Antibody correlates of protection against Delta infection after vaccination: A nested case-control within the UK-based SIREN study

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SUMMARY

Objectives: To investigate serological correlates of protection against SARS-CoV-2 B.1.617.2 (Delta) infection after two vaccinations.

Methods: We performed a case-control study, where cases were Delta infections after the second vaccine dose and controls were vaccinated, never infected participants, matched by age, gender and region. Sera were tested for anti-SARS-CoV-2 Spike antibody levels (anti-S) and neutralising antibody titres (nAbT), using live virus microneutralisation against Ancestral, Delta and Omicron (BA.1, B.1.1.529). We modelled the decay of anti-S and nAbT for both groups, inferring levels at matched calendar times since the second vaccination. We assessed differences in inferred antibody titres between groups and used conditional logistic regression to explore the relationship between titres and odds of infection.

Results: In total, 130 sequence-confirmed Delta cases and 318 controls were included. Anti-S and Ancestral nAbT decayed similarly between groups, but faster in cases for Delta nAbT (p = 0.02) and Omicron nAbT (p = 0.002). At seven days before infection, controls had higher anti-S levels (p < 0.0001) and nAbT (p < 0.0001; all variants) at matched calendar time. A two-fold increase in anti-S levels was associated with a 29% ([95% CI 14–42%]; p = 0.001) reduction in odds of Delta infection. Delta nAbT > 40 were associated with reduced odds of Delta infection (89%, [69–96%]; p < 0.0001), with additional benefits for titres > 100 (p = 0.009) and > 400 (p = 0.007).

Conclusions: We have identified correlates of protection against SARS-CoV-2 Delta, with potential implications for vaccine deployment, development, and public health response.

Introduction

The SARS-CoV-2 immunity and reinfection evaluation (SIREN) study – one of the world's largest cohort studies of HCW – has followed UK participants since June 2020 and has enabled the identification and detailed analysis of SARS-CoV-2 reinfections, as well as evaluating vaccine effectiveness (Wallace et al., 2021)[1]. Throughout the pandemic, SIREN has captured different patterns of antigenic

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exposure,[2] and has demonstrated that SARS-CoV-2 vaccination played a major role in protection against Alpha (B.1.1.7) infection or reinfection, in line with other studies.[3,4]

It is, however, known that vaccine effectiveness throughout COVID-19 pandemic has been impacted by the coalescence of emerging variants and waning responses from antecedent vaccination. Decreases in protection from infection have been seen since the emergence of Delta (B.1.617.2) and even more pronounced during the Omicron era.[5,6] Regarding serological correlates of protection, it has been previously reported that increasing anti-SARS-CoV-2 spike (S) levels are correlated with protection against Ancestral and Alpha infection, although neutralising antibody titres (nAbT) seem to offer a more accurate estimation of protection before vaccination.[7,8] Following Alpha, later SARS-CoV-2 variants have been correlated with reduction of neutralising activity resulting from previous infection or vaccination,[9–12] although accurate titres associated with protection remain to be identified.

The aims of this study are to investigate serological correlates of protection (CoP) against Delta infection by determining differences in antibody responses between cases infected with SARS-CoV-2 Delta variant and non-infected controls after two vaccine doses.

Methods

Study population and design

We conducted a matched case-control study, nested within the SIREN study - a prospective cohort of healthcare workers across the UK who undergo regular SARS-CoV-2 antibody and PCR testing.[13] The SIREN study is REC approved (20/SC/0230), and an NIHR priority study. All participants included in this analysis had received two vaccinations and were not previously infected at the start of the study period.

Case and Control Definition

Cases were defined as individuals who had received two doses of vaccination and had a primary Delta infection (confirmed by sequencing) at least 14 days after their second vaccine dose, between April and October 2021.

Controls were individuals that had no evidence of primary SARS-CoV-2 infection by 31st October 2021, had two vaccine doses before April 2021 (prior to Delta variant emergence) and for which we had at least 4 sera samples available between April and October 2021.

We have applied the following exclusion criteria for cases and controls: vaccination with single dose regimen, prior infection at onset of study date (indicated by prior PCR positive or anti-N positive at start of period, April 2021), individuals who did not have sequence data available or had less than four serological samples between April and October 2021.

Matching criteria

Cases and controls were matched, initially in a 1:3 ratio by gender (male/female), age (< 25, 25–34, 35–44, 45–54, ≥55 years) and region (England: South, London, Midlands, North, Devolved Administrations). Furthermore, controls must have had one sample taken within minus 30 days and plus 15 days from the PCR data of the correspondent matched case.

