The duration, dynamics and determinants of SARS-CoV-2 antibody responses in individual healthcare workers

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Summary:

Serially-measured SARS-CoV-2 anti-nucleocapsid IgG titres from 452 seropositive healthcare workers demonstrate levels fall by half in 85 days. Levels fall faster in younger adults and following asymptomatic infection. Anti-spike IgG remains elevated in most seropositive individuals up to 6 months.

Abstract

Background

SARS-CoV-2 IgG antibody measurements can be used to estimate the proportion of a population exposed or infected and may be informative about the risk of future infection. Previous estimates of the duration of antibody responses vary.

Methods

We present 6 months of data from a longitudinal seroprevalence study of 3276 UK healthcare workers (HCWs). Serial measurements of SARS-CoV-2 anti-nucleocapsid and anti-spike IgG were obtained. Interval censored survival analysis was used to investigate the duration of detectable responses. Additionally, Bayesian mixed linear models were used to investigate anti-nucleocapsid waning.

Results

Anti-spike IgG levels remained stably detected after a positive result, e.g., in 94% (95% credibility interval, CrI, 91-96%) of HCWs at 180 days. Anti-nucleocapsid IgG levels rose to a peak at 24 (95% credibility interval, CrI 19-31) days post first PCR-positive test, before beginning to fall. Considering 452 anti-nucleocapsid seropositive HCWs over a median of 121 days from their maximum positive IgG titre, the mean estimated antibody half-life was 85 (95%CrI, 81-90) days. Higher maximum observed anti-nucleocapsid titres were associated with longer estimated antibody half-lives. Increasing age, Asian ethnicity and prior self-reported symptoms were independently associated with higher maximum anti-nucleocapsid levels and increasing age and a positive PCR test undertaken for symptoms with longer anti-nucleocapsid half-lives.

Conclusion

SARS-CoV-2 anti-nucleocapsid antibodies wane within months, and faster in younger adults and those without symptoms. However, anti-spike IgG remains stably detected. Ongoing longitudinal studies are required to track the long-term duration of antibody levels and their association with immunity to SARS-CoV-2 reinfection.

Keywords:

SARS-CoV-2; Covid-19; Serology; Antibody; Waning; Longitudinal

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Introduction

Measurable IgG antibodies to SARS-CoV-2 antigens develop after many, but not all, SARS-CoV-2 infections.[1–4] Serological responses are typically detectable within 1-3 weeks.[5–8] This allows antibody assays to be used to estimate the proportion of a population exposed or infected. Additionally, although the extent of immunity associated with different antibody titres and other immune responses is yet to be fully determined, it is probable that antibody levels will provide some information about the risk and/or severity of future infection.

However, SARS-CoV-2 IgG antibody levels are dynamic over time.[9] This has implications for epidemiological studies, e.g., if IgG levels fall below detection thresholds before they are measured, past infections may be under ascertained. Similarly, it has implications for estimating population protection if antibodies are a marker for protective immunity.

Contrasting data have been made available on the longitudinal trajectory and longevity of antibodies induced by SARS-CoV-2 infection. For example, a US study showed IgG antibody levels to trimerised spike were relatively stable in 121 individuals around 110 days post symptom onset.[10] Similarly, data from 1215 individuals in Iceland suggest IgG responses to nucleocapsid and the S1 component of spike were sustained for 100-125 days.[11] However, others have noted declines in neutralizing antibodies over similar time periods.[12–14]

We have recently undertaken baseline serological testing in a cohort of >10,000 HCWs.[15] We now describe serial SARS-CoV-2 antibody measurements, demonstrating quantitative anti-nucleocapsid responses fall over time and vary with age, ethnicity and previous symptoms, but anti-spike levels antibodies remain stably detected in most individuals.

Methods

Setting and participants

Oxford University Hospitals (OUH) offers both symptomatic and asymptomatic SARS-CoV-2 testing programmes to staff at its four teaching hospitals in Oxfordshire, UK. 12,411 healthcare workers (HCWs) have undergone serological testing to date; data on HCWs who attended more than once for antibody testing are presented.

SARS-CoV-2 PCR testing of nasal and oropharyngeal swabs for all symptomatic (new persistent cough, fever ≥37.8°C, anosmia/ageusia) staff was offered from 27-March-2020 onwards. Asymptomatic HCWs were invited to participate in voluntary staff testing for SARS-CoV-2 by nasal

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and oropharyngeal swab PCR and serological testing from 23-April-2020 onwards. The cohort, associated methods and findings from the first test per individual have been previously described.[15] Following initial PCR and antibody testing, asymptomatic HCWs were invited to optionally attend for serological testing up to once every two months, with some offered more frequent screening as part of related studies. Asymptomatic staff were also offered optional SARS-CoV-2 PCR tests every two weeks.

Laboratory assays

Serology for SARS-CoV-2 IgG to nucleocapsid protein was performed using the Abbott Architect i2000 chemiluminescent microparticle immunoassay (CMIA; Abbott, Maidenhead, UK). Antibody levels ≥1.40 manufacturer's arbitrary units were considered positive, 0.50-1.39 equivocal (following Abbott Diagnostics Product Information Letter PI1060-2020) and <0.5 negative. Anti-trimeric-spike IgG levels were measured using an ELISA developed by the University of Oxford,[2] using net-normalised signal cut-off of ≥8 million units to determine antibody presence and defining 4.0-7.9 million units as equivocal.[16] Details on PCR assays are provided in the Supplement.

