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Annalan M D Navaratnam , Madhumita Shrotri , Vincent Nguyen ,
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Affiliations

Institute of Health Informatics, University College London, UK (AMDN, MS, VN, SB, IB, TB, EF, WF, CG, PP, AA, AY, RWA); Institute of Epidemiology and Health Care, University College London, London, UK (JK, SB, ACH); Department of Infectious Disease Epidemiology, London School of Hygiene and Tropical Medicine, Keppel Street, London, UK (EF); Institute for Global Health, University College London, London, UK (AR).

*Correspondence to: Prof Robert W Aldridge, r.aldridge@ucl.ac.uk

**Virus Watch Collaborative:

Susan Michie, Pia Hardelid, Linda Wijlaars, Eleni Nastouli, Moira Spyer, Ben Killingley, Ingemar Cox, Vasileios Lampos, Rachel A McKendry, Tao Cheng, Yunzhe Liu, Jo Gibbs, Richard Gilson, Anne M Johnson.

Centre for Behaviour Change, University College London, London, UK (SM); Department of Population, Policy and Practice, UCL Great Ormond Street Institute of Child Health, London, UK

(PH, LW, EN, MS, BK); Francis Crick Institute, London, UK (EN, MS); Health Protection and
Influenza Research Group, Division of Epidemiology and Public Health, University of
Nottingham School of Medicine, Nottingham, UK (BK); University College London Hospital,
London, UK (BK); Department of Computer Science, University College London, London, UK (IC,
VL); London Centre for Nanotechnology and Division of Medicine, London, University College
London (RM); SpaceTimeLab, Department of Civil, Environmental and Geomatic Engineering,
University College London, London, UK (TC, YL); Institute for Global Health, University College
London, London, UK (JG, RG). Institute for Global Health, University College London, London, UK
(AMJ).

Corresponding author

Prof Robert W Aldridge, r.aldridge@ucl.ac.uk,

T: 0207692000 ext 65541

Institute of Health Informatics, University College London, London, WC1E 6BT

Highlights

- If confirmed infection, 80% of individuals were positive for nucleocapsid antibody.
- Median peaked between days 90 and 119 after infection.
- Waning levels after 120 days, but earlier for females and younger age categories.
- Vaccination status prior to infection may reduce positivity.

Abstract

Objectives:

Seroprevalence studies can provide a measure of SARS-CoV-2 cumulative incidence, but a better
understanding of spike (anti-S) and nucleocapsid (anti-N) antibody dynamics following
infection is needed to assess longevity of detectability.

Methods:

Adults aged ≥18 years old, from households enrolled in the Virus Watch prospective community cohort study in England and Wales, provided monthly capillary-blood samples which were tested for anti-S and anti-N. Participants self-reported vaccination dates and past medical history. Prior polymerase chain reaction (PCR) swabs were obtained through Second Generation Surveillance System (SGSS) linkage data. Primary outcome variables were seropositivity and total anti-N and anti-S levels after PCR confirmed infection.

Results:

A total of 13,802 eligible individuals provided 58,770 capillary blood samples. 537 of these had a prior positive PCR confirmed SARS-CoV-2 infection within 0-269 days of antibody sample date, with 432 (80.45%) having a positive anti-N result. Median anti-N levels peaked between days 90 and 119 post PCR results and then began to decline. There is evidence of anti-N waning from 120 days onwards, with earlier waning for females and younger age categories.

Conclusion:

Our findings suggests that anti-N have around 80% sensitivity for identifying previous COVID-19 infection and duration of detectability is affected by sex and age.

Keywords

Anti-N; anti-S; serosurveillance; COVID-19; corona virus

Introduction

Antibodies produced following natural infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the virus which causes COVID-19, are known to provide some protection against reinfection for at least 6 months in the early stages of the pandemic.(Hansen

et al., 2021) The proportion of infected individuals who are N-antibody (anti-N) and S-antibody (anti-S) positive and the stability of the antibody response over time are not well established.(Siggins et al., 2021) In the UK, surveillance has been largely through symptomatic testing with reverse transcriptase polymerase chain reaction (PCR) assays or asymptomatic testing through Lateral Flow Device (LFD) tests. In large scale population surveys for the Office for National Statistics (ONS) COVID Infection Study and the REal-time Assessment of Community Transmission (REACT) study and UK Health Security Agency (UKHSA) (formerly Public Health England) blood donation surveys, monitoring via seroprevalence is being carried out.(Coronavirus (COVID-19) Infection Survey, UK, n.d.; Sero-surveillance of COVID-19, n.d.; Riley et al., 2021) Meta-analyses of the proportion of infections that are asymptomatic show approximately one third of cases do not develop symptoms at any point during acute infection.(Beale et al., 2020)

