Longitudinal SARS-CoV-2 Nucleocapsid Antibody Kinetics, Seroreversion, and Implications for Seroepidemiologic Studies

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Given widespread use of spike antibody in generating coronavirus disease vaccines, SARS-CoV-2 nucleocapsid antibodies are increasingly used to indicate previous infection in serologic surveys. However, longitudinal kinetics and seroreversion are poorly defined. We found substantial seroreversion of nucleocapsid total immunoglobulin, underscoring the need to account for seroreversion in seroepidemiologic studies.

Estimating the incidence of infections caused by SARS-CoV-2 that are frequently asymptomatic is challenging when using routine passive surveillance methods. Antibodies can provide a record of previous infection, whether symptomatic or asymptomatic, and serologic surveys that measure antibodies across populations are routinely used for a variety of pathogens and are believed to provide key insights into the epidemiology and transmission of SARS-CoV-2 (*1*–*3*). However, antibody contraction and seroreversion (loss of previously documented antibodies) may lead to false-negative results, a potential issue as we move into the third year of the SARS-CoV-2 pandemic (*4*). Seroepidemiologic studies can adjust for seroreversion when kinetics are well characterized, but limited data are available from >1 year after infection. To characterize seroreversion and ease interpretation of seroepidemiologic studies, we measured SARS-CoV-2 nucleocapsid antibodies, a marker of previous infection even among populations vaccinated with spike-based COVID-19 vaccines, in a longitudinal study of healthcare workers in the United States.

The Study

The Mass General Brigham Institutional Review Board approved the study protocol (2020P000849). Participants provided written consent to participate in the study.

During April 28–September 30, 2020, a total of 2,358 employees of the Brigham and Women's Hospital (Boston, MA, USA) were enrolled in a longitudinal SARS-CoV-2 cohort study. Blood samples were collected at baseline, monthly for 3 months, and then every 3 months through February 2022, up to 21 months after enrollment. Sociodemographic characteristics (Appendix, https://wwwnc.cdc.gov/EID/article/28/9/22-0729-App1.pdf) were collected on electronic questionnaires.

We tested serum samples by using the Diagnostics Elecsys SARS-CoV-2 N Immunoassay (Roche, https://www.roche.com), a double-antigen sandwich electrochemiluminescence total immunoglobulin immunoassay that detects antibodies against viral nucleocapsid protein. We performed assays based on a cutoff index (≥1.0) defined according

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to manufacturer's guidance. An independent in-house assay performance study that included 832 prepandemic negative controls and 251 PCR-positive samples showed a specificity of 99.6% (95% CI 98.9%–100%) and a sensitivity of 90.8% (95% CI 81.3%–95.7%) for samples collected >14 (range 15–68) days after symptom onset (*5*).

We extracted SARS-CoV-2 PCR test results and dates from the Brigham Health electronic medical record. PCR-positive date was defined as the date of the first registered SARS-CoV-2 PCR-positive test result. For 4 employees who had a positive SARS-CoV-2 PCR test result outside the Brigham Health system, the date of the COVID-19–positive result, generated for all SARS-CoV-2–positive employees in the Brigham Health electronic medical record, was used as the PCR-positive date. To assess antibody kinetics, we used a generalized additive mixed-effect model (GAMM) with the natural logarithm of antibody levels modeled as a function of time from first positive PCR test result. To estimate the half-life, we used a linear mixed-effects model (LMM) and assumed constant exponential decay after the peak level (Appendix).

A total of 125 (5.3%) of 2,358 study participants enrolled in the healthcare worker cohort study were positive for nucleocapsid antibodies during April 2020–January 2021, during predominantly wild-type SARS-CoV-2 circulation (*6*). Of these participants, 110 (88%) had ≥1 samples collected after an index seropositive sample and were included in the analyses. A total of 687 unique samples were collected from the 110 participants (mean 6.3 samples/participant). The median age of participants was 33 (range 20–71) years; 94 (86%) were female, 97 (88%) White, and 6 (5%) Hispanic. The median body mass index was 24 kg/mm^2 (range 19–42 kg/mm^2).