Statistical methods

For anti-nucleocapsid antibodies, individuals with ≥1 positive antibody result (titre ≥1.40) and ≥2 antibody results were classified as showing rising titres only, falling or stable titres only, or both. Those with only one measurement could not be classified and were excluded. In those with falling/stable titres we estimated the duration of antibody responses following the maximum observed result using Bayesian linear mixed models and their association with age, gender, ethnicity, previous self-reported symptoms and PCR results (allowing correlated random intercept and slope terms, Supplement, Table S1). We assumed antibody levels fell exponentially, and so modelled log2 transformed antibody levels over time (observed data and fitted models demonstrated close congruence, Supplementary File). The incidence of Covid-19 in our hospital fell after a peak in March and April 2020,[15] such that re-exposure of HCWs was uncommon; we therefore had insufficient data to study boosting of antibody responses.

We additionally modelled the antibody trajectory from a first positive PCR test using a similar approach but allowing for non-linear effects of time rather than assuming an exponential decline.

It was not possible to model anti-spike IgG titres over time in those with a positive result as most positive readings were above the upper limit of quantification of the assay (9 million units). Therefore, we considered changes in binary results for both anti-spike and anti-nucleocapsid using Bayesian interval censored regression to estimate the proportion of individuals remaining antibody

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positive (as opposed to equivocal or negative) at varying times following their maximum antibody result (Supplement).

Ethics

Deidentified data from staff testing were obtained from the Infections in Oxfordshire Research Database (IORD) which has generic Research Ethics Committee, Health Research Authority and Confidentiality Advisory Group approvals (19/SC/0403, 19/CAG/0144).

Results

A total of 3276 HCWs provided ≥2 samples for serological testing between 23-April and 20-October-2020 (3217 had anti-nucleocapsid results [Table 1], 3123 anti-spike results, 3064 had both) (Figure 1, Table S2). For both assays the median (IQR) [range] number of samples tested was 2 (2-2) [2-8] and time from the first to last sample was 124 (95-144) [3-174] days.

Observed IgG antibody trajectories are shown in Figure 2. 522/3217 (16%) HCWs had ≥1 sample with detected anti-nucleocapsid antibodies (Figure 2A,2B) and another 90 (3%) had ≥1 sample with an equivocal titre (Figure 2C). Antibody titres in those with consistently negative results were broadly stable (Figure 2D), whereas falls were observed in 438/466 (94%) initially anti-nucleocapsid antibody positive individuals, and in 61/83 (73%) with an initial equivocal titre (Figures 2A,2C). 560/3123 (18%) HCWs had a positive anti-spike IgG (Figure 2E,2F) and 209 (7%) an equivocal result. Amongst 457 HCWs with an initially positive anti-spike titre, 362 (79%) had a final titre that remained above the upper limit of quantification and 49 (11%) had a fall in titre (Figure 2E).

Anti-nucleocapsid IgG trajectories after a positive antibody result

Among 522 individuals with ≥1 anti-nucleocapsid IgG-positive sample, 70 (13%) seroconverted with rising titres only and so were excluded from analyses of the duration of response following a peak IgG result (39/70 had a PCR test and are included in a separate analysis below). In the remaining 452 (87%), the median (IQR) [range] number of samples tested was 2 (2-3) [2-5] and time from the first to last sample was 121 (83-143) [4-171] days. Only 3/120 (3%) individuals with ≥3 measurements had a final titre above the minimum observed, i.e. potential evidence of boosting, and titre increases were all <5%. The median (IQR) age was 41 (29-50) years and 75% of participants were female (Table 1). The most common self-reported ethnic groups were White (302, 67%) and Asian (89, 20%; predominately south Asian and Filipino). 274 (61%) recalled self-identified Covid-19-like symptoms between 01-February-2020 and testing. 95 (21%) had a positive SARS-CoV-2 PCR following symptomatic testing and 59 (13%) a positive PCR during asymptomatic screening. It is likely that

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many of the remainder were infected prior to widespread availability of testing. The first positive PCR in each individual was prior to or on the day of their maximum antibody titre in all but 5/154 (3%, tested 3-17 days later).

Using a Bayesian statistical model, the trajectory of anti-nucleocapsid IgG levels following the maximum measured titre in each individual is shown in Figure 3A. The estimated mean antibody half-life was 85 (95% credibility interval, CrI 81-90) days and estimated mean maximum antibody level 4.3 (95%CrI 4.1-4.4) arbitrary units. The mean trajectory crossed the diagnostic threshold of 1.40, switching from a positive to equivocal result at 137 (95%CrI 127-148) days. IgG half-lives and maximum titres varied between individuals (Figures 3B,3C). Higher maximum observed anti-nucleocapsid levels correlated with longer IgG half-lives, i.e. slower rates of decline over time (Figure 3D; Spearman's rank R^2=0.65, p<0.0001). Findings were similar in a sensitivity analysis investigating the impact of starting with each individual's maximum result on half-life estimates (see Supplement).