Seroprevalence studies can provide a measure of cumulative incidence that accounts for asymptomatic infections, but more information on antibody waning is needed to aid the interpretation of these studies. The widespread use of COVID-19 vaccines in England and Wales (e.g. Pfizer, AstraZeneca and Moderna) that only stimulate anti-S means that distinguishing antibodies derived from natural infection from those derived through vaccination requires measurement of both anti-N and anti-S.(Favresse et al., 2020) A challenge with seroprevalence studies is that it is still unclear how long anti-S and anti-N remain in circulation post-infection. The duration of which antibodies are detectable can also inform modelling approaches guiding the pandemic response, especially in countries where vaccine roll-out is in early stages. Much of the current evidence on antibody response or duration of detection is focused on specific occupational or institutional sub-groups, with reversion of anti-N at 242 days (e.g healthcare, university, nursing homes).(Shrotri et al., 2021; Vusirikala et al., 2021, Krutikov et al) Assessing longer-term antibody responses across the population is critical to evaluate immune protection at the population level. Furthermore, anti-S are produced in response to both vaccination or previous infection and are therefore not an accurate measure of previous infection in countries

that have rolled out widespread COVID vaccination programmes. Anti-N, which is only produced in response to SARS-CoV-2 infection or vaccines not available in the UK, may be a reliable option for serosurveillance, but little is known about the timeline of seroconversion nor duration of detectable levels of antibodies. (Bochnia-Bueno et al)

To improve our understanding of the longevity of anti-N and anti-S responses over time, we evaluated both antibody detection and titres to establish factors that contribute to seropositivity and waning post infection, defined by previous positive PCR. Specifically, we aimed to investigate:

1. The proportion of individuals who are anti-N positive, within 269 days of PCR confirmed infection, and associated demographic and clinical characteristics.

2. Anti-N and anti-S detection and titres from 0-540 days since infection.

3. Comparison of anti-N and anti-S detection and titres based on infection, vaccination or both.

Methods

Study design and setting

The Virus Watch study is a household community cohort of acute respiratory infections in England and Wales that started recruitment in June 2020.(Hayward et al., 2021) As of 31 August 2021, 50,773 participants were recruited using a range of methods including post, social media and SMS messages and letters from their General Practice. Participants provided information on age, sex, ethnicity, household information (e.g. number of household members, postcode) and medical history (e.g. underlying medical conditions, medication history). Participants were followed-up weekly by email with a link to a survey that captured information about vaccination status and SARS-CoV-2 infection. Between February and May 2021, invitations to participate in

monthly antibody testing were sent to enrolled eligible households. Consenting participants

provided capillary blood samples on a monthly basis up until 31st August 2021.

Samples

Capillary blood samples (400-600µL) were self-collected by participants using an at-home kit,

manufactured by the company Thriva. (Thriva, n.d.) Completed kits were returned by

participants using pre-paid envelopes and priority postage boxes to United Kingdom

Accreditation Service (UKAS)-accredited laboratories, for serological testing using Roche's

Elecsys Anti-SARS-CoV-2 electrochemiluminescence assays targeting total immunoglobulin (Ig)

to the Nucleocapsid (N) protein, or to the receptor binding domain in the S1 subunit of the Spike

protein (S) (Roche Diagnostics, Basel, Switzerland). Results for anti-N were reported as numeric

values in the form of a cut-off index (COI). The detection limit is determined by the ratio of the

luminescence of the sample relative to the predefined negative threshold. This threshold was

determined by calibrating against known negative samples.(Egger et al., 2020) At the

manufacturer-recommended seropositivity thresholds (≥1•0 cut-off index [COI] for N and

≥0•8 units per millilitre [U/ml] for S), the N assay has a sensitivity of 97·2%-99•5% and

specificity of 99•8%, while the S assay has a sensitivity of 97•9%-98•8% and a specificity of

100% with high agreement between the assays for samples from previously infected

individuals. (Ainsworth et al., 2020;

Evaluation_of_Roche_Elecsys_anti_SARS_CoV_2_PHE_200610_v8.1_FINAL.pdf, n.d.; Riester et al.,

2021) All samples processed between 24th February 2021 (first samples) to 31st August 2021

were included in the analysis of anti-N and, depending on vaccination status, anti-S results. For

anti-S levels, samples processed before the 1st July (with the exception of a 2 day pilot) had an

upper limit of 250U/ml. After this date, if sample results exceeded the upper limit of the

analytical measuring interval they underwent dilution with diluent universal of 1:10 up to

1:100, providing an upper limit of 25,000 U/ml. (download.pdf, n.d.)

Covariates

Age, sex, ethnicity, underlying health conditions, body mass index (BMI) and whether

participants were taking immunosuppressants were self-reported during study registration.

Age was grouped into the following categories: 18-34, 35-49, 50-64, 65-79 and ≥80 years.

Ethnicity data were grouped into Black, White, South Asian, Other Asian, Mixed categories

and Other/missing. Sex was limited to male and female categories. Participants were asked

to report specific health conditions, which were grouped as part of the analysis (further

details in Appendix Table 1).

The primary source of data was the Virus Watch dataset linked to the Second Generation

Surveillance System (SGSS), which contains SARS-CoV-2 PCR test results. The linkage period for

SGSS encompassed data from April 2020 until August 2021 due to data availability. The earliest

positive test result was taken as the date for PCR-antibody time analysis. We assumed that

participants who did not have a positive PCR result in the SGSS never had virologically

confirmed SARS-CoV-2 infection, so were therefore excluded from analysis.