A total of 74 (67%) of 110 participants had a positive SARS-CoV-2 RT-PCR test in the Mass General Brigham electronic health record before the first seropositive sample; 96% were symptomatic (Appendix) and 1 required hospitalization. Symptomatic status was not assessed for study participants who did not have a positive PCR test result because timing of infections was unknown.

The mean peak cutoff index level for nucleocapsid antibodies among PCR-positive participants was 37 (95% CI 27–50) and occurred 72 days after the index PCR-positive test result (Figure 1). Assuming constant linear antibody contraction with the LMM approach, antibody half-life was estimated at 128 (95% CI 114–146) days and mean time to seroreversion as 737 (95% CI 680–793) days. Antibody contraction and seroreversion kinetics diverged between the LMM and GAMM models; the nonlinear GAMM model suggested more rapid contraction up to 1 year postinfection, followed by slower contraction and seroreversion thereafter (Table; Figures 1, 2). We observed a stepwise but nonsignificant trend toward a slower relative decrease in concentration of nucleocapsid antibodies among older age groups but not across body mass index or sex (Appendix).

Conclusions

We report on the kinetics of SARS-CoV-2 nucleocapsid antibodies (total immunoglobulin) up to 21 months after infection and estimate peak antibody

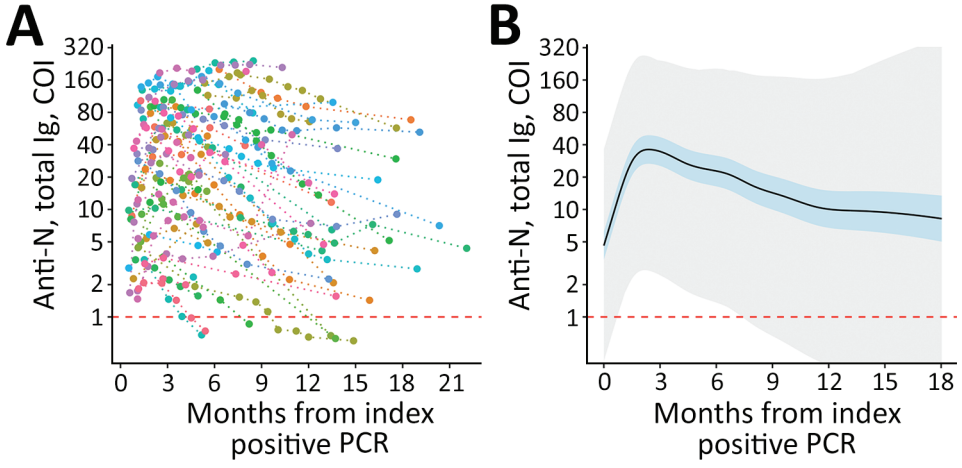

Figure 1. Longitudinal SARS-CoV-2 anti-N total immunoglobulin kinetics among SARS-CoV-2 PCR-positive participants in a study of healthcare workers in Boston, Massachusetts, USA, 2020). A) Individual study participant (n = 74) total anti-N levels from time of index PCR-positive test result. Individual levels are indicated by colored points connected by a dotted line. B) Fitted generalized additive mixed-effect model depicting estimate (solid black line), 95% CI (blue shaded area), and 95% prediction interval (gray shaded area). Estimates are truncated at 18 months given sparsity of later data points. Horizontal dashed red lines indicate the COI for seropositive (above) and seronegative (below) results. The lower limits of detection (COI 0.07) are outside the figure frame. Anti-N, nucleocapsid antibodies; COI, cutoff index.

levels, kinetics, and rates of seroreversion by using 2 modeling methods. Both models suggest substantial seroreversion by 18 months postinfection, and half-life–based estimates suggest seroreversion of ≈50% at 2 years.