Effect of demographics and other covariates on anti-nucleocapsid trajectories

Within this cohort of HCWs of working age, age, self-reported ethnicity, prior symptoms compatible with Covid-19 and a positive SARS-CoV-2 PCR were independently associated with changes in anti-nucleocapsid trajectories (Table 2, Figures S1-S5, Table S3). Increasing age was independently associated with higher maximum anti-nucleocapsid levels and a longer half-life, 0.17 (95%CrI 0.07-0.25) arbitrary units and 3.40 (1.79-4.62) days per 10 years respectively (Table 2, Figure S5). HCWs of Asian ethnicity had higher maximum anti-nucleocapsid levels with adjusted increases of 0.54 (0.18-0.95) arbitrary units compared to White HCWs, with marginal evidence for longer half-lives, by 6.28 (-0.44 to 14.8) days. Within the limits of the power of the study, there was no strong statistical evidence that antibody trajectories varied in other ethnic groups.

Prior self-reported symptoms were associated with a higher starting maximum anti-nucleocapsid levels (adjusted increase 0.40 [95%CrI 0.12-0.69]), but not changes in half-lives. There was moderate evidence that a positive PCR result undertaken for symptoms, independently increased the starting maximum level by 0.30 (-0.04 to 0.67) arbitrary units, and half-life by 9.56 (2.35-19.09) days compared to those with no positive PCR. We observed no effect of gender on either maximum level or antibody half-life.

Anti-nucleocapsid trajectories following a positive PCR test

245 of the 3217 HCWs with ≥2 anti-nucleocapsid samples had a positive PCR test. 114/128 (89%) symptomatic HCW seroconverted (maximum IgG titre ≥1.40), including 11/12 (92%) who required hospital treatment, all other infections were mild. 79/117 (68%) identified through asymptomatic

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screening seroconverted. In the 52 individuals not showing evidence of seroconversion, all but 1 (98%) had ≥1 antibody test ≥14 days after their PCR-positive test, and in 29 (56%) this was before 90 days. PCR cycle threshold values were lower in individuals who seroconverted (Table S4).

Data from PCR-positive individuals who seroconverted were used to model antibody trajectories relative to a first positive PCR test. Antibody levels rose to a peak at 24 (95%CrI 19-31) days post-first positive PCR test, before beginning to fall (Figure S6). Comparing with the antibody trajectory estimated in the main analysis, the estimated rates of waning were consistent between the two models. Antibody trajectories were similar in those being tested following symptoms or during asymptomatic screening (Figure 4).

Anti-spike trajectories

To enable comparison with anti-spike results and to facilitate comparison with studies reporting only categorical antibody results, we considered the proportion of seropositive individuals remaining antibody-positive (as opposed to equivocal or negative) when observed at varying time intervals (Figure 5A,5C) and using an interval censored survival analysis approach (Figure 5B,5D). Consistent with the model in Figure 4, the median time remaining anti-nucleocapsid IgG-positive was 166 (95%CrI 139-214) days. In contrast, anti-spike IgG levels remained above positive threshold in most seropositive HCWs for the duration of the study (Figure 5C,5D), by 180 days post maximum anti-spike IgG level an estimated 94% (95%CrI 91-96%) remained positive.

Discussion

Most epidemiological outbreak models assume that SARS-CoV-2 infection leads to the development of post-infection immunity for a defined duration. Here we show contrasting antibody trajectories in 608 symptomatic and asymptomatic HCWs seropositive for anti-nucleocapsid and/or anti-spike antibodies followed for a median of 4 months from their maximum IgG titre. We show anti-nucleocapsid IgG levels wane with an estimated half-life of 85 (95%CrI 81-90) days. We observed variation between individuals; higher maximum observed anti-nucleocapsid titres were associated with longer half-lives. Increasing age, Asian ethnicity and prior self-reported symptoms were independently associated with higher maximum anti-nucleocapsid levels, and increasing age and a positive PCR test undertaken for symptoms with longer antibody half-lives. In contrast, although we could not quantitively follow titres of anti-spike IgG, levels remained stably above the threshold for a positive result in 94% at 180 days post maximum titre.

IgG waning and reinfection within a year is reported for seasonal coronaviruses[17] whereas IgG remains detectable against SARS-CoV and MERS-CoV 1-3 years later.[18] The differences we observe in SARS-CoV-2 antibody trajectories may be antigen and/or assay dependent, e.g., stable anti-spike antibodies with waning of anti-nucleocapsid IgG using the same Abbott platform as in our study was also seen in an earlier smaller study, but total anti-nucleocapsid antibodies assayed using a Roche platform remained stable.[14] To some extent these findings are conditional on assay cut-offs which can be tuned to prioritise sensitivity or specificity, with inherently more specific assays having potential to also be set more sensitively, resulting in apparently longer durations of detectable antibody responses.

For anti-nucleocapsid, we observe higher IgG titres with longer durability occurring after symptomatic PCR-positive infection, consistent with data from Long et al. where 40% of asymptomatic individuals and 13% of the symptomatic group became negative for IgG in the early convalescent phase[19] and consistent with emerging coronaviruses, where antibody titres remained detectable longer after more severe illness,[18] waning more rapidly after asymptomatic infection.