Vaccination status was collected through the weekly Virus Watch questionnaire. This question

was added to the weekly surveys on 11th January 2021, where individuals reported dates of

their vaccination. Vaccination type was recorded in data collection and used to establish

national licensure date for each type of vaccine (e.g. Pfizer 2nd December 2020, AstraZeneca

30th December 2020 and Moderna 8th January 2021). Results were excluded from analysis if

the reported vaccination date preceded the national licensure date or if a second dose was

reported but not a first dose. This was specifically relevant for comparison of anti-S and anti-N

responses post-infection among those who had not been vaccinated or provided a sample pre-vaccination. When analysing response to vaccination in those with no evidence of prior infection only samples with negative anti-N were included.

Primary outcome(s)

The main outcome variable was detection of anti-N expressed as a binary variable (≥1•0 COI), and anti-N level, expressed as the semi-quantitative COI, and subsequently log-transformed to base 10.

Secondary outcomes

Secondary outcome was comparison of seropositivity to anti-S and anti-N, and levels in relation to prior infection and vaccination.

Participants

Within the Virus Watch cohort, eligible households were defined as having at least one adult aged 18 years and over, a valid England or Wales postcode, and a complete postal address registered.(Hayward et al., 2021) Individuals that were 18 years and over within eligible households could consent to participate through provision of valid, electronic consent.

Individuals were included in this analysis if they had provided at least one finger prick blood sample. Evidence of prior infection was defined as seropositivity to the Nucleocapsid protein (COI ≥ 1•0) among vaccinated individuals, or seropositivity to either Spike (≥0.8 U/ml) or

Nucleocapsid protein among unvaccinated individuals. Samples with void anti-N or void anti-S results were excluded from analyses.

Analysis

Analyses were carried out using two approaches: at individual level and at sample level. At individual level, samples obtained between 0-269 days post positive SARS-CoV-2 PCR were aggregated, selecting the sample with the first positive anti-N result if there was more than one sample per participant. Individual level data was used to compare age, sex, ethnicity, body mass index (BMI) or underlying medical conditions and if taking immunosuppressants. Proportions of individuals positive for anti-N or anti-S were calculated with 95% confidence intervals. To calculate odds of seroconversion after infection, and explore which explanatory variables influence this, univariable and multivariable logistic regression models were created using individual-level data from those with a previous positive PCR result. The multivariable model was adjusted for age and sex at birth, when they were not the explanatory variable being investigated. The explanatory variables included age categories, sex at birth, conditions associated with severe COVID-19 infection, obesity and whether taking any immunosuppressants or not (see Supplementary files; Table 1).

For antibody levels, as they are not normally distributed, median values for each group were calculated with interquartile ranges. Due to the available assay platform and dilution capabilities, the dynamic range of the anti-S assay was limited to 0•4-250 U/ml for samples processed before between February and June 2021. Only samples that underwent further dilution, from July 2021 onwards, were included in anti-S levels analysis. As dilution capabilities did not affect the threshold for a positive result, all anti-S samples were included

when calculating proportion of positivity. For analysis of antibody positivity and levels by days post PCR confirmed infection (e.g. 0-29, 30-59, 60-89, 90-119, 120-269 and 270+ days after a positive PCR), all samples were included. The proportion of anti-N positive samples, with 95% confidence intervals, and median antibody levels were calculated for each time period category. Anti-N positivity and levels over time were stratified by age groups (18-49 and 50+) and sex. Comparison of anti-N and anti-S positivity and levels were made for unvaccinated individuals or those who provided a sample before vaccination.

Comparison of anti-N and anti-S positive levels were made between those with prior infection only (no vaccination) and those who had both vaccination and prior infection. Further comparison was made on anti-S positivity over time for those with prior infection only (no vaccination) and prior vaccination only (no infection), using the date of PCR test or vaccination date as day 0. Antibody levels were not normally distributed, therefore non-parametric tests were used to compare groups: the Mann-Whitney U (Wilcoxon Rank Sum) test for two groups, and the Kruskal-Wallis test for more than two groups with Benjamini & Hochberg correction. For comparison of binary variables, the Chi-Square test was used. All analyses were carried out with R studio (R 4.0.5.) using packages: 'tidyverse', 'ggplot2', 'flextable' and 'rstatix'. Results

15,534 participants were consented and eligible to take part in the study, of which 13,802 participants provided 58,770 valid anti-N samples (Figure 1). The median age of participants was 63 (IQR 46, 80), and of 13,765 with a recorded sex at baseline survey, 57•07% (n = 7,856) were female. 5,331 samples from 1,479 individuals were anti-N positive.

Of the 13,802 individuals with valid antibody results, 596 had a prior positive PCR result from the SGSS linkage data. Of those who did not have a prior positive PCR result in the SGSS linkage data, 8.2% (999/13206) were positive for anti-N. 537 of those individuals had samples within 269 days (59 excluded) of their PCR result, and were therefore included in the individual level analysis. 80•44% of individuals (432/537) were seropositive for anti-N after PCR confirmed infection (see Table 1 for groups by age, gender and vaccination status and supplementary file Table 2 and 3 for breakdown by medical conditions). A univariable logistic regression model showed no evidence for a difference in being seropositive by age, sex or health condition. A multivariable logistic regression found higher odds of being seropositive if aged between 35-49 compared to age 18-34 (adjusted OR 1•98; p = 0•04), but no association was found for sex, obesity, conditions related to severe COVID19 infection or if taking any immunosuppressant (Supplementary file;, Table 4).