Serological testing

All sera samples from cases prior to infection and after second vaccinations were tested. For controls, we selected all samples between the second and third vaccines. Serum samples were tested using the quantitative Roche Elecsys anti-SARS-CoV-2 spike (anti-S) assay (Roche AVOC2S, product code: 09289275190) with reporting in

BAU/mL derived from a two-point calibration and a reagent specific master curve; the semi-quantitative Roche Elecsys anti-SARS-CoV-2 nucleocapsid (anti-N) assay (Roche ACOV2, product code: 09203079190) reported as a cut-off index (COI) value based on the electrochemiluminescence signal of a two-point calibration, with COI > 1.0 classified as positive. Both Roche assays were performed on the automated Roche COBAS e801 and for anti-S > 2500 U/mL [range 0.4–2500 U/mL], these samples were automatically diluted by the analyser. Detailed laboratory methodology has been described previously.[8]

Samples were also tested using live virus neutralisation (LV-N) assays against variants Ancestral, Delta (B.1.617.2) and Omicron (BA.1, B.1.1.529) variants. From LV-N assays, we report neutralising antibody titres (nAbT) as the reciprocal of the serum dilution that achieves 50% inhibition of viral infection (no specific units), based on scipy-fitted dose: response curves of 8 data points per sera (from duplicates of 1:40, 1:160, 1:640 and 1:2560 dilutions). For nAbT, the quantitative range is 40–2560.

Laboratory staff were blinded to whether the sera were from cases and controls, to reduce measurement bias.

Data analysis

Modelling antibody decay and estimating antibody titres

For modelling antibody decay, we included sera samples taken from 14 days after second dose until third doses for controls, whereas for cases the interval began at the same time and was censored at Delta infection. In total 2256 samples were included, from all 448 selected participants (130 cases and 318 controls).

For each antibody metric, we modelled the trajectories of the logarithm of titres over time by a linear mixed-effect model,[7,14] with an interaction between time and case/control status, which allowed us to test for the difference in average decay rates (slopes). After testing for significance (likelihood ratio), we included (correlated) intercepts and slopes at participant level, to account for repeated sampling and intrinsic heterogeneity among participants. For anti-S, we used a standard linear model, and for LV-N a Tobit model was used to accommodate both left- and right-censored titres (where the live virus neutralisation assay reported no, weak or complete inhibition, at either side of its numerical range).

Given the ~5–10% decay in neutralising antibody titre each week from 14d after second doses, we used linear models to infer titres at matched calendar times after second doses between cases and controls. For each case, we inferred titres at 7 days (7d) before the positive PCR test confirming the infection (hereafter T−7dPCR$^+$). We assumed an average of 7d potential delay in PCR positivity, as PCRs were taken every fortnight, and therefore T−7dPCR$^+$ reflects a pre-infection nadir, rather than any early antibody response to infection. For each case's matched controls, we inferred their titres at the same time since second dose as their matched case (i.e. date of dose 2$_{matched\ control}$ + (date of T−7dPCR$^+$ - date of dose 2$_{matched\ case}$)). This procedure allowed us to compare titres accounting for waning at the same time interval after second dose and before cases' Delta infection.

Comparison of anti-S levels and nAb titres

We compared differences in geometric means between cases (inferred at 7d before infection) and control titres (inferred at matched time after second dose) fitting random effect linear models to data grouped according to the matching cases and controls. This allowed us to accommodate matching of multiple controls to each case to use all 448 selected participants. For analysis purposes, nAbT were reported as IC50, which provides estimated values for 50% of inhibition of infection in vitro. For nAbT, we used an analogous tobit model, to include left- and right- censored values, above (> 2560) or below (< 40) our LV-N assay's quantitative range.

Conditional logistic models

We modelled the probability of reinfection as a function of antibody titres. For all antibody assays, we used a conditional logistic regression, compatible with the matched design of the study. For anti-S, we used the logarithm in base 2 of the antibody level as a continuous predictor. For nAbT, we categorised the titres into < 40 (below the quantifiable range), 40–100, 100–200, 200–400, 400–800, 800–1600, 1600–2560, > 2560 (above the quantifiable range). We coded the resulting ordinal predictor using staircase approach[8,15] that allowed us to probe threshold values of nAbT associated with protection from infection. After identifying categories associated with a significant *change* in odds of infection, we calculate and report odds ratios for those categories compared to the lowest-titre group (taken as reference).

We performed this analysis using titres before infection for cases (inferred titres at 7 days before their positive PCR) and controls' titres (inferred at matching intervals after their second dose) as well as using titres inferred at 6 weeks after each participant's second dose.

Statistical software

Stata (version 17, StataCorp) was used for statistical modelling. Python's scipy was used to report live-virus micronuetralisation titres as IC50 (described previously).[8] Data were visualised in R (v 4.2.2), using ggplot2 (v 3.4.0). Table 1 was generated using gtsummary.[16]

Results

We identified 163 potential SARS-CoV-2 infection cases confirmed by sequencing that occurred at least 14 days after second vaccine dose between April and October 2021. We excluded 17 cases of reinfection, to leave 146 potential first infection cases. Of those, we selected 137 SARS-CoV-2 B.1.617.2 infections confirmed by sequencing, however three of these were excluded as had less than four sera samples available between October and April 2021. Furthermore, one case, who had received the Janssen COVID-19 vaccine (Ad26. COV2-S), was excluded, given this is a single-dose primary vaccination. After analysing serology results, we have further excluded three cases who had detectable anti-(N levels before infection date. Therefore, 130 SARS-CoV-2 Delta infection cases were included in our analysis (Fig. 1).

As per selection criteria, 404 controls were initially selected. Of those, we have excluded 64 that had detectable anti-N results upon serological screening and 22 that had their matched sera sample taken after a third vaccine dose. Consequently, a total of 318 controls were included in this study.