Half-life–based approaches that assume constant exponential contraction might underestimate seroreversion through the first year postinfection and overestimate seroreversion at later timepoints (Table). Using a GAMM model that tolerates variable antibody contraction over time, we estimated that seroreversion was 1.4%/month during 4–12 months after infection, but 95% CIs were wide. Assuming contraction remains relatively constant after an initial rapid decrease, an assumption supported by previous studies on SARS-CoV-2 and other common human coronaviruses (*4*), seroreversion would be 19% at 2 years and 35% at 4 years.

These findings suggested that serologic surveys conducted >1 year after widespread SARS-CoV-2 transmission will be markedly affected by seroreversion. The total immunoglobulin immunoassay used for this study shows more durable detection of nucleocapsid antibodies than other formats, particularly single isotope assays, and rates of seroreversion might be higher across other assay designs (*5*,*7*). Total immunoglobulin immunoassays specific for spike antibodies versus nucleocapsid antibodies appear to provide improved durability of antibody detection, and single isotype antispike assays provide similar or lower durability than total nucleocapsid immunoglobulin assays (*7*). However, given the widespread use of spike-based COVID-19 vaccines, the utility of spike antibodies for detection of previous infection in population-level surveys is limited.

Table. SARS-CoV-2 nucleocapsid total immunoglobulin seroreversion by GAMM and half-life methods, by months from infection, among healthcare workers in Boston, Massachusetts, USA, 2020*

Months	GAMM, % (95% CI)	Half-life, % (95% CI)
4	0 (0–4.9)	0.6 (0.6–0.6)
6	2.7 (3.0–9.5)	1.2 (1.0–1.3)
8	5.5 (1.5–13.4)	2.1 (1.8–2.6)
10	9.6 (3.9–18.8)	3.8 (2.9- 4.8)
12	11 (4.9–20.5)	6.2 (4.7–8.3)
14	12.3 (5.8–22.1)	9.8 (7.2–13.3)
16	13.7 (6.8–23.8)	14.8 (10.6–20.1)
18	15.1 (7.8–25.4)	21.1 (15.0–28.7)
24	NC	48.9 (36.1–62.1)

*The GAMM method estimates are restricted to observational data and therefore not calculated more than 18 months postinfection. Half-life method represents constant log linear antibody contraction with an estimated peak total nucleocapsid antibody level that had a cutoff index of 37 and a half-life of 128 days. GAMM, generalized additive mixed model; NC, not calculated.

This study has several strengths, including a mean of 6.3 unique sample time points/participant that enables more precise demarcation of antibody dynamics and a long study interval that includes samples collected up to 21 months postinfection. However, our cohort was based in the United Sates, enrolled only adults, and overrepresented women and White participants. Therefore, our findings might not be generalizable. Peak antibody levels were estimated for PCR-positive persons; lower peak levels might be observed among seropositive persons who do not provide a PCR-positive test result, which would cause more rapid seroreversion than that we report. Data points >18 months