We examined anti-N positivity and titres over time after infection, with PCR test date as day 0 (Figure 2). The minimum and maximum days post PCR were 1 day and 520 days (median 168•5 days, IQR 103•63 - 233•38), respectively. The proportion of samples with detectable levels of anti-N increased from 42•6% at days 0-29 post PCR (66/155, 95% CI 35-50) to 76% at 30-59 days (95/125, 95% CI 69-83), and then remained relatively stable at between 80-85% at 60-89, 90-119, 120-269 and 270+ days. Median anti-N titres reached a peak at around 60 COI between days 30 and 120, before beginning to decline. Patterns were similar

in both sexes and different age groups although with some evidence of a faster decline in younger adults (18-49 years old) and in women (Figure 2 and 3). Men had a higher proportion of samples with detectable levels of anti-N on days 120-269 (91%, 95%CI 88-94) and 270+ (88%, 95% 82-94) compared to women (80%, 95%CI 77-83 and 76%, 95%CI 70-82, respectively). Men also had a higher median anti-N levels than days 120-269 (39.9, IQR 13.32-98.95) compared to women (30.6, IQR 3.01 - 116, p = 0.01). Over 50 year olds had a higher proportion of samples with detectable levels of anti-N on days 270+ (85.1%, 95%CI 81-89) compared to 18-49 year olds (65.4%, 95%CI 55-76). Median anti-N levels were higher on days 120-269 and 270+ for samples from over 50 year olds (48.6COI, IQR 8.21-128 and 46.5COI, IQR 4.9-113.75, respectively) compared to 18-49 year olds (18.8COI, IQR 3.08-65.98, p <0.001, and 6.5 COI, IQR 0.18-31.79, p <0.001, respectively).

Of the 2,046 samples, 244 belonged to 124 individuals with only previous infection and no prior vaccination or only previous infection who were vaccinated after providing a capillary blood sample. At 0-29 days, 60% (95% CI 30-90%) of samples had both detectable anti-S and anti-N but anti-S detectability had a sharper increase than anti-N and remained above 90% during the follow up period (Figure 4).

Anti-N positivity was generally higher for samples from those with no prior vaccination (N = 244), compared to samples from those with vaccination prior to infection (N = 305) with the most notable difference on days 90-119; 87•8% (95% CI 78-98) vs 55% (95% CI 33-77, p = 0•011). Anti-N titres from samples from non-vaccinated individuals had higher median levels than in samples from those who had aprior vaccination (39•6 COI vs 6•53 COI, p < 0•0001).

The proportion of anti-S positivity was generally higher for samples taken from people with prior vaccination (N = 301) compared to those without prior vaccination (N = 244), with the

most notable difference on days 0-29; 60% (95% CI 30-90) vs 98•5% (95% CI (96-100), p <

0•0001. The proportion of anti-S positivity was higher in samples from individuals with only

prior vaccination (N = 46,647), in comparison to infection only (N = 244), from 0 - 269 days.

There were no results for 270+ days post vaccination and therefore were not included in this

section of the analysis. The median age in the vaccination only group was higher than the

infection only group (65 vs 43 years old, p < 0•0001).

Discussion

We present the findings of a large community cohort study demonstrating anti-S and anti-N

trends in participants with antibody results from 1 to 540 days since PCR confirmed infection.

Our study found that approximately 4 out of 5 individuals were seropositive for anti-N at any

point between 0 to 269 days after a PCR confirmed SARS-CoV-2 infection. Logistic regression

models, both univariable and multivariable, only showed higher odds of a positive ant-N result

for 35-49 year olds, compared to 18-34 year olds. The peak proportion in positivity for anti-N and

median antibody level was 120-269 days and 90-119, respectively, after infection. Proportion of

seropositivity and median antibody levels were significantly higher among men than women from

120 days onwards, which was a similar pattern observed in ≥50 year olds compared to 18-

49 year olds. Samples of those who were vaccinated prior to PCR confirmed infection, had a lower

median anti-N and higher anti-S 30-269 days post infection. The proportion of anti-S positive

samples were higher for vaccination only, compared to infection only, over time.

Previous studies have found that anti-N positivity after PCR confirmed infection ranges from 84•7% at 28 days to 68•2% at 293 days, with proportions being highest among those with severe symptom profiles. (Alfego et al., 2021; Thiruvengadam et al., 2021) Antibody positivity may be influenced by the viral load during infection, with higher viral loads causing higher levels of antigen exposure and more severe symptoms. (Ali et al., 2021) As our data is from a community cohort study it can be assumed that the symptom profiles may be less severe among our participants in comparison to hospital based longitudinal studies, and therefore leading to lower antibody levels.