Demographic details of cases and controls are shown in Table 1. Staff role and work setting were not significantly different between cases and controls (consistent with the non-significant likelihood ratios in the conditional logit), nor were pre-existing comorbidities. Individuals with Asian background were more likely to be cases (8.5%) than controls (4.1%). We found that AZD1222 was more frequently used in cases (8.5%) than controls (1.7%). There was no difference in intervals between first and second doses between cases and controls.

Dynamics of antibody levels and nAb titres for cases and controls

To explore waning after second dose, we plotted anti-S and nAbT against time since 14d after second dose (Fig. 2), including a median of 4 sera for each case and control. Averaged slopes and intercepts from fitted trajectories are plotted Fig. 2. We observed decays for all 4 serological parameters: anti-S, Ancestral, Delta and Omicron nAbT (Fig. 2A-D respectively). For Delta and Omicron nAbT, we found that

Table 1
Demographics of Delta infection cases (*n* = 130) and controls (*n* = 318).

Characteristic	Case, N = 130	Control, N = 318
Sex		
Female	115 (88%)	284 (89%)
Male	15 (12%)	34 (11%)
Age		
Under 25	4 (3.1%)	8 (2.5%)
25–34	12 (9.2%)	26 (8.2%)
35–44	40 (31%)	100 (31%)
45–54	54 (42%)	131 (41%)
Over 55	20 (15%)	53 (17%)
Region		
South West	26 (20%)	74 (23%)
North East & Yorkshire and the Humber	19 (15%)	47 (15%)
North West	15 (12%)	37 (12%)
Scotland	14 (11%)	36 (11%)
East Midlands	13 (10%)	36 (11%)
London	11 (8.5%)	24 (7.5%)
South East	9 (6.9%)	20 (6.3%)
West Midlands	10 (7.7%)	18 (5.7%)
East of England	7 (5.4%)	13 (4.1%)
Northern Ireland	3 (2.3%)	9 (2.8%)
Wales	3 (2.3%)	4 (1.3%)
Ethnic group		
White	109 (84%)	278 (87%)
Asian	11 (8.5%)	13 (4.1%)
Mixed race	6 (4.6%)	12 (3.8%)
Black	4 (3.1%)	5 (1.6%)
Other ethnic group	0 (0%)	9 (2.8%)
Prefer not to say	0 (0%)	1 (0.3%)
Role[a]		
Clinical	99 (76%)	236 (74%)
Administrative	19 (15%)	47 (15%)
Other/Support	12 (9.2%)	35 (11%)
Setting		
Other	33 (25%)	89 (28%)
Outpatient	31 (24%)	73 (23%)
Office	28 (22%)	62 (19%)
Inpatient Wards	18 (14%)	33 (10%)
Intensive Care/Theatres	6 (4.6%)	30 (9.4%)
Patient facing (non-clinical)	7 (5.4%)	21 (6.6%)
Ambulance/Emergency Department/ Maternity/Labour Ward	7 (5.4%)	10 (3.1%)
Patient facing		
Yes	113 (87%)	278 (87%)
No	17 (13%)	40 (13%)
Comorbidities		
No medical condition	95 (73%)	218 (69%)
Chronic respiratory conditions	18 (14%)	52 (16%)
Chronic non respiratory conditions	15 (12%)	45 (14%)
Immunosuppression	2 (1.5%)	3 (0.9%)
Vaccines		
Pfizer-BioNTech Pfizer-BioNTech	119 (92%)	313 (98%)
Oxford-AstraZeneca Oxford-AstraZeneca	11 (8.5%)	5 (1.6%)
Dose 1 & 2 interval		
> 4 weeks	117 (90%)	274 (86%)
≤4 weeks	13 (10%)	44 (14%)

[a] 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.