Relatively short-term anti-nucleocapsid IgG responses have two epidemiological consequences. Firstly, antibody waning may lead to under-ascertainment of previous infections within the current pandemic, particularly in younger individuals following asymptomatic/mild infection. Additionally, IgG testing is unlikely to determine whether SARS-CoV-2 has circulated historically, e.g. in a particular geographic region.

Older age (within this cohort of working age HCWs, up to 69 years) was associated with higher maximum observed anti-nucleocapsid IgG titres and longer half-lives, with similar findings associated with Asian ethnicity (many of the Asian HCWs in our study came to work in the UK healthcare system as adults). It is possible to hypothesise that this could arise from boosting of cross-reactive anti-nucleocapsid antibodies from prior exposure, e.g. to a previously circulating or geographically-restricted human coronavirus. However, anti-nucleocapsid cross-reactivity between endemic coronaviruses and other epidemic coronaviruses, SARS-CoV and MERS-CoV, is uncommon.[18,21]

Limitations

The assays we used could only measure quantitative trajectories for anti-nucleocapsid IgG, further studies, e.g. using multiple serum dilutions, are required to quantify anti-spike IgG over time. We therefore cannot say whether the longer duration of positive anti-spike responses is due to slower waning or higher initial levels relative to assay cut-offs. Another limitation of our study was that our cohort of individuals consisted of adults of working age (17-69 years); further longitudinal studies will be required to investigate younger and older age groups. The small numbers of self-reported Black (n=25) and Other (n=36) ethnicities reduced/limited power to detect an association between these ethnicities and antibody trajectories. We also do not account for mediators, e.g. socioeconomic inequalities, that may link ethnicity to antibody responses in the absence of a direct causal relationship. Due to many of our staff developing symptoms before widespread SARS-CoV-2 PCR testing was available, only 34% of the anti-nucleocapsid-positive cohort had a documented positive PCR, and as a proportion of the cohort were asymptomatic throughout, we modelled time from maximum positive antibody test rather than time from first positive PCR or time from symptom onset in our main analysis of antibody durability. However, under an exponential assumption, half-lives can be unbiasedly estimated from any measurements taken after a maximum; we excluded individuals with only evidence of rising titres to avoid underestimating half-lives. Further, data from those that were PCR positive were consistent with this analysis (Figure 4).

Multiple different assays are in use globally to characterize antibody responses to SARS-CoV-2. Here we study only two; however, other antibody classes and targets, and aspects of immunity, including the innate and cellular responses are important in conferring post-infection immunity.[22] When comparing longitudinal studies of antibody durability, care must be taken, as the various assays have not yet been cross-calibrated, and implications for protective immunity are not fully understood.

Implications

It is widely recognized that pathogen-specific IgG levels decline after the acute phase of an infection. After the initial humoral response in which short-lived plasmablasts secrete high titres of antibody, long-lived plasma cells and memory B cells then contribute to longer-term antibody-mediated protection.[23] Although declines in IgG titres are expected, understanding the assay-dependent rate of decline, whether and when titres fall below assay positive cut-offs, and how these titres relate to protection from subsequent asymptomatic and symptomatic re-infection is crucial.

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Serological testing also helps quantify the extent of infection in populations, informing epidemiological models and public health strategies. However waning antibody levels may lead to underestimated exposure due to loss of seropositivity. For example, using the anti-nucleocapsid assay an estimated 33% of individuals seroreverted (i.e. fell below the positive cut off for the Abbott assay) within 3 months of IgG detection and an estimated 53% by 6 months (Figure 5B). Therefore, depending on the assay used, sero-epidemiological surveys performed several months into this pandemic may have underestimated prior exposure, especially in younger adults who lose detectable antibody faster. Our findings contrast with the repeated cross-sectional REACT2 study,[24] which reported greater reductions over time at a population-level in the proportion of adults ≥65 years testing antibody-positive, and more sustained responses in those 18-24 years. Nearly all HCWs in our study were <65 years, other differences may arise from study design, the assay used, and the potential for new infections predominantly in younger people[25] to replace others who had sero-reverted, supporting population-level seroprevalence (in our study each individual was followed-up separately).

Future work

Antibody dynamics have significant implications for the course and management of pandemics. Durability of immunity post-infection and post-vaccination will dictate the overall course of the pandemic. Further work is required to determine how prior infection and/or vaccination impacts the probability of future infection and severity of subsequent disease, determine the antibody-based correlates of this protection, and therefore the ability of serological tests to identify those who are immune. Longitudinal cohorts with baseline immunology are required to determine immune correlates of protection, to determine whether measurement of the current antibody status is enough to infer whether an individual have functional immunity or not, whether waning IgG titres are representative of waning immune protection, or whether protection remains even after an individual sero-reverts.

Conclusion

We demonstrate that the half-life of SARS-CoV-2 anti-nucleocapsid IgG antibody responses in a cohort of adult HCWs is 85 days and varies between individuals by age, ethnicity and prior symptom history. In contrast anti-spike IgG responses were sustained in most HCWs up to 180 days. The extent and duration of immunity to SARS-CoV-2 infection following Covid-19 and its association with antibody titres remains a key question to be answered.

