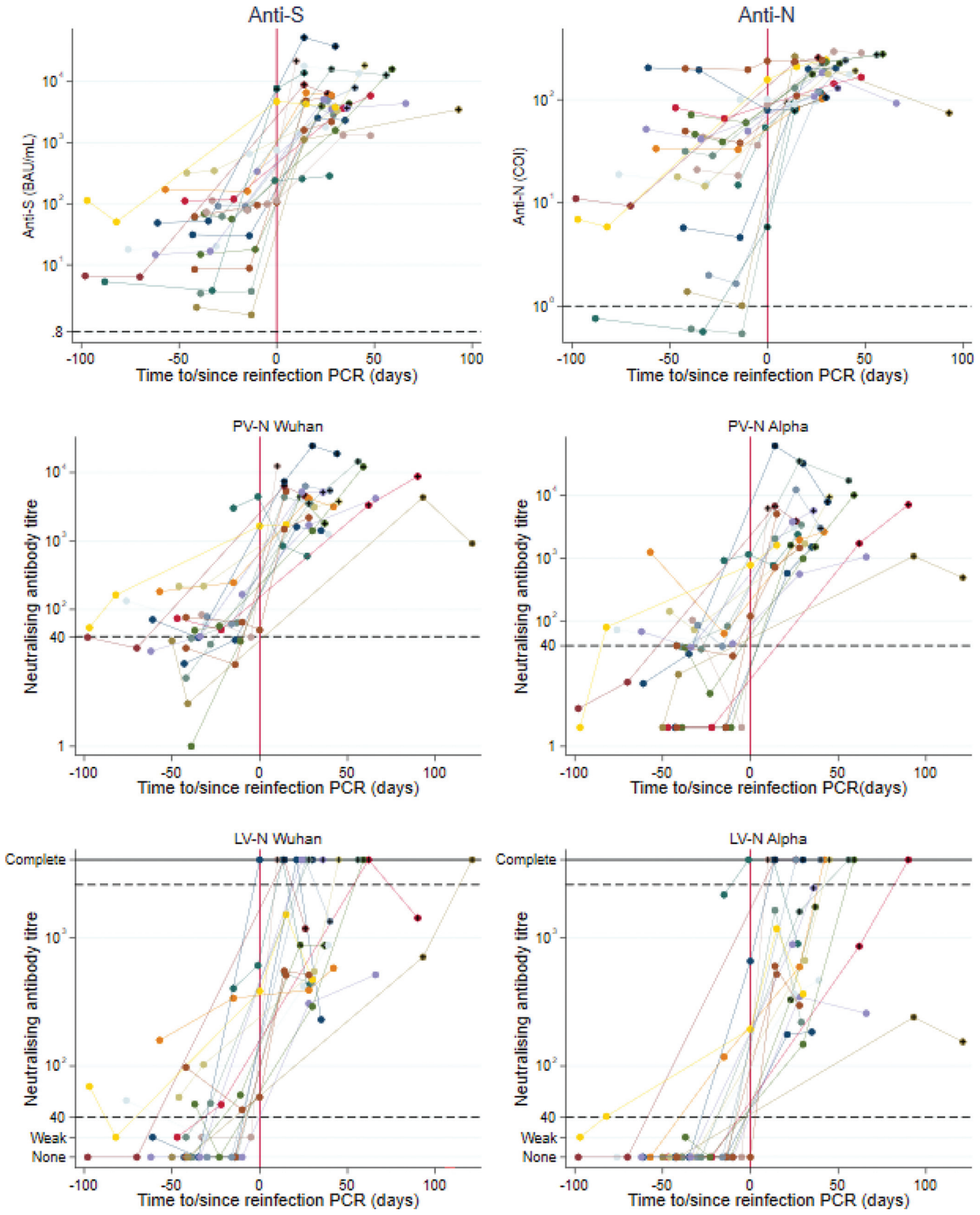

Fig. 1. Trajectories of antibody levels and neutralisation titres in cases before and after reinfection. The vertical red line at Time=0 is the date of the PCR test detecting reinfection. Points with a plus (+) sign refer to samples collected after vaccination. Dashed lines indicate detection thresholds of assays, except the upper dashed lines in panels E and F that indicate the upper end of the quantitative range of the LV-N assay. Same colour used for same participant across panels, but panels A and B have 3 more participants.