Increasing age and sex in some studies have been associated with higher anti-S and anti-N IgG responses. (Alfego et al., 2021; Choudhary et al., 2021) The only factor independently associated with seropositivity in the logistic regression model was age, with 35-49 year olds more likely to be seropositive than older groups, when compared to 18-34 year olds. Yet when assessing anti-N response over time, there was a difference between age categories 18-49 and 50+ on days 120-269 and 270+, with the former having an earlier peak and earlier antibody waning. Older people have a higher frequency of comorbidities, putting them at higher risk of severe disease, which may explain longer duration of anti-N positivity. (Zheng et al., 2020) This being said, there was not a higher or lower odds of being seropositive if individuals have medical conditions associated with higher risk of COVID19 related mortality or taking immunosuppressant therapy. This is likely due to a small number of participants in these groups in our analyses (see Supplementary files: Table 5). Although limited to 269 days post infection because of disparity in length of follow up between individuals, time interval between PCR and anti-N results was not accounted for in our models. Seropositivity has been shown to be affected by days since PCR, which may also explain why some differences were not found in our regression model. (Alfego et al., 2021)

The proportion of seropositive anti-N samples was above 80% from 30 days onwards, but only 42•6% of samples were positive between days 0-29. Although this is lower than other studies, where seroconversion rates have been 84•7% at 10-28 days respectively, these studies followed individuals who had been hospitalised due to COVID19. (Thiruvengadam et al., 2021) When hospitalised individuals were stratified by symptom severity, the cumulative percentage of anti-N positive asymptomatic individuals was 60% at 22-28 days. (Thiruvengadam et al., 2021) Other studies in hospitalised individuals have also demonstrated symptom severity is associated with an earlier peak in anti-N titres, as well as determining longer duration of anti-N positivity. (Feng et al., 2021; Van Elslande et al., 2021) Difference in anti-N over time was seen when the data was disaggregated by sex, with earlier peak and waning in females. This may also be related to risk of disease severity, with a meta-analysis showing men are 2•41 times more at risk of developing severe disease in comparison to women. (Barek et al., 2020) Higher ACE2 expression, decreased B cell and NK cell-specific transcripts in men is suggested to be the cause of higher viral loads and therefore more severe symptoms. (Bwire, 2020; Lieberman et al., 2020) Although we have not used symptom severity in our analysis, as Virus Watch is a community cohort study, it can be assumed that in comparison the symptom profiles are likely to be milder on average. This would explain a lower percentage of anti-N positive samples at 0-29 days, but provides a more accurate representation of anti-N longevity in the community setting.

After excluding post-vaccination samples, there was evidence of anti-N waning in both proportion of detectable anti-N in samples and median anti-N titres, which was not the case for

anti-S. Earlier anti-N waning has also been observed in other studies, which monitored IgG. (Alfego et al., 2021; Choudhary et al., 2021) A higher proportion of non-vaccinated samples were anti-N positive compared to samples from participants who had vaccination prior to infection. This trend was also reflected in median anti-N and ant-S titres. This finding is important when considering the use of anti-N as an alternative to anti-S for seroprevalence studies in highly vaccinated populations. The anti-S response seen between vaccinated and non-vaccinated is in keeping with the immunological mechanism of the vaccines, which induces an anti-S specific response. The proportion of anti-S positive results in the vaccination only cohort was higher at days 120-269 than anti-S response in the infection only group, indicating a more sustained anti-S response from vaccination. Although anti-S levels may differ between these groups, this is not necessarily an indication of risk of future infection. Comparison of infection risk between vaccination and natural immunity has provided conflicting information, with pooled results of randomised control trials showing no difference and observational studies favouring natural immunity. (Choudhary et al., 2021) Our results may be the outcome of confounding however, as the median age of the vaccine only group was higher than infection only.

The strengths of this study include a large sample size that spans various age groups and captures multiple underlying health conditions (see Supplementary file Table 6. We present serial antibody measurements using a highly sensitive, widely used validated commercial assay that provides quantitative readings.
(Evaluation_of_Roche_Elecsys_anti_SARS_CoV_2_PHE_200610_v8.1_FINAL.pdf, n.d.) There are limitations however, as a large proportion of the data is self-reported and therefore susceptible to reporting bias, as participants may only volunteer information they feel is relevant or necessary. Self-reporting is also susceptible to data entry errors. This led to some samples being excluded from any analyses based on erroneous reporting of vaccination status. Furthermore, a large proportion of the data in this analysis (e.g. medical conditions, medications) is only collected at registration, so did not account for changes in participant's health or medications.

The dates of PCR confirmed infection however, were SGSS data, allowing accurate calculation of time between infection and blood test. Those who did not have a positive PCR confirmed infection according to the linkage data were excluded from the analysis, therefore further reducing the sample size. The earliest positive PCR result was used in the analysis and subsequent PCR results were excluded. Therefore we are unable to report on subsequent asymptomatic reinfections/re-exposure which may boost antibody levels. (Ali et al., 2021; Dispinseri et al., 2021)

Conclusion

As only 4 in 5 participants with prior PCR confirmed infection were anti-N positive at any time point up to 269 days after infection, seroprevalence studies on anti-N alone may underestimate the true cumulative incidence of infection. We have demonstrated a decline in anti-N levels from 120 days onwards, providing a better understanding of the limitations of seroprevalence studies. Duration of anti-N positivity is affected by age and sex; therefore serosurveillance may require shorter time window of testing post-infection.