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Declaration of interests

DWE declares lecture fees from Gilead, outside the submitted work. RJC is a founder shareholder and consultant to MIROBio, work outside the submitted work. GS reports scientific advisory board fees from GSK Vaccines, outside the submitted work. M.A. reports personal fees from Prenetics, outside the submitted work. JC reports personal fees from Nuffield Department of Medicine, University of Oxford, outside the submitted work. TF reports personal fees from NHS Professionals, outside the submitted work. No other author has a conflict of interest to declare.

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References

1. Deeks JJ, Dinnes J, Takwoingi Y, et al. Antibody tests for identification of current and past infection with SARS‑CoV‑2. Cochrane Db Syst Rev **2020**; 6:CD013652.

2. The National SARS-CoV-2 Serology Assay Evaluation Group. Performance characteristics of five immunoassays for SARS-CoV-2: a head-to-head benchmark comparison. Lancet Infect Dis **2020**

3. Wellinghausen N, Plonné D, Voss M, Ivanova R, Frodl R, Deininger S. SARS-CoV-2-IgG response is different in COVID-19 outpatients and asymptomatic contact persons. J Clin Virol **2020**; 130:104542.

4. Fafi-Kremer S, Bruel T, Madec Y, et al. Serologic responses to SARS-CoV-2 infection among hospital staff with mild disease in eastern France. Ebiomedicine **2020**; 59:102915.

5. Suhandynata RT, Hoffman MA, Kelner MJ, McLawhon RW, Reed SL, Fitzgerald RL. Longitudinal Monitoring of SARS-CoV-2 IgM and IgG Seropositivity to Detect COVID-19. J Appl Laboratory Medicine **2020**; 5:jfaa079-.

6. Xiang F, Wang X, He X, et al. Antibody Detection and Dynamic Characteristics in Patients with COVID-19. Clin Infect Dis **2020**;

7. To KK-W, Tsang OT-Y, Leung W-S, et al. Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV-2: an observational cohort study. Lancet Infect Dis **2020**; 20:565–574.

8. Long Q-X, Liu B-Z, Deng H-J, et al. Antibody responses to SARS-CoV-2 in patients with COVID-19. Nat Med **2020**; 26:845–848.

9. Adams ER, Ainsworth M, Anand R, et al. Antibody testing for COVID-19: A report from the National COVID Scientific Advisory Panel. Wellcome Open Res **2020**; 5:139.

10. Wajnberg A, Amanat F, Firpo A, et al. Robust neutralizing antibodies to SARS-CoV-2 infection persist for months. Science **2020**; :eabd7728.

11. Gudbjartsson DF, Norddahl GL, Melsted P, et al. Humoral Immune Response to SARS-CoV-2 in Iceland. New Engl J Med **2020**;

12. Seow J, Graham C, Merrick B, et al. Longitudinal evaluation and decline of antibody responses in SARS-CoV-2 infection.

13. Robbiani DF, Gaebler C, Muecksch F, et al. Convergent antibody responses to SARS-CoV-2 in convalescent individuals. Nature **2020**; 584:437–442.

14. Muecksch F, Wise H, Batchelor B, et al. Longitudinal analysis of serology and neutralizing antibody levels in COVID19 convalescents. J Infect Dis **2020**; :jiaa659-.

15. Eyre DW, Lumley SF, O'Donnell D, et al. Differential occupational risks to healthcare workers from SARS-CoV-2 observed during a prospective observational study. Elife **2020**; 9:e60675.

16. Eyre DW, Lumley SF, O'Donnell D, et al. Stringent thresholds for SARS-CoV-2 IgG assays result in under-detection of cases reporting loss of taste/smell. MedRxiv **2020**; https://doi.org/10.1101/2020.07.21.20159038.

17. Edridge AWD, Kaczorowska J, Hoste ACR, et al. Seasonal coronavirus protective immunity is short-lasting. Nat Med **2020**; :1–3.

18. Huang AT, Garcia-Carreras B, Hitchings MDT, et al. A systematic review of antibody mediated immunity to coronaviruses: kinetics, correlates of protection, and association with severity. Nat Commun **2020**; 11:4704.

19. Long Q-X, Tang X-J, Shi Q-L, et al. Clinical and immunological assessment of asymptomatic SARS-CoV-2 infections. Nat Med **2020**; 26:1200–1204.

20. Lipsitch M, Grad YH, Sette A, Crotty S. Cross-reactive memory T cells and herd immunity to SARS-CoV-2. Nat Rev Immunol **2020**; 20:709–713.

21. Iyer AS, Jones FK, Nodoushania A, et al. Dynamics and significance of the antibody response to SARS-CoV-2 infection. Medrxiv Prepr Serv Heal Sci **2020**;

22. Tan AT, Linster M, Tan CW, et al. Early induction of SARS-CoV-2 specific T cells associates with rapid viral clearance and mild disease in COVID-19 patients. Biorxiv **2020**; :2020.10.15.341958.

23. Stephens DS, McElrath MJ. COVID-19 and the Path to Immunity. Jama **2020**; 324:1279–1281.

24. Ward H, Cooke G, Atchison C, et al. Declining prevalence of antibody positivity to SARS-CoV-2: a community study of 365,000 adults. Medrxiv **2020**; https://doi.org/10.1101/2020.10.26.20219725.