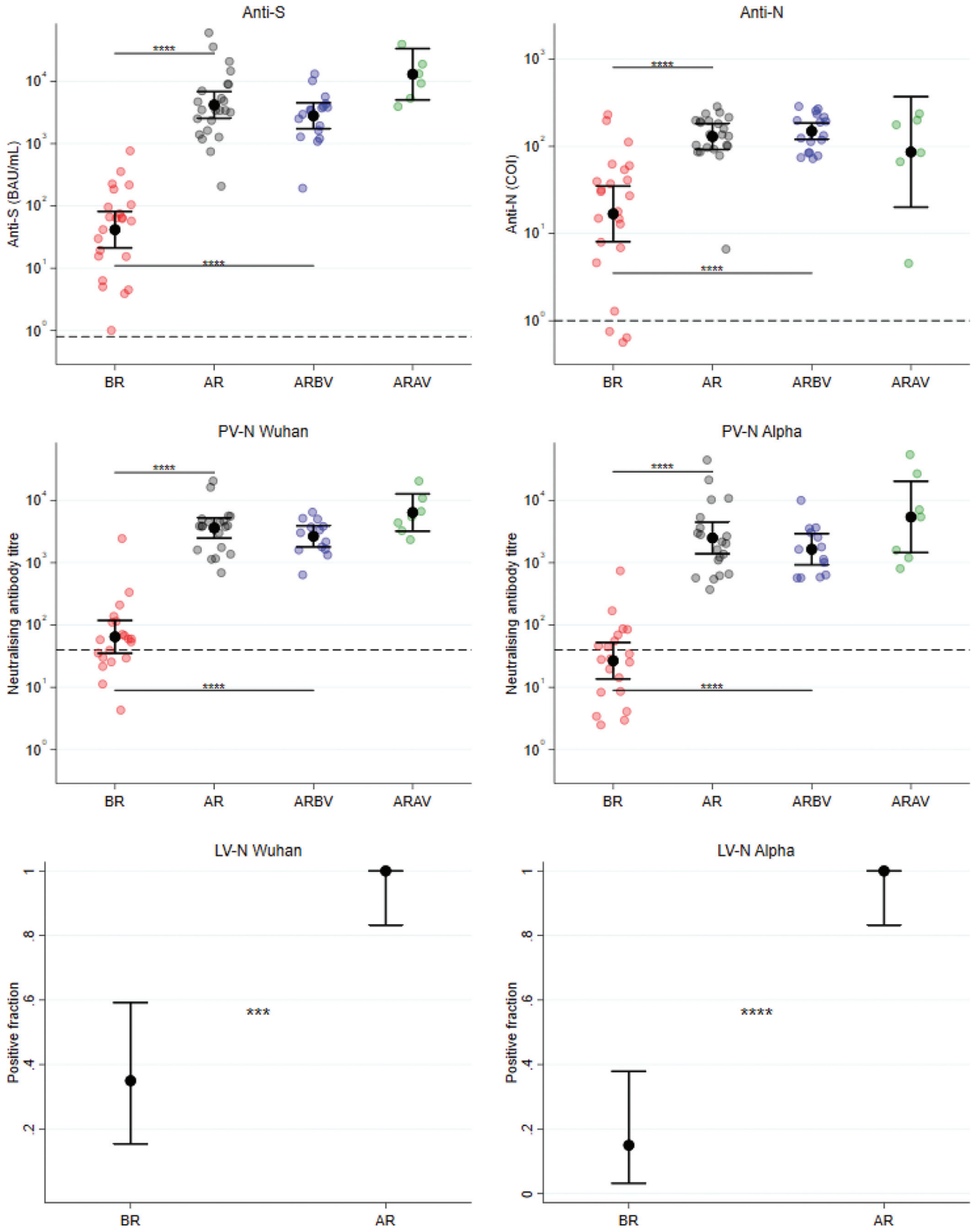

Fig. 2. Comparison of antibody levels and neutralisation titres before and after reinfection for cases. Top and middle rows: antibody levels and neutralisation titres after reinfection (AR, black) are significantly higher than before reinfection (BR, red) for anti-S ($p < 10^{-4}$, paired t-test), anti-N (=10^{-4}, Wilcoxon signed-rank), anti-PV-N Wuhan ($p < 10^{-4}$, paired t-test) and anti-PV-N Alpha ($p < 10^{-4}$, random effect tobit model). The same effects and similar significance levels are obtained when considering only samples after reinfection but before vaccination (ARBV, blue). Bottom row: among cases, the fraction of LV-N with nAb titres >40 is significantly higher (McNemar's test) after reinfection than before, for LV-N Wuhan ($p = 0.001$) and LV-N Alpha ($p < 10^{-4}$). Dashed lines indicate positivity threshold of the assay.