decay rates were faster in cases than in controls (p = 0.02 and 0.001, respectively). For Delta nAbT, a week's progression since second dose resulted in an 8.1% reduction in cases [95% CI 7.2–9.0%] compared to 6.9% [6.5–7.3%] reduction in controls. For Omicron nAbT, a week's progression resulted in 5.2% [4.3–6.0%] and 3.7% [3.3–4.0%] reductions in titres in cases and controls, respectively.
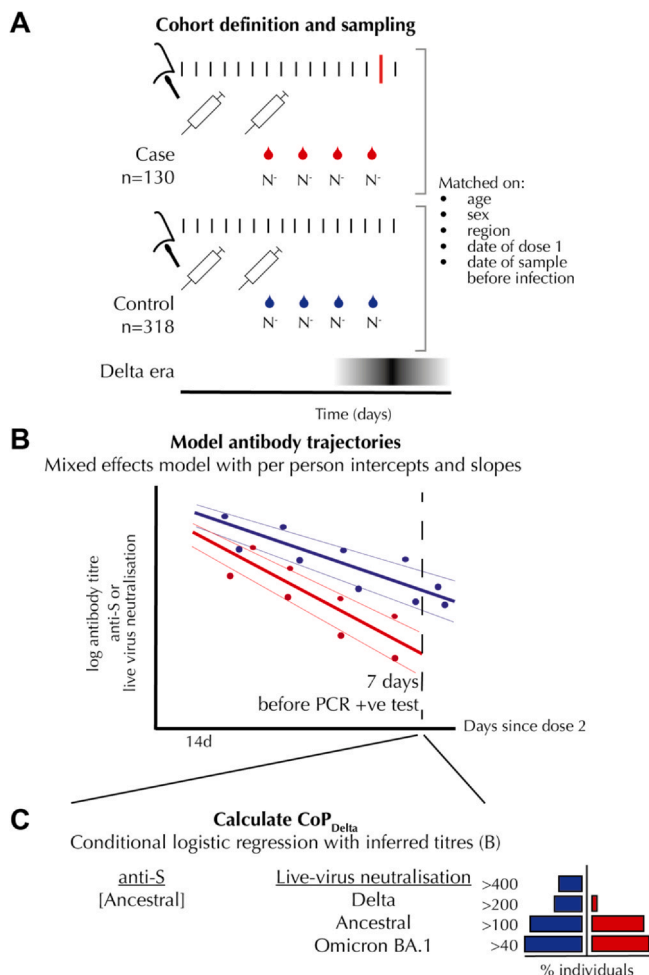
Fig. 1. A SIREN nested case:control study to determine serological correlates of protection for Delta infection after two doses. (A) A nested SIREN case:control study of 130 cases with 318 matched controls. Individuals perform fortnightly PCR tests, with 8-weekly venepuncture. All individuals were vaccinated twice before the start of the study period. (B) Schematic of the mixed effects regression strategy used to assess antibody trajectories after dose 2. From 14d after dose 2, the logarithm of antibody titres decayed linearly. At the participant-level, random effects were included for intercepts and slopes, and the overall trend line for cases and controls is shown. For anti-S antibodies a mixed effect regression model was fitted. For live-virus neutralisation a mixed effect tobit model was fitted for each variant (Delta, Ancestral and Omicron BA.1) separately. In both kinds of models, pairing between case and controls was maintained. (C) Conditional logistic regression for anti-S (with anti-S as a continuous variable), and discretised live-virus neutralisation titres (i.e. staircase regression). This allows the change of odds of infection of with each step to be calculated.

Identifying correlates of protection against SARS-CoV-2 Delta infection 7 days prior to infection

In order to identify a correlate of protection (CoP) against Delta infection, we first considered inferred antibody levels and nAbT 7 days before the cases' infection and at matching times since second dose for controls. We found that cases had lower inferred anti-S levels and nAbT against all variants than controls ($p < 0.0001$ for all metrics). According to the conditional logistic model, each two-fold increase in anti-S levels was associated with a 29% [14–42%] reduction in odds of infection ($p = 0.001$).

We next considered whether nAbT inferred at 7 days before the cases' infection was associated with a reduction in odds of infection (Fig. 3). Firstly, we examined the distributions of titres in cases and controls (Fig. 3A-C), which suggested there may tiered benefits with increasing nAbT. For Delta nAbT, and considering < 40 as a reference, values in the range 40–100 were associated with an odds reduction

of 76% (30–92%, $p = 0.009$); values in 100–200 were associated with a 91% [72–97%] reduction ($p < 0.0001$); values > 400 were associated with a 99% [94–99.9%] reduction ($p < 0.0001$; Fig. 3D). Alternatively, the conditional regression model can report effects from each tier of Delta nAbT in relation to the category below (Fig. 3E). When comparing to < 40, Delta nAbT between 40 and 100 were associated with decreased odds of infection ($p = 0.009$). We observed additional benefit for titres in 100–200 (compared to 40–100, $p = 0.0009$) and further benefit for titres > 400 (compared to 200–400, $p = 0.007$) (Fig. 3E). Overall, Delta nAbT above 40 was associated with an 89% [69–96%] reduction in odds of infection ($p < 0.0001$).

Having established a correlate of protection against Delta infection with Delta nAbT, we repeated the analysis with nAbT against Ancestral and Omicron BA.1 inferred at 7 days before the cases' infection. Comparing the distributions of Ancestral nAbT between cases and controls (Fig. 3B), we found – when compared to titres between 0 and 100 – that values between 200 and 400 were associated with a 90% [39–98%] reduction ($p = 0.01$) and values between 400 and 800 were associated with a 99.5% [95–99.9%] reduction in odds of infection ($p < 0.0001$; Fig. 3D). Considering these odds in relation to the nAbT category below, we found that when compared to values 100–200, Ancestral nAbT between 200 and 400 were associated with a significant reduction in the odds of infection ($p < 0.0001$, Fig. 3E). There was additional benefit for titres between 400 and 800 ($p < 0.0001$) and no further benefit for titres above 800 (Fig. 3E). For Ancestral nAbT, overall titres > 200 were associated with an 84% [70–91%] reduction in the odds of infection ($p < 0.0001$).

We repeated this procedure for Omicron nAbT. Comparing distributions of Omicron nAbT inferred at 7 days before the cases' infection (Fig. 3 C), we found – when compared to titres below 40 – titres between 40 and 100 were associated with a 91% (74–97%, $p < 0.0001$) reduction in odds of infection, whilst titres > 100 were associated with a 99% [97–99.2%] reduction ($p < 0.0001$; Fig. 3D). Omicron nAbT between 40 and 100 were associated with decreased odds of infection ($p < 0.0001$) when compared to < 40, with additional benefit for titres > 100 ($p < 0.0001$, Fig. 3E). Overall, nAbT > 40 were associated with 94% [83–98%] reduction in odds of infection ($p < 0.0001$).