25. Pouwels KB, House T, Prtichard E, et al. Community prevalence of SARS-CoV-2 in England during April to September 2020: Results from the ONS Coronavirus Infection Survey. Medrxiv **2020**; https://doi.org/10.1101/2020.10.26.20219428.

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Figure legends

Figure 1. SARS-CoV-2 antibody trajectory cohorts.

Figure 2. SARS-CoV-2 anti-nucleocapsid (panels A-D) and anti-spike (panels E-H) IgG antibody trajectories. Panels A and B show anti-nucleocapsid trajectories for HCWs with a positive result (≥1.40 arbitrary units) at some time. Panel A shows those whose first measurement was positive (n=466, only data from 100 randomly sampled individuals is shown to assist visualisation) and Panel B the remainder (n=56) in whom seroconversion was observed. Panel C shows those with a maximum titre that was equivocal (0.50-1.39, n=90). Panel D shows results from HCWs with a maximum titre that was negative (<0.50, n=2605, 100 randomly sampled individuals are shown). The dashed and dotted lines indicate the thresholds for a positive and equivocal result, note the different y-axis scales in panels A and B versus panels C and D. Similarly, panels E-H show anti-spike trajectories in million net normalised units for individuals who start positive (≥8 million units, n=457), seroconvert (n=103), have a maximum equivocal result (4.0-7.9 million units, n=209 [100 shown]) and only negative results (<4 million units, n=2354 [100 shown]). Anti-spike assay values above the upper limited of quantification, i.e. >9 million, are plotted as 9 million.

Figure 3. SARS-CoV-2 anti-nucleocapsid IgG antibody trajectories in 452 SARS-CoV-2 seropositive HCWs. Panel A shows the overall mean trajectory of anti-nucleocapsid IgG antibody levels from the maximum observed level (i.e. the model fixed effect). The posterior mean and 95% credibility interval are shown as a solid line and shaded area. The dashed red line represents the diagnostic threshold of 1.40 arbitrary units. Panel B shows the estimated anti-nucleocapsid IgG half-life with 95% CrI by days for all participants, ranked by its value. The solid red line indicates the overall mean. Credibility intervals exceeding 500 days are truncated at 500 days. Panel C shows the estimated maximum anti-nucleocapsid IgG antibody level with 95% CrI for all participants, ranked by its value. The solid red line indicates the overall mean. Panel D shows a comparison of maximum observed anti-nucleocapsid IgG antibody level and the estimated anti-nucleocapsid IgG half-life per individual.

Figure 4. Comparison of SARS-CoV-2 anti-nucleocapsid IgG antibody levels following a positive PCR test and the maximum IgG level per individual in those with a positive PCR test. Panel A shows those with a positive PCR undertaken for symptoms; Panel B shows those with a positive PCR for

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asymptomatic screening. The x-axis value for the model starting from the maximum IgG level is aligned to the maximum point from the model starting with a positive PCR test. The model starting from a positive PCR is fitted with a 5-knot spline (3 interior knots at t=10, t=30, and t=50, locations chosen based on model fit).

Figure 5. Proportion of HCWs remaining anti-nucleocapsid IgG (panels A-B) and anti-spike IgG (panels C-D) antibody positive by days following their maximum antibody level. Panels A and C show the observed proportion in 30-day intervals with binomial 95% confidence intervals. The number of individuals tested and the number of individuals remaining antibody positive is shown at the base of each bar. Panels B and D shows the results of Bayesian interval censored survival analyses, the posterior mean and 95% credibility interval are shown.

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Tables

Characteristic	Whole cohort n (%) or median (IQR) [Range]	452 HCWs with a positive antibody result and ≥1 subsequent sample n (%) or median (IQR) [Range]
Age		
Age, years	39 (29-50) [16-76]	41 (29-50) [17-69]
Gender		
Female	2542 (79)	340 (75)
Male	673 (21)	112 (25)
Not disclosed	2 (<1)	
Self-reported ethnicity		
White	2473 (77)	302 (67)
Black	90 (3)	25 (6)
Asian	440 (14)	89 (20)
Other	214 (7)	36 (8)
Covid-19-like symptoms between 01 February 2020 and testing		
Yes	898 (28)	274 (61)
No	2319 (72)	178 (39)
Previous positive SARS-Cov-2 PCR		
Symptomatic	128 (4)	95 (21)
Asymptomatic	117 (4)	59 (13)
None	2972 (92)	298 (66)

Table 1. Baseline cohort demographics for 3217 HCWs and 452 HCWs with ≥1 positive SARS-CoV-2 anti-nucleocapsid IgG result and ≥1 subsequent follow up sample.