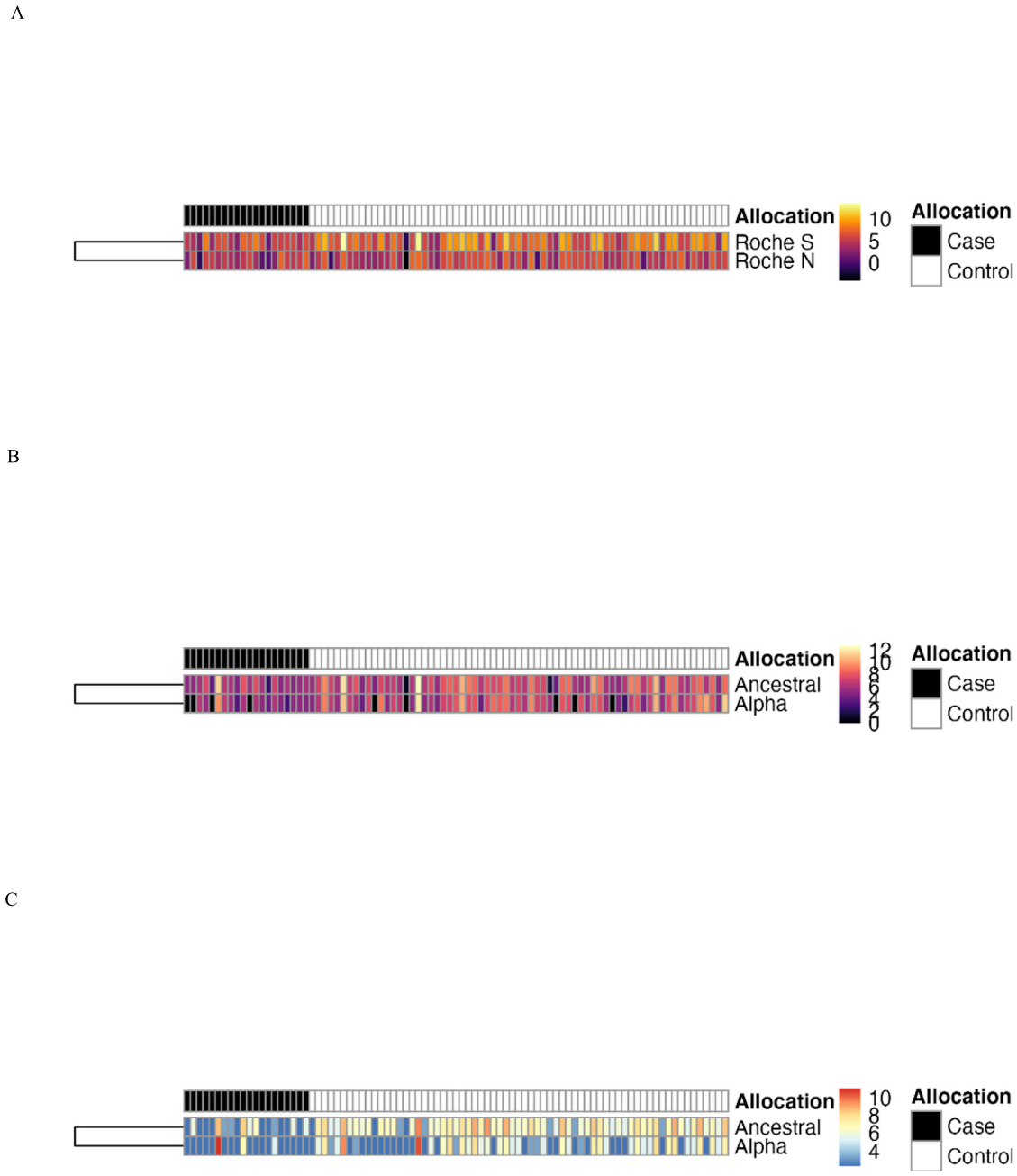

Fig. 3. Serological status of single infection controls and reinfection cases (A-C). Supervised heatmaps with pre-reinfection sera from cases and temporally matched samples from controls. For (A), Log_2 anti-S and log_2 anti-N are shown. Log_2 PV-N and log_2 LV-N are shown in (B) and (C), respectively.

Despite strong positive correlations between PV-N and LV-N with anti-S, the reinfection cases were frequently outliers in these correlations (Tables 3 and 4).

Mixed models with participant-specific intercept and slopes were used to assess if LV-N (PV-N) positivity inferred from anti-S, anti-N or PV-N (LV-N) positivity, considering all samples from cases and controls (Table 5). Increasing anti-S levels or positive nAbs (regardless of assays or variants) were associated with significantly higher odds of positivity to all nAb assays, particularly for LV-N (PV-N) positivity.

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Discussion

In this unique cohort of early SARS-CoV-2 reinfections prior to vaccination, levels of anti-S and nAb titres offered substantial discrimination between cases and controls. The absence of an observed association with anti-N may reflect assay characteristics, anti-N rapidly declining post-infection or that the antibody-mediated neutralisation of spike is the mechanism by which immune sera confer protection.[26–28] We were able to identify protection thresholds for nAB which correlate with protection against SARS-CoV-2 reinfection. LV-N titres above the quantitative threshold appear sufficient to protect against SARS-CoV-2 reinfection, whilst PV-N titres above 100 were required. For anti-S, increasing levels were associated with reduced risk of reinfection, although we were not able to determine a specific quantitative range of pro-