Declaration of interests

☒ 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.

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Conflicts of interest

ACH serves on the UK New and Emerging Respiratory Virus Threats Advisory Group. AMJ was a Governor of Wellcome Trust from 2011-18 and is Chair of the Committee for Strategic Coordination for Health of the Public Research.

Ethical Approval Statement:

This study has been approved by the Hampstead NHS Health Research Authority Ethics Committee. Ethics approval number - 20/HRA/2320.

Data availability:

We aim to share aggregate data from this project on our website and via a "Findings so far" section on our website - https://ucl-virus-watch.net/. We will also be sharing individual record level data on a research data sharing service such as the Office of National Statistics Secure Research Service. In sharing the data we will work within the principles set out in the UKRI Guidance on best practice in the management of research data. Access to use of the data whilst research is being conducted will be managed by the Chief Investigators (ACH and RWA) in accordance with the principles set out in the UKRI guidance on best practice in the management of research data. We will put analysis code on publicly available repositories to enable their reuse.

Table 1: Proportion of those with positive anti-N result, grouped by age, sex and vaccination status

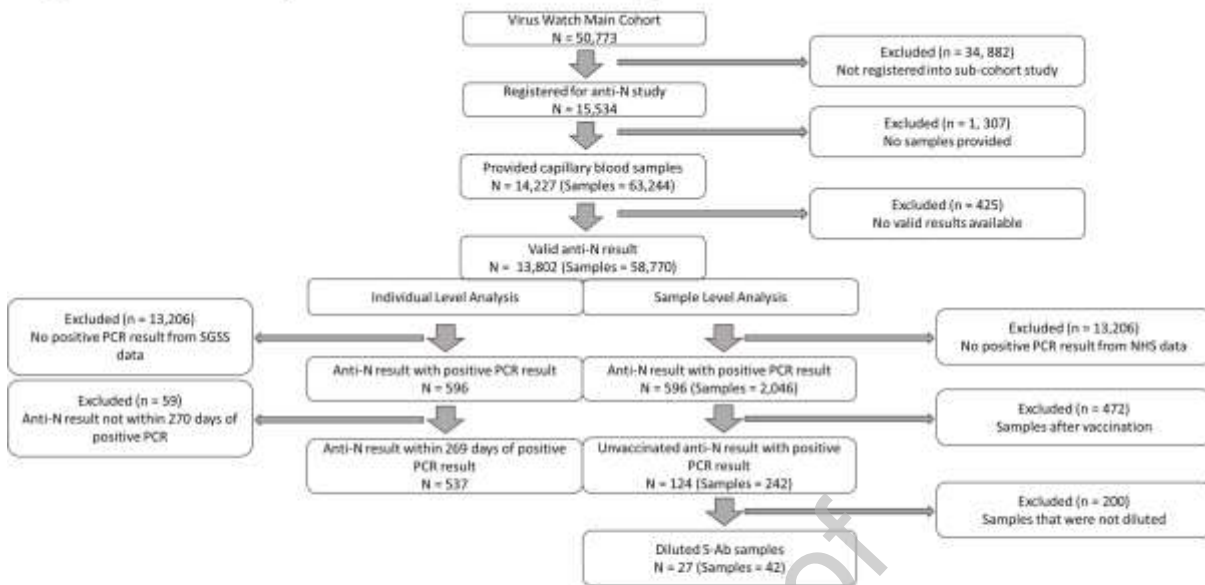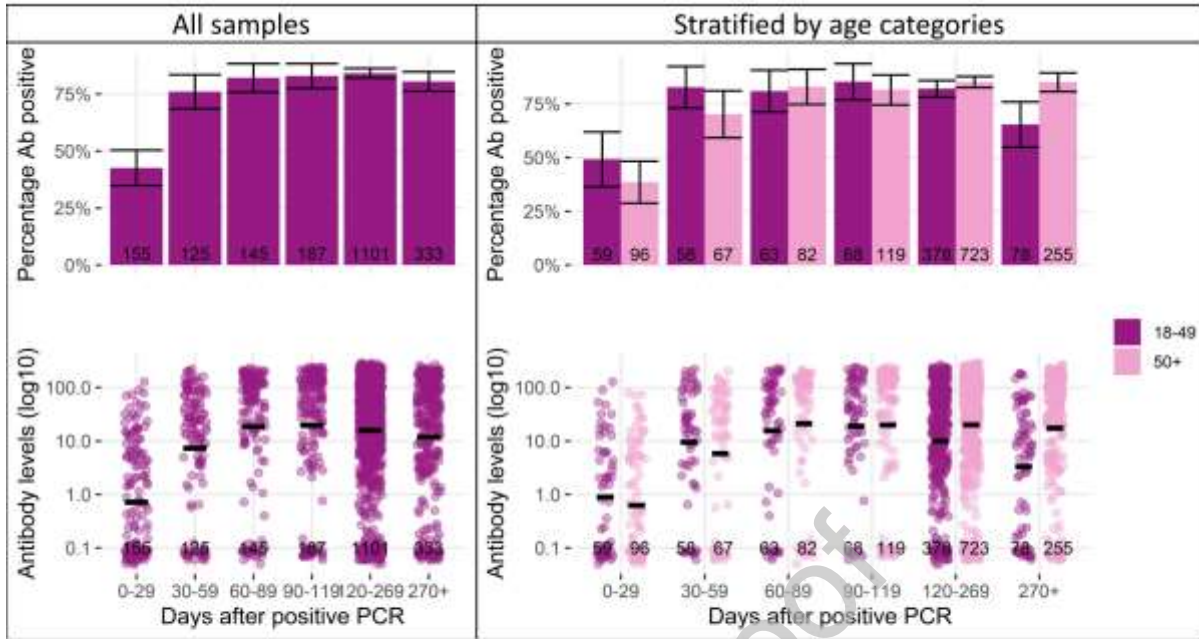|	Anti-N Positive (n / N)	Percentage Positive (%)	95% CI
Age category			
18-34	58 / 79	73·42%	(63·68, 83·16)
35-49	121 / 143	84·62%	(78·7, 90·53)
50-64	151 / 193	78·24%	(72·42, 84·06)
65-79	100 / 120	83·33%	(76·66, 90)
80+	2 / 2	100%	(100, 100)
Sex			
Female	253 / 324	78·09%	(73·58, 82·59)
Male	177 / 211	83·89%	(78·93, 88·85)
Missing data	2 / 2	100%	(100, 100)
Vaccinated			
No	101 / 116	87·07%	(80·96, 93·18)
Yes	308 / 396	77·78%	(73·68, 81·87)
Missing data	23 / 25	92%	(81·37, 102·63)