		Univariable model			Multivariable model		
		Posterior mean	95% CrI		Posterior mean	95% CrI	
Baseline model	Maximum antibody level (Intercept)	4.26	4.07	4.44	3.33	3.02	3.67
	Antibody level half-life	85.38	81.17	90.01	69.94	63.74	77.19
Gender model	Maximum antibody level (Intercept): Female	4.24	4.04	4.46			
	Antibody level half-life: Female	84.88	80.14	90.25			
	Change in intercept: Male	0.03	-0.40	0.47	-0.05	-0.34	0.25
	Change in half-life: Male	1.95	-8.26	13.12	-0.40	-5.66	5.30
Age model	Maximum antibody level (Intercept): 41 years (median)	3.82	3.56	4.10			
	Antibody level half-life: 41 years (median)	75.27	69.71	81.46			
	Change in intercept: per 10-year older	0.21	0.10	0.30	0.17	0.07	0.25
	Change in half-life: per 10-year older	4.07	2.52	5.17	3.40	1.79	4.62
Ethnicity model	Maximum antibody level (Intercept): White	4.03	3.82	4.24			
	Antibody level half-life: White	81.48	76.69	86.66			
	Change in intercept: Black	0.48	-0.30	1.37	0.37	-0.21	1.03
	Change in intercept: Asian	0.83	0.3	1.3	0.54	0.1	0.9

|							
			2	4		8	5
	Change in intercept: Other	0.59	0.10	1.37	0.44	0.07	1.00
	Change in half-life: Black	7.03	-11.41	31.13	2.63	-6.92	15.03
	Change in half-life: Asian	14.64	1.54	29.29	6.28	-0.44	14.76
	Change in half-life: Other	14.85	-3.12	38.26	8.24	-1.13	20.55
Prior symptom model	Maximum antibody level (Intercept): No	3.90	3.64	4.16			
	Antibody level half-life: No	83.57	77.38	90.61			
	Change in intercept: Yes	0.60	0.24	0.96	0.40	0.12	0.69
	Change in half-life: Yes	2.85	-5.99	11.59	0.26	-4.52	5.19
PCR model	Maximum antibody level (Intercept): No	4.09	3.87	4.32			
	Antibody level half-life: No	80.23	75.48	85.28			
	Change in intercept: Positive (symptomatic)	0.65	0.17	1.16	0.30	-0.04	0.67
	Change in intercept: Positive (asymptomatic)	0.23	-0.31	0.81	0.14	-0.24	0.55
	Change in half-life: Positive (symptomatic)	19.60	7.22	35.66	9.56	2.35	19.09

Change in half-life: Positive (asymptomatic)	13.49	- 0.3 1	30. 37	6.45	0.8 6	- 15. 85