Association between antibody levels, titres and odds of Delta infection 6 weeks after vaccination

We performed the same analysis as above on inferred antibody levels and nAbT at 6 weeks after the second dose of each participant. Whilst this is a retrospective analysis, with inferred antibody levels, we wanted to establish if, in principle, odds of infection could be forecasted based on post-dose measurements. Consistently, we have found that controls have higher titres anti-S levels ($p = 0.001$) and higher nAbT against all variants ($p < 0.0001$ for all). Again, each two-fold increase in anti-S levels was associated with a 22% [9–33%] reduction in odds of infection ($p = 0.002$).

Again, we assessed if there were additional gains from higher nAbT, as the distributions between cases and controls (Fig. 4A-C) once more suggested there may be further benefit with higher inferred titres at 42d after the participant's second vaccination. Comparing the distributions of Delta nAbT between cases and controls (Fig. 4A), we found – taking 0–100 as the reference – that values between 100 and 200 and above 800 were associated with, respectively, 64% (6–86%, $p = 0.04$) and 94% (80%–98%, $p < 0.0001$) reductions in odds of infection, respectively (Fig. 4D). Titres between 100 and 200 were associated with decreased odds of infection ($p = 0.04$) when compared to < 100, with additional benefit for titres > 800 ($p = 0.01$) (Fig. 4E). Overall, Delta nAbT > 100 were associated with an 82% [54–93%] reduction in odds of infection ($p = 0.0004$).

Once more we repeated this analysis of protection against Delta infection using nAbT against Ancestral and BA.1. When considering
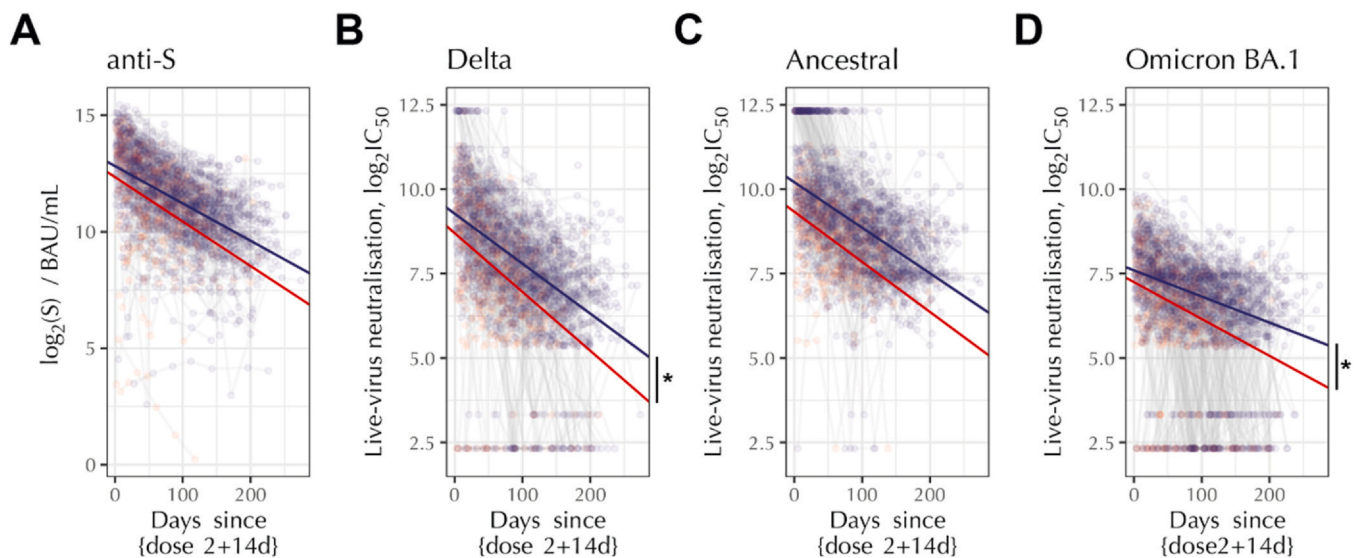
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Fig. 2. Trajectories for anti-S and neutralising antibodies after second SARS-CoV-2 vaccinations. (A) Logarithm of anti-S antibodies (S) are plotted against time in days since second doses for participants who develop Delta infections (cases, red) and uninfected participants (controls, blue). (B-D) Logarithm of neutralising antibody titres (nAbTs), measured by live virus microneutralisation and expressed as IC50, plotted against time in days since second doses for cases and controls (as in A). IC50 is the reciprocal of the dilution of sera at which 50% of viral infection is inhibited in vitro. Ancestral SARS-CoV-2 (B), Delta (C) and Omicron BA.1 (D) variants are shown. In (A-D), samples from the same individual are linked by a grey line, and average slopes and intercepts from mixed effects linear model (A) or mixed effects tobit models (B-D) are plotted. In (A-D), there are 496 sera from 130 cases and 1748 sera from 318 controls. The rate of decay (slopes) between cases and controls are similar for anti-S and Ancestral nAbTs, but faster in cases for Delta nAbTs (p = 0.02) and Omicron BA.1 nAbTs (p = 0.002).

the distribution of Ancestral nAbT between cases and controls (Fig. 4B), we found – taking 0–200 as the reference – that titres between 400 and 800 were associated with an 82% [15–96%] reduction in odds of infection (p = 0.03), whilst values > 800 correlated with a 96% [82–99%] reduction (p < 0.0001) (Fig. 4D). nAbT between 400 and 800 were associated with a significant reduction in the odds of infection (p = 0.03), when compared to values between 200 and 400, with additional benefits for titres > 800 (p < 0.0001) (Fig. 4E). Overall, Ancestral nAbT > 400 were associated with 80% [62%–89%] reduction in the odds of infection (p < 0.0001).