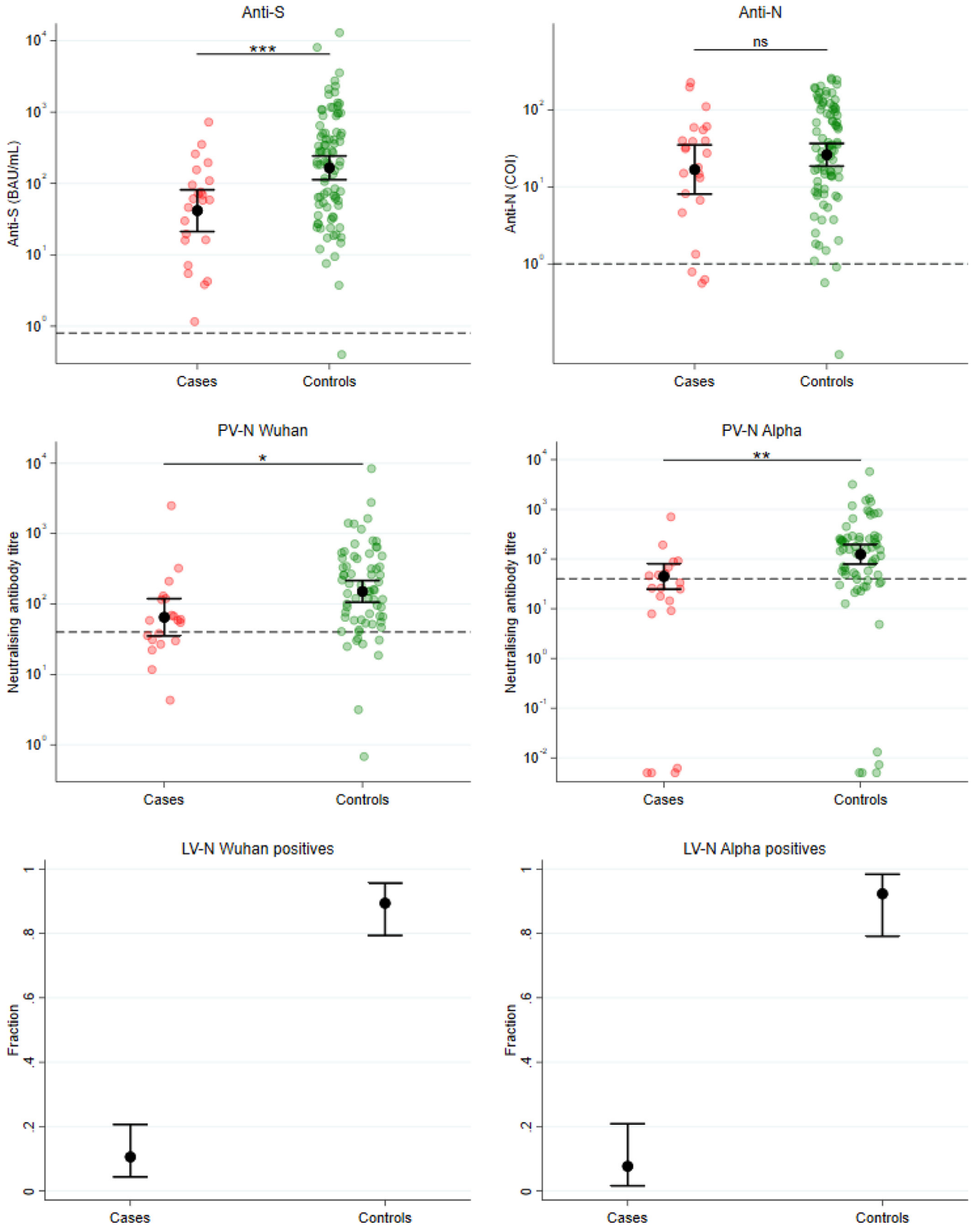

Fig. 4. Comparison between case and control antibody levels and neutralisation titres in last sample before reinfection (cases) and the closest corresponding sample in calendar time (controls), with p-values obtained from fixed effect linear regression. Top row: geometric mean of anti-S levels is significantly higher in cases ($p = 0.001$) than in controls, while no significant difference is observed in geometric means of anti-N levels ($p = 0.29$). Middle row: geometric means of PV-N titres are significantly higher in cases than in controls for Wuhan ($p = 0.01$) and Alpha ($p = 0.0044$, random effect tobit model). Bottom row: among participant nAb titres > 40 with LV-N Wuhan and LV-N Alpha, the proportion of controls is higher than that of cases, with disjoint confidence intervals. Dashed lines indicate positivity threshold of the assay.