Figure 1: Study inclusion flow diagram

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Figure 2 Anti-N percentage positivity and levels, stratified by days post positive PCR test and age.

Figure 2. Anti-N percentage and levels, stratified by days post positive PCR test and age.
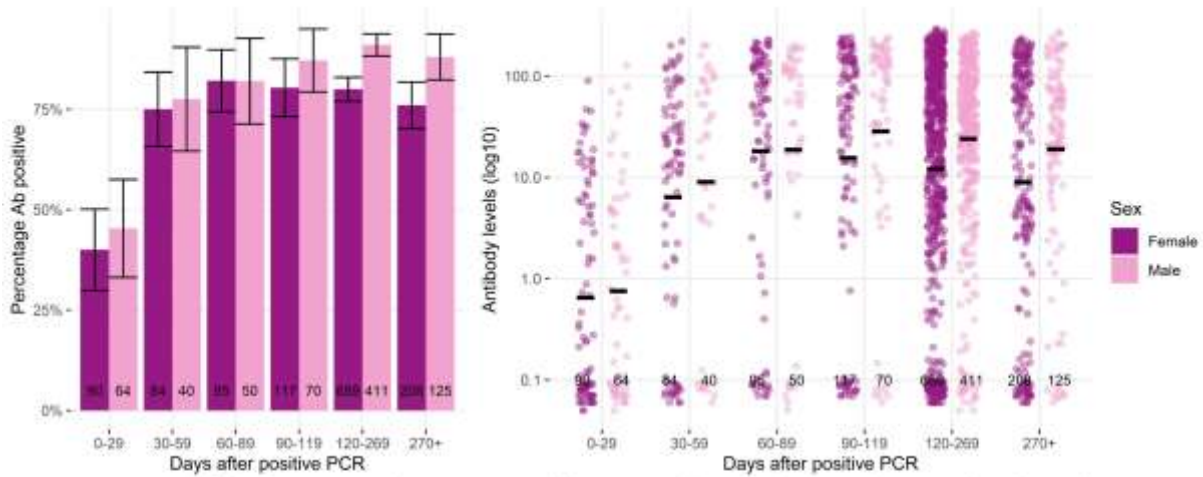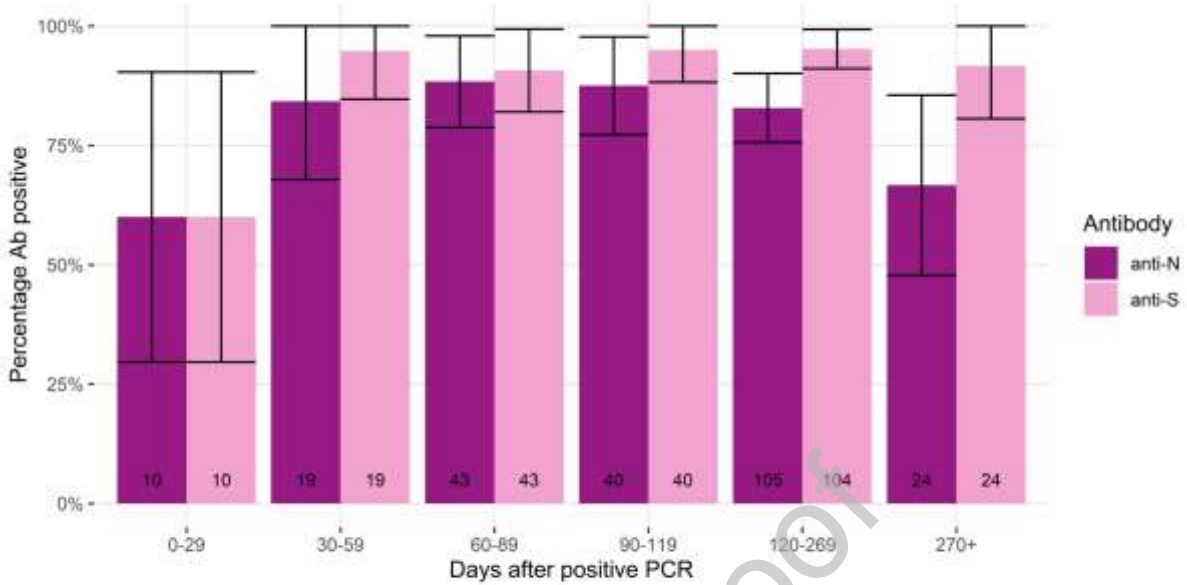
Notes: 95% confidence intervals represented by vertical cross bars and anti-N levels underwent Log10 transformation with bars representing the logarithmic mean. PCR = polymerase chain reaction