Finally, we assessed the Omicron nAbT distribution between cases and controls (Fig. 4C), and nAbT between 100 and 200 were associated with decreased odds of infection (p = 0.002) when compared to < 100 (Fig. 4D). We found trends of further small reductions in the odds of infection with increasing Omicron nAbT, which improve the overall odds reduction estimates (Fig. 4D), with the effect of crossing each tier not reaching significance (Fig. 4E). Overall, Omicron nAbT > 100 were associated with a 69% [44–82%] reduction in odds of infection (p = 0.0001) when compared to < 100.

We next assessed whether titres could be forecasted with later post-dose 2 measurements. As our earliest Delta infection occurred 71 days after the second vaccine dose, we have performed the same analysis looking into protective antibody titres at 63 days (9 weeks) after dose 2. We have found that similarly to results 6 weeks after vaccination, Delta nAb > 100 (p = 0.01) is associated with significant reduction of risk when compared to < 100, although additional benefits start from above 200 (p = 0.01, Figure S1).

Discussion

This analysis on SARS-CoV-2 Delta infections after vaccination highlighted key serological differences among cases and controls, including lower peak response after seconds dose and faster waning of Delta and Omicron nAbT post-vaccination in cases with Delta infection, which likely is related to susceptibility to infection as per previous findings. As demonstrated on samples pre-vaccine roll-out,[8] cases had lower inferred anti-S levels and nAbT against all variants before infection when compared to controls.

The experimental determination of a serological CoP has proven challenging throughout COVID-19 pandemic.[7,8] It requires considerable number of serum samples, enhanced PCR testing and sequencing capacity to accurately detect/confirm SARS-CoV-2 infection, assign its variant, and the identification of appropriate controls. Using a case-control design nested within the SIREN study, we have been able to address these challenges, and provide here a serological CoP against Delta infection at 7 days before infection, as well as forecast odds of infection from 42 days after second vaccine dose.

Increasing anti-S levels offer a continuous reduction in odds of infection (around 30% reduction with a two-fold increase), whereas odds of infection predicted by nAbT were more tiered (Delta nAbT > 40 are associated with an 89% reduction in odds of infection), and therefore, a much more robust CoP as previously demonstrated.[8,17,18] Although correlated with neutralising activity,[8,19] our findings emphasise that widely available binding assays to Ancestral Spike protein may not offer an accurate prediction of protection against VOC infection. When comparing nAbT against different variants, having Delta nAbT as reference, we demonstrated that higher Ancestral nAbT (> 200) were required to neutralise Delta variant, whereas Omicron nAbT > 40 were sufficient. These findings are consistent with antigenic differences in the spike protein in emergent variants, which require higher neutralisation titres to prevent infection.[20–22]

When forecasting odds of infection 42 days after vaccination based on nAbT, we found that Delta nAbT > 100 is associated with around 82% of protection against infection, however large confidence intervals (54–93%) indicate this prediction may not be as strong as for nAb 7 days before infection. This type of analysis may be especially relevant to predict the need of booster doses, however, it would require a large number of sera, and a live isolate of the prevailing variant available in the laboratory, and recommendations would require rapid implementation which are currently not feasible in clinical practice.