Table 2. Univariable and multivariable models of determinants of SARS-CoV-2 anti-nucleocapsid antibody trajectories. Posterior mean and 95% credibility intervals for the maximum antibody level (model intercept) and antibody half-life (model slope) are shown. See Supplementary Table S3 for other model parameters and statistical analysis quality metrics.
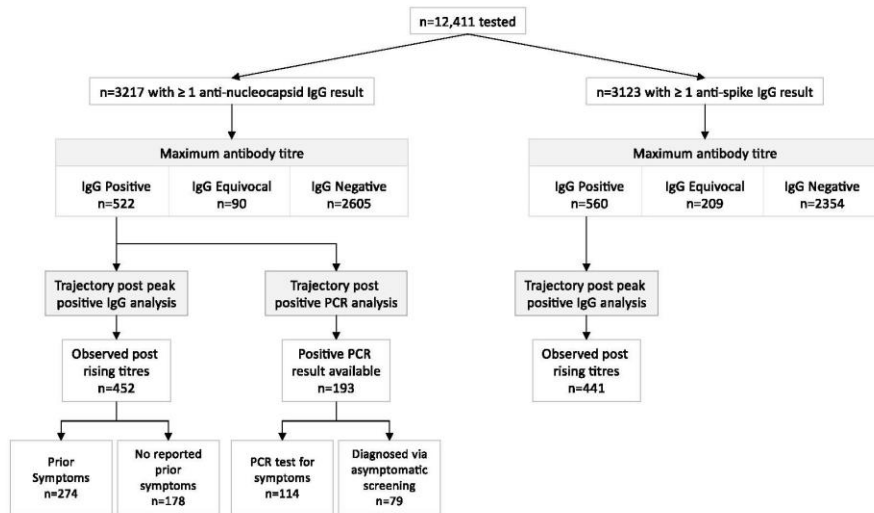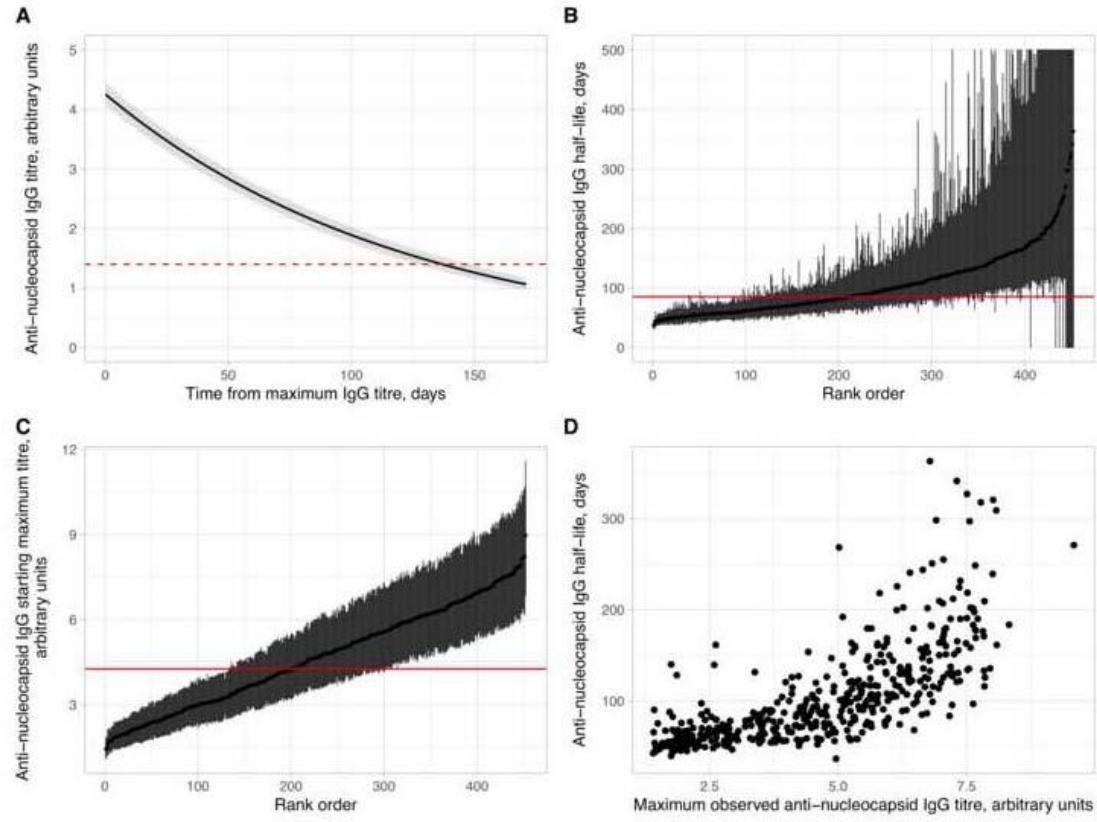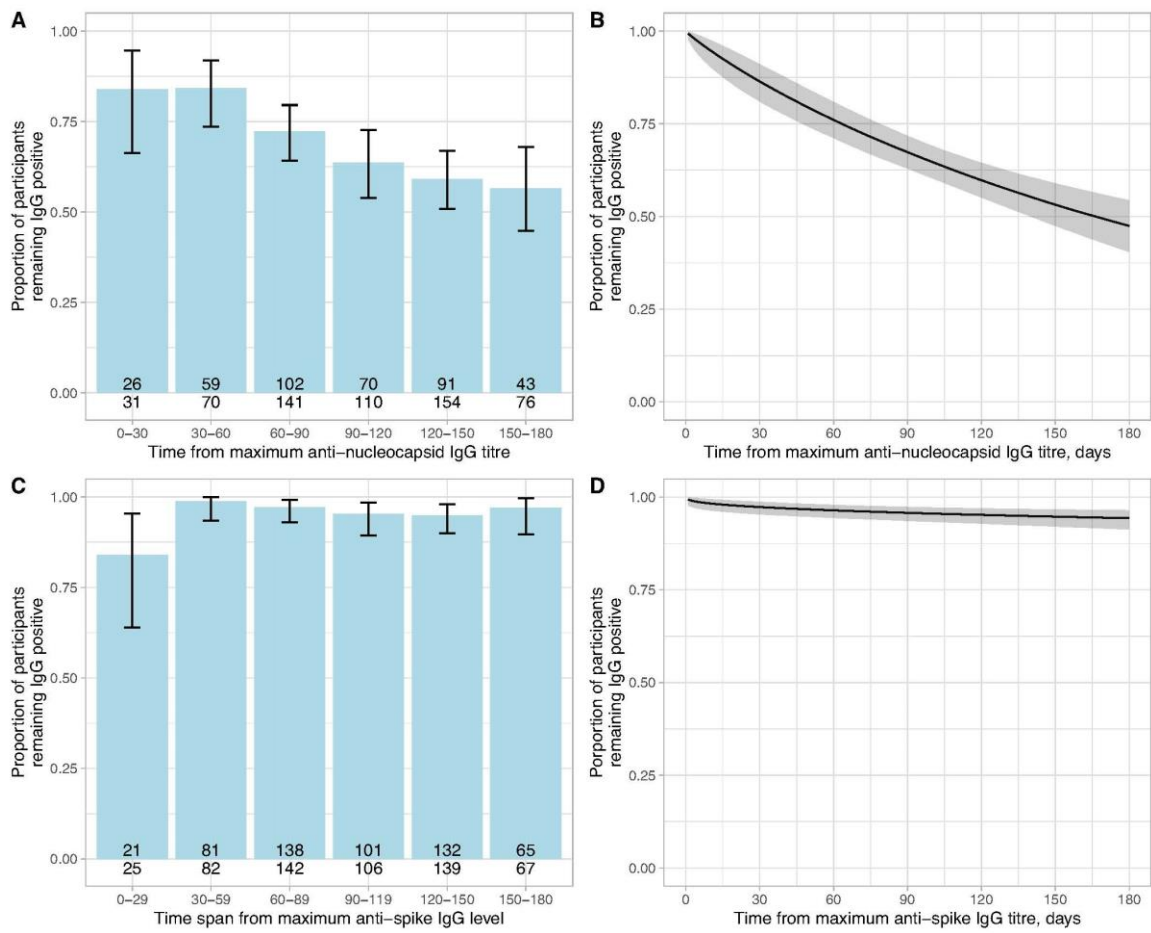
Figure 1

Figure 2

Figure 3

Figure 4

Figure 5