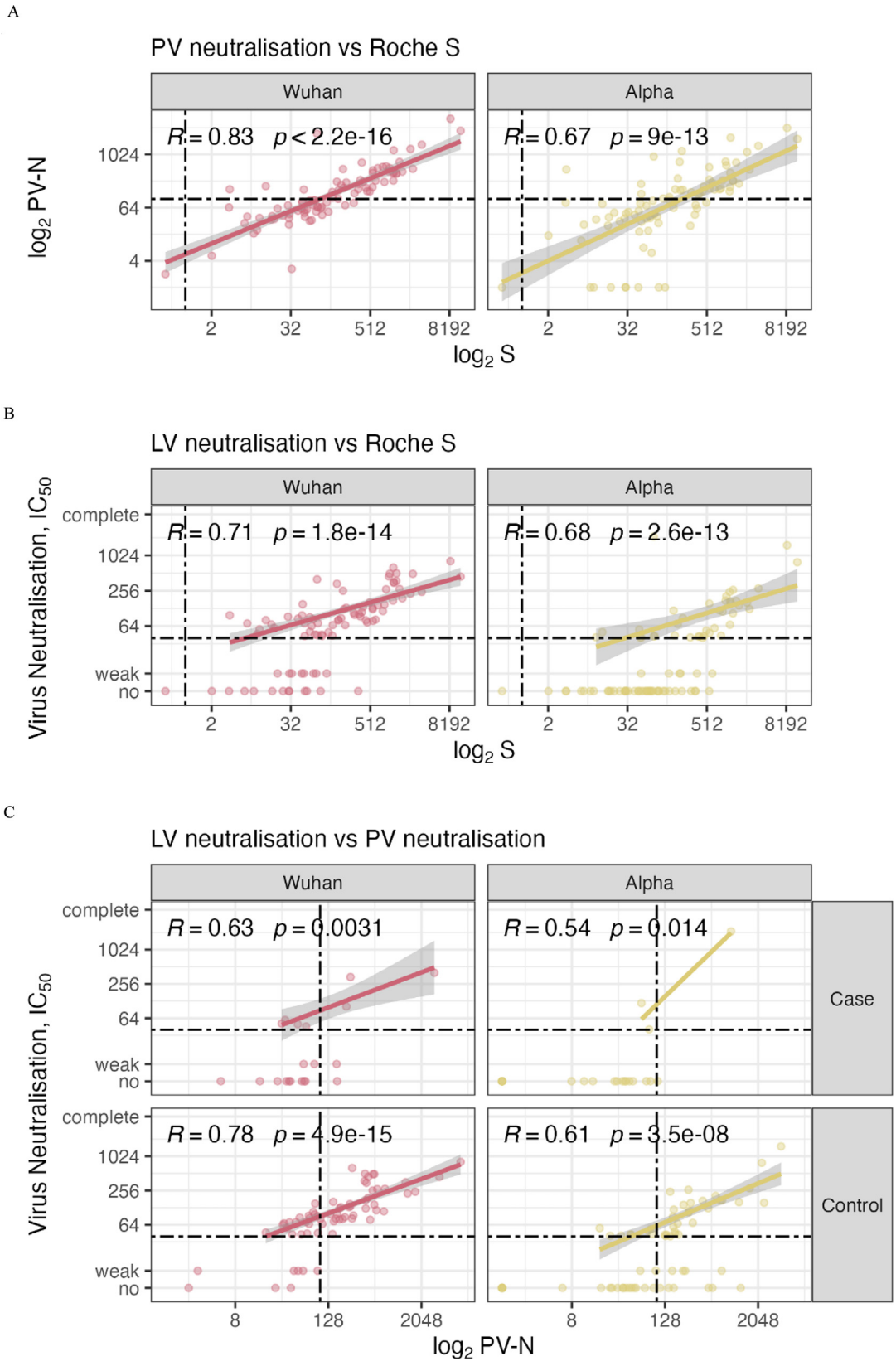

Fig. 5. Correlation between neutralisation assays and binding anti-S levels.
(A) PV-N titres against Wuhan and Alpha in pre-reinfection sera and temporally matched control samples, plotted against binding anti-S antibodies.
(B) LV- titres, reported as IC_{50}, plotted against binding anti-S antibodies.
(C) PV-N titres against Wuhan and Alpha in pre-reinfection sera and temporally matched control samples, plotted against LV-N titres, reported as IC_{50}.
In (A) and (B), binding antibodies are plotted as $\log_2$, PV-N titres as $\log_2(x+1)$ and LV-N titres as $\log_2$, after assigning 5, 10 or 5120 as no, weak or complete inhibition, respectively. In (A) correlation coefficient and P value are from Spearman's correlation, and a regression line is shown using all data. In (B) and (C), all data are used for Spearman's correlation, whereas the regression line uses only data within the quantifiable range (40-2560). Dashed lines indicate an anti-S level of >0.8U/mL (considered "positive" by the manufacturer), and a PV-N or LV-N titre of 100 or 40 respectively, as described in the Results section.

Table 3
Relationship between PV-N titres and anti-S levels before reinfection events.

	Wuhan			Alpha		
	S+ PV-N>100	S+ PV-N<100	S- PV-N<100	S+ PV-N>100	S+ PV-N<100	S- PV-N<100
Cases (n=20)	5 (25%)	15 (75%)	0	2 (10%)	18 (90%)	0
Controls (n=67)	39 (58%)	27 (40%)	1 (1%)	38 (57%)	28 (42%)	1 (1%)

The table is complementary to the findings on nAb titres and their correlation with anti-S levels. Using anti-S > 0.8U/mL (manufacturer's positive threshold) and a PV-N titre of >100 (defined here), the distribution of pre-reinfection sera and temporally matched controlled samples is shown. Most cases lack neutralisation against Alpha.

Table 4