There are some limitations to our study. Firstly, we have a small number of AZD1222 recipients, and caution should be taken in
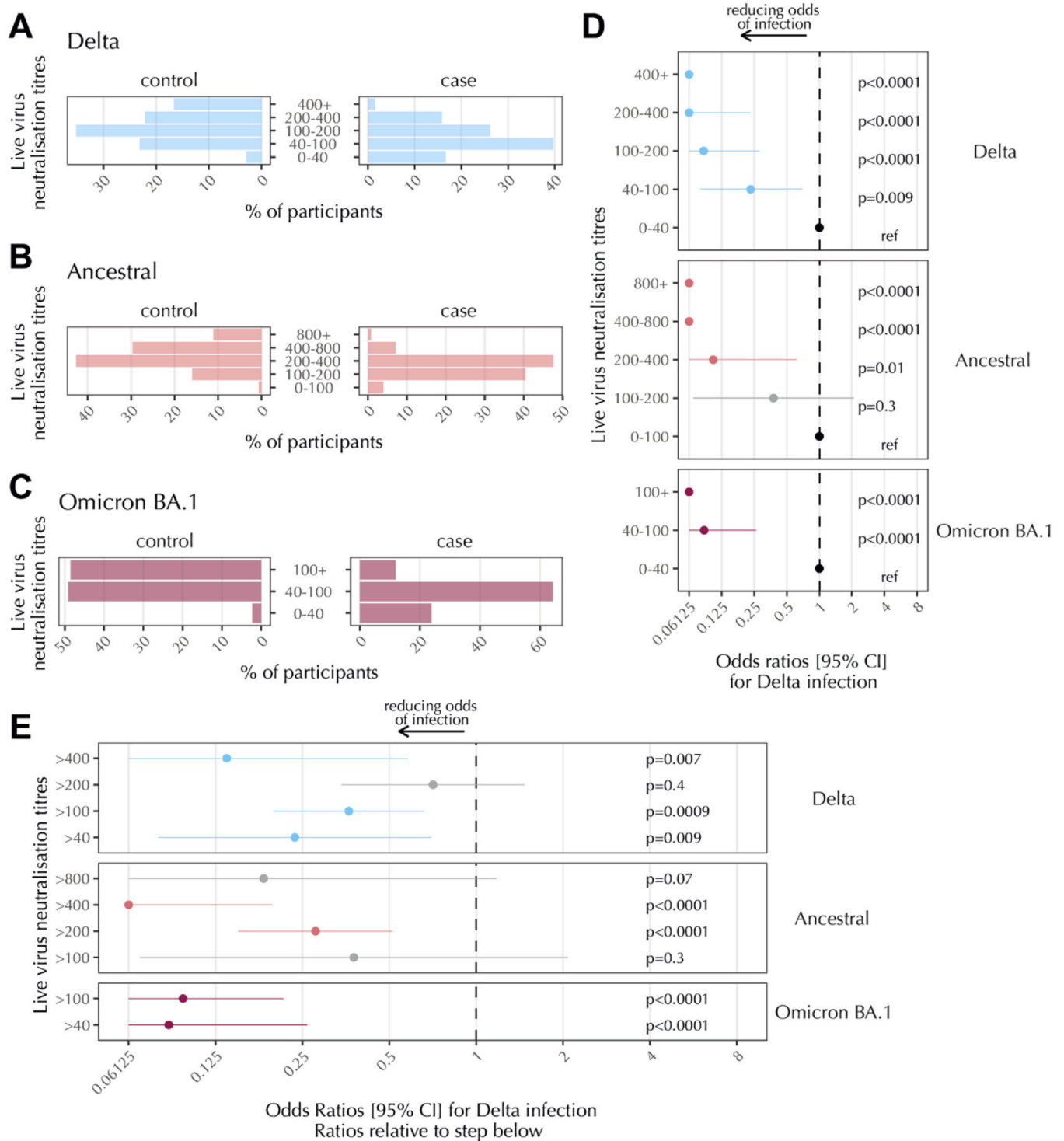
Fig. 3. Antibody titres inferred at 7 days prior to Delta PCR positivity correlate with protection. (A-C) Pyramid plots of the percentages of cases and controls within each tier of neutralising antibody titres (nAbTs) against Delta (A), Ancestral (B) and Omicron BA.1 (C), using nAbTs inferred at 7 days before PCR test positivity in cases and a corresponding time of waning in each matched control. (D) Forest plots of odds ratios of Delta infection using inferred titres summarised in (A-C) for Delta, Ancestral and Omicron BA.1, compared to the lowest category. For each variant, the reference group is shown. (E) As in (D), with stepwise odds ratios calculated as the reduction in odds for each ascending step in inferred titres, compared to the lower category. In (D) and (E), p values for each odds ratio are shown and odds ratio with 95% CI are plotted. If p > 0.05, the odds ratio is plotted in grey. In all logistic models, we included 126 cases and 307 controls because some participants remained unmatched after the exclusions detailed in the Methods, and the conditional models can only include groups of matched participants.

generalising their waning trajectories. Given the differences in vaccine effectiveness between AZD1222 and BNT162b2, it seems unlikely that AZD1222 serological responses would wane more slowly than our estimates. Secondly, these results might not be directly applicable to the wider population in several ways: it is possible that