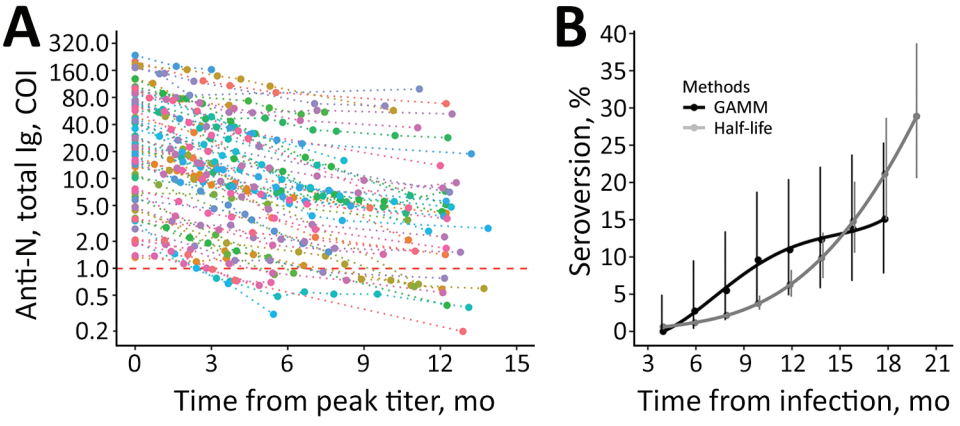

Figure 2. Longitudinal SARS-CoV-2 total anti-N immunoglobulin contraction and estimated seroreversion over time in a study of healthcare workers in Boston, Massachusetts, USA, 2020. A) Consistency in contraction of individual-level total anti-N immunoglobulin levels over time when indexed against participants' highest recorded sample value. Includes seropositive participants with and without registered SARS-CoV-2 PCR-positive test results and with ≥1 sampling time points after peak level (n = 90). Individual levels are indicated by colored points connected by a dotted line. Horizontal dashed red line indicates the cutoff index for seropositive (above) and seronegative (below) results. The lower limit of detection (COI 0.07) is outside the figure frame. B) Points indicating estimated rates of seroreversion from total anti-N immunoglobulin seropositive to seronegative by 2-month intervals. The half-life estimate is based on peak total anti-N immunoglobulin level of 37 COI and half-life of 128 days. Solid lines indicate quadratic polynomial trend; error bars indicate 95% CIs. Anti-N, nucleocapsid antibodies; COI, cutoff index; GAMM, generalized additive mixed-effect model.

postinfection are sparse, and antibody dynamics might differ outside our measurement window. Infections accrued during wild-type predominance, in the prevaccination setting, are assumed to reflect a single infection. Antibody kinetics might differ for infections caused by other SARS-CoV-2 strains or after vaccine breakthrough or repeat infections (H.J. Whitaker et al., unpub. data, https://www.medrxiv.org/content/10.1101/2021.10.25.21264964v1.full-text; D. Follman et al., unpub. data, https://www.medrxiv.org/content/10.1101/2022.04.18.22271936v1.full).

In summary, antibody seroreversion and the global rollout of vaccines are increasingly useful considerations when planning and interpreting SARS-CoV-2 seroepidemiologic studies. Immunoassays targeting the nucleocapsid protein can detect previous infection among populations vaccinated with spike protein vaccines, but antibody contraction and seroreversion are likely to be substantial as we move into the third year of the pandemic. By characterizing seroreversion after SARS-CoV-2 infection, this study provides provisional format-specific considerations for interpreting and adjusting estimates of previous infection for seroepidemiologic purposes.

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About the Author

Dr. Loesche is an emergency medicine resident at Brigham and Womens Hospital and Massachusetts General Hospital, Boston, MA. His primary research interests are microbiology, epidemiology, and bioinformatics.

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Appendix

Methods

Generalized Additive Mixed-Effect Model

A generalized additive mixed-effect model (GAMM) was generated to model SARS-CoV-2 antibody titers over time. The natural logarithm of antibody titers was modeled as a function of time from first positive SARS-CoV-2 PCR test result, which was incorporated as a nonparametric smoothing function with a cubic regression basis function. A random intercept and smoothing function was used with subjects as the grouping variable. The default value for knots was used. The model was fit by maximum likelihood with a Gaussian link function. The fitted GAMM was used to estimate peak antibody titers and time until peak. Final model specifics and measures of model fit are provided.

Linear Mixed-Effects Model

To estimate the rate of decay of antibody levels, we assumed that there was a constant exponential decay following peak titer. A linear mixed-effects model (LMM) was fitted on antibody titer levels centered on the peak measured antibody date. Random intercept and slope were use with subjects as the grouping variable. This model was used to calculate the half-life interval. The LMM was fitted by restricted maximum likelihood with a Gaussian link function. Estimated seroreversion is computed using the GAM fit which incorporated the nonlinear pattern of slower waning 12 months post infection plus a LMM fit (which estimates individual deviations from the GAMM).

Statistical analyses were performed by using the R statistical programming language (R Core Team 2021, https://www.r-project.org). The mgcv (https://cran.r-project.org/web/packages/mgcv/index.html) and LME4 (https://cran.r-

project.org/web/packages/lme4/index.html) packages were used for the GAMM and LMM models respectively.