Relationship between LV-N titres and anti-S levels in sera before reinfection events.

	Wuhan			Alpha		
	S+ LV-N>40	S+ LV-N<40	S- LV-N<40	S+ LV-N>40	S+ LV-N<40	S- LV-N<40
Cases (n=20)	7 (35%)	13 (65%)	0	3 (15%)	17 (85%)	0
Controls (n=67)	59 (88%)	7 (10%)	1 (1%)	36 (54%)	30 (45%)	1 (1%)

The table is complementary to the findings on nAB titres and their correlation with anti-S levels. Using S>0.8U/mL (manufacturer's positive threshold) and a PV-N titre of >100, the distribution of pre-reinfection sera and temporally matched controlled samples is shown. Most cases lack neutralisation against Alpha.

Table 5
Predicted positivity of neutralising antibody titres against different variants.

	LV-N Wuhan + Odds Ratio (95% Confidence Interval)	LV-N Alpha +	PV-N Wuhan +	PV-N Alpha +
Anti-S (log_2)	2·3 (1·80–3·01) [r]	3·6 (2·66–4·97)	3·4 (2·28–5·06)	2·6 (2·10–3·44)
LV-N Wuhan +	_	68·3 (19·1–244·8) [s]	84·5 (10·0–716·7) [s]	16·2 (6·5–40·7) [s]
LV-N Alpha +	30·8 (8·7–109·1) [r]	_	257·9 (5·7-11646·1) [s]	402·9 (9·7–16672·7) [s]
PV-N Wuhan +	13·1 (5·4–31·8) [s]	17·5 (6·9–44·6) [s]	_	81·0 (31·6–207·4)
PV-N Alpha +	9·5 (3·8- 24·1) [s]	20·4 (8·7–47·91) [s]	1929·1 (53·9–69043·5) [s]	_

Odds ratios for positivity to one assay (column) given another assay (row), from logistic regression with random effects at participant level. The first row (Anti-S) gives the increase in odds of positivity to the nAB assay in that column for doubling of anti-S levels (unit increase in log_2). All other rows give the increase in odds of positivity (+) to the nAB assay in that column, knowing positivity to the assay in the row. Model selection is explained in the statistical methods section.
[r] indicates that the selected model has random intercepts and slopes, with uncorrelated random effects.
[s] stand for correlated random effects; no symbol stands for a random intercept model.