Figure 3: Anti-N percentage positivity and levels by sex and days after positive PCR

Notes: 95% confidence intervals represented by vertical cross bars and anti-N levels underwent Log10 transformation with bars representing the logarithmic mean.

Figure 3: Anti-N percentage positivity and levels by sex and days after positive PCR

Notes: 95% confidence intervals represented by vertical cross bars and anti-N levels underwent Log10 transformation with bars representing the logarithmic mean. PCR = polymerase chain reaction

Figure 4: Percentage antibody positivity anti-N and anti-S among pre-vaccinated samples or samples taken from unvaccinated individuals, stratified by days post virological confirmed infection.
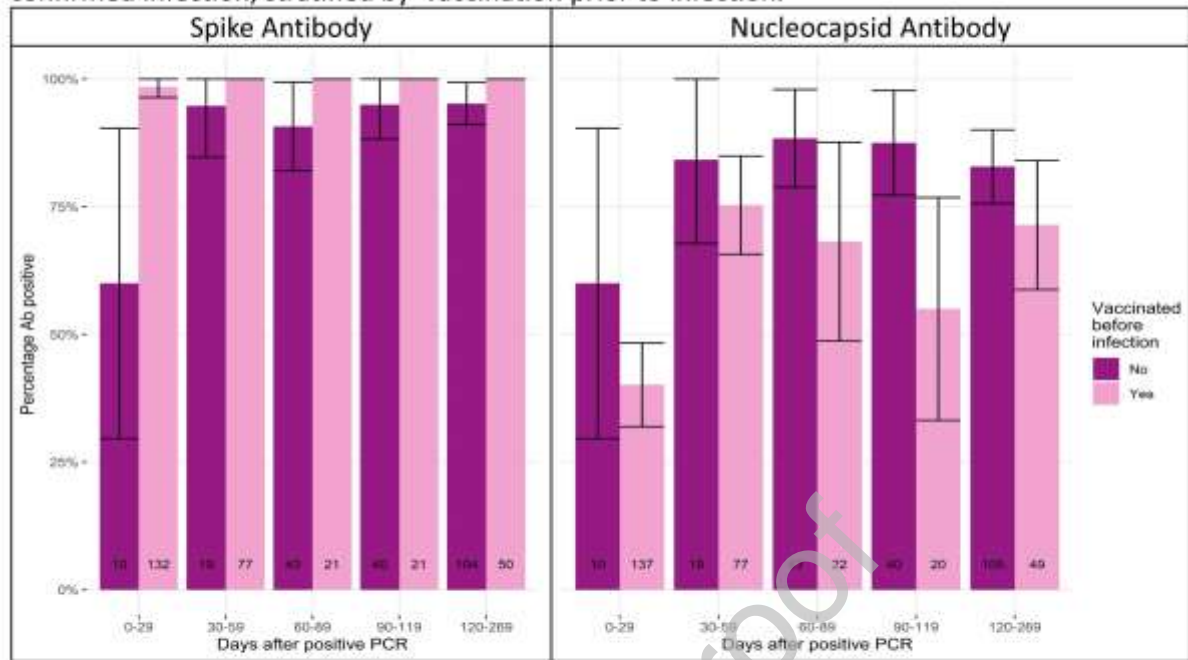
Notes: 95% confidence intervals represented by vertical cross bars

Figure 4: Percentage antibody positivity anti-N and anti- among pre-vaccinated samples or

samples taken from unvaccinated individuals, stratified by days post virological confirmed

infection.

Notes: 95% confidence intervals represented by vertical cross bars

Figure 5. Proportion of antibody positivity for anti-S and anti-N by days post virological confirmed infection, stratified by vaccination prior to infection.

Notes: 95% confidence intervals represented by vertical cross bars

Figure 5. Proportion of antibody positivity for anti-S and anti-N by days post virological

confirmed infection, stratified by vaccination prior to infection.

Notes: 95% confidence intervals represented by vertical cross bars