exposure and risk behaviours among healthcare workers may differ to the general population; by design, we have considered only vaccine-induced immunity, and it is conceivable that infection-induced immunity might have more potent immunological memory at respiratory mucosae reducing a serological CoP; and this analysis
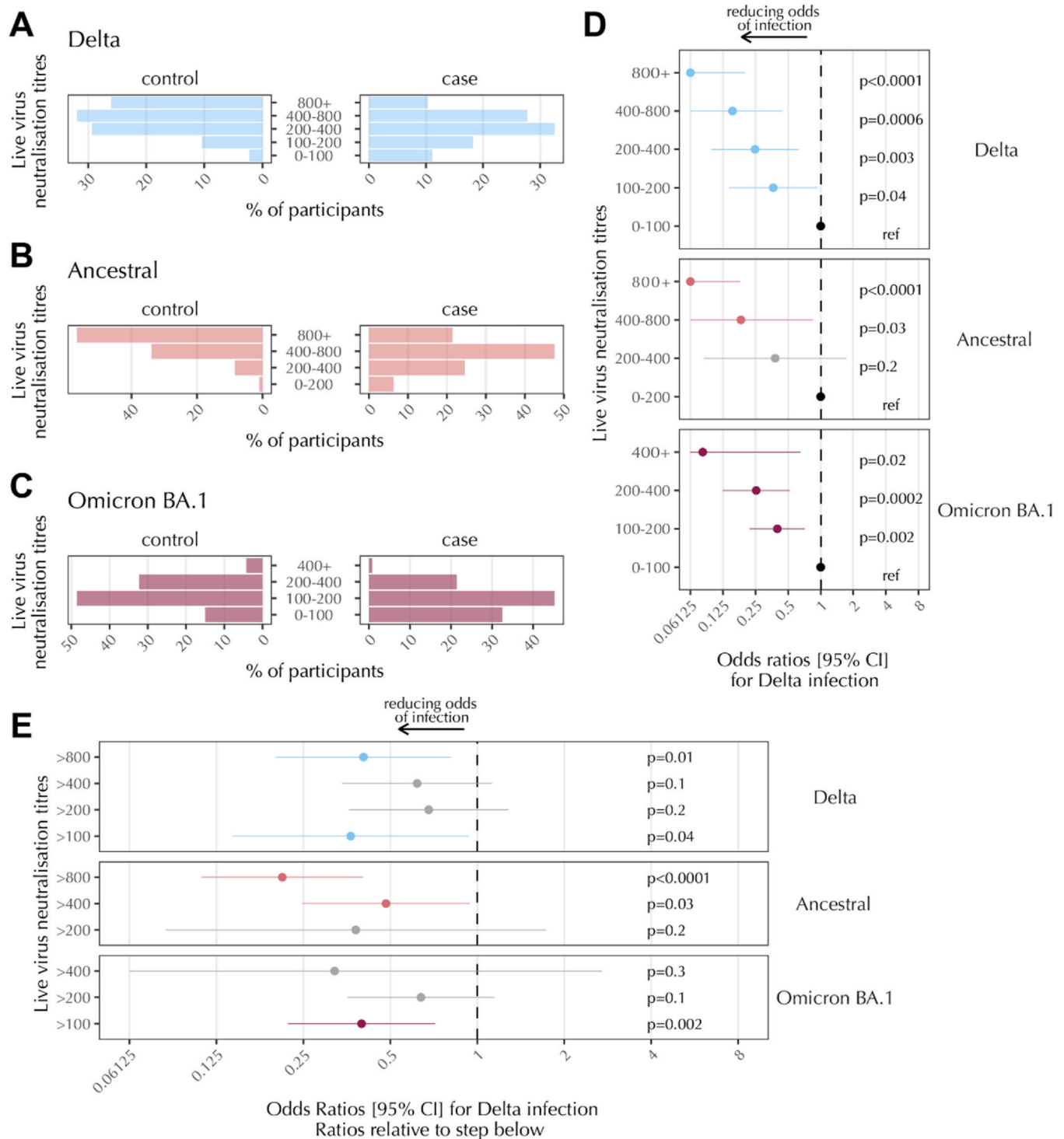
Fig. 4. Antibody titres inferred at 42 days after second vaccinations correlate with protection. (A-C) Pyramid plots of the percentages of cases and controls each tier of neutralising antibody titres (nAbTs) against Delta (A), Ancestral (B) and Omicron BA.1 (C), using nAbTs inferred at 42 days after second doses in both groups. (D) Forest plots of odds ratios of Delta infection using inferred titres summarised in (A-C) for Delta, Ancestral and Omicron BA.1, compared to the lowest category. For each variant, the reference group is shown. (E) As in (D), with stepwise odds ratios calculated as the reduction in odds for each ascending step in inferred titres, compared to the lower category. In (D) and (E), p values for each odds ratio are shown and odds ratio with 95% CI are plotted. If p > 0.05, the odds ratio is plotted in grey. In all logistic models, we included 126 cases and 307 controls because some participants remained unmatched after the exclusions detailed in the Methods, and the conditional models can only include groups of matched participants.

includes few older, or immunocompromised, vulnerable participants, such as those receiving haemodialysis[23]. Thirdly, live virus microneutralisation assays are not widely available at scale, in part due to BSL3 and in part due to technical expertise, however, we illustrate that nAbT could offer benefits, for example, for targeted vulnerable groups. Finally, we have used inferred titres for the

experimental determination of CoP for a given variant; however, the use of experimentally measured titres would require even more frequent prospective serum, not feasible since it places extreme demands on participants and resources.

In conclusion, we identified serological CoP against Delta infection after two vaccinations, from inferred neutralising antibody

titres 7 days before infection. Furthermore, we find that serological responses at 42 days after second vaccination provide predictive odds of infection. Our results are informative in two ways: firstly, it shows there is in fact a numerical threshold around which change in risk can be measured upon which vaccinology research should focus; and secondly, it demonstrates that there is no simple route to translate this into a risk prediction model applicable to widespread clinical practice, even in a resource unlimited setting – deployment should target vulnerable groups.

Author Contributions

AA, EJC, and FI designed the analysis plan and wrote the paper. AA, FI, EJC, ADO, AS, TB, SH, AC, RB and VH contributed to study design. AA, SF and FI managed and finalised the dataset for analysis. EJC and FI analysed data. FI performed the statistical modelling, supervised by AC. AA did the literature search. EJC, ADO, MYW, MJC, EL, AS, TB and RB contributed to sample testing. AA, EJC, FI, ADO, SF and VH had full access to all the data in the study. All authors reviewed, approved the manuscript for publication and accept responsibility to submit for publication.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. All authors confirmed they have no completing interest to declare.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jinf.2023.07.007.

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