tection as estimated previously.[9] On investigation of correlation between assays, we found an association between neutralising activity across different variants and different assays, and with anti-S levels.[29]

Pre-reinfection LV-N and PV-N titres were significantly lower in cases than controls, supporting the mounting evidence that neutralising activity is critical for protection against SARS-CoV-2 infection.[11–13,30] It is know that titres and longevity of nAb are directly associated with clinical presentation of the primary COVID-19 episode, given asymptomatic SARS-CoV-2 infections induce lower levels and a more rapid decline of nAb titres over time when compared to moderate or severe infection.[31–33] However, we were unable to investigate this here as both groups in our study overwhelmingly reported mild or asymptomatic primary infections and none were hospitalised.

Our findings corroborate with the growing evidence base on SARS-CoV-2 correlates of protection, particularly the role of neutralising antibodies in treatment and as prophylaxis. For LV-N, any titre within the quantitative range (a dilution of 1:40) conferred protection against reinfection, which is similar to what was previously reported with conventional LV-N assays, although a different cut-off was considered (>20).[11,12] For PV-N, whilst we demonstrated that a titre above 100 is protective, another study reported that a titre of 26 IU/ml was associated with 80% of protection against infection, when assessing neutralisation levels 28 days after second ChAdOx1 nCoV-19 vaccine dose.[34] Differences in thresholds between LV-N and PV-N are unsurprising, given their different underlying characteristics.[35]

Comparing assays, neutralising activity was correlated with anti-S levels, as previously demonstrated.[29] Some cases and controls, however, appear discordant for anti-S positivity and nAb titres, lacking the expected neutralisation predicted by their Wuhan titres. This is particularly important given viral evolution and the emergence of different SARS-CoV-2 variants, as most assays in clinical use only detect antibodies against Wuhan.

Our study has some limitations. Considering limited PCR capacity and sequencing in early 2020, some primary infection dates were approximated. Our case definitions required an increase in anti-S levels after reinfection to select true reinfection events. This may have excluded reinfections without boosting, therefore interpreting post-reinfection boosts requires caution. The timing of available pre-reinfection sample was heterogenous, taken up to 82 days before the event (median 16 days, range 10–82 days). Given waning, antibody levels and neutralisation titres at reinfection may have subsequently decreased, and differences between cases and controls more pronounced.

For LV-N, the low number of samples prior to reinfection within the quantitative range (>40) might have limited our ability to confidently assign a numerical value as correlate of protection. Regarding PV-N, the protection threshold against reinfection (>100) requires careful interpretation, as our statistical approach included pre-determinate values. In addition, the use of the anti-RBD binding assay (anti-S) to infer neutralising ability of individual sera samples should be cautioned. Although our study was focused on humoral immune response to SARS-CoV-2 infection, we have not considered the role of mucosal antibodies. Furthermore, our study

has not analysed the T-cell response, which can provide an additional level of protection.[36,37]

Ultimately, our design as a large prospective public health trial is a critical strength, allowing us to scale up participation to provide sufficient power to detect rare reinfection events early in the pandemic and conduct a robust analysis using a case-control design.

Conclusions

We have identified a quantifiable range of neutralisation titres that protects against SARS-CoV-2 reinfection in the Alpha era, and its correlation with anti-S levels. We have demonstrated that infections with Wuhan conferred some cross-neutralisation activity against early subsequent variants. These findings provide relevant insights for clinical practice and highlight discrepancies between binding anti-S levels and neutralisation titres. Our cohort will also allow similar studies to assess the impact of antibodies in protection considering different vaccination status and exposures to different SARS-CoV-2 variants, which will be vital for future vaccination strategies and population COVID-19 management.

Data sharing

The metadata will be available through the Health Data Research UK CO-CONNECT platform and available for secondary analysis once the SIREN study has completed reporting.

Declaration of Competing Interest

All authors declare no competing interests.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2022.09.004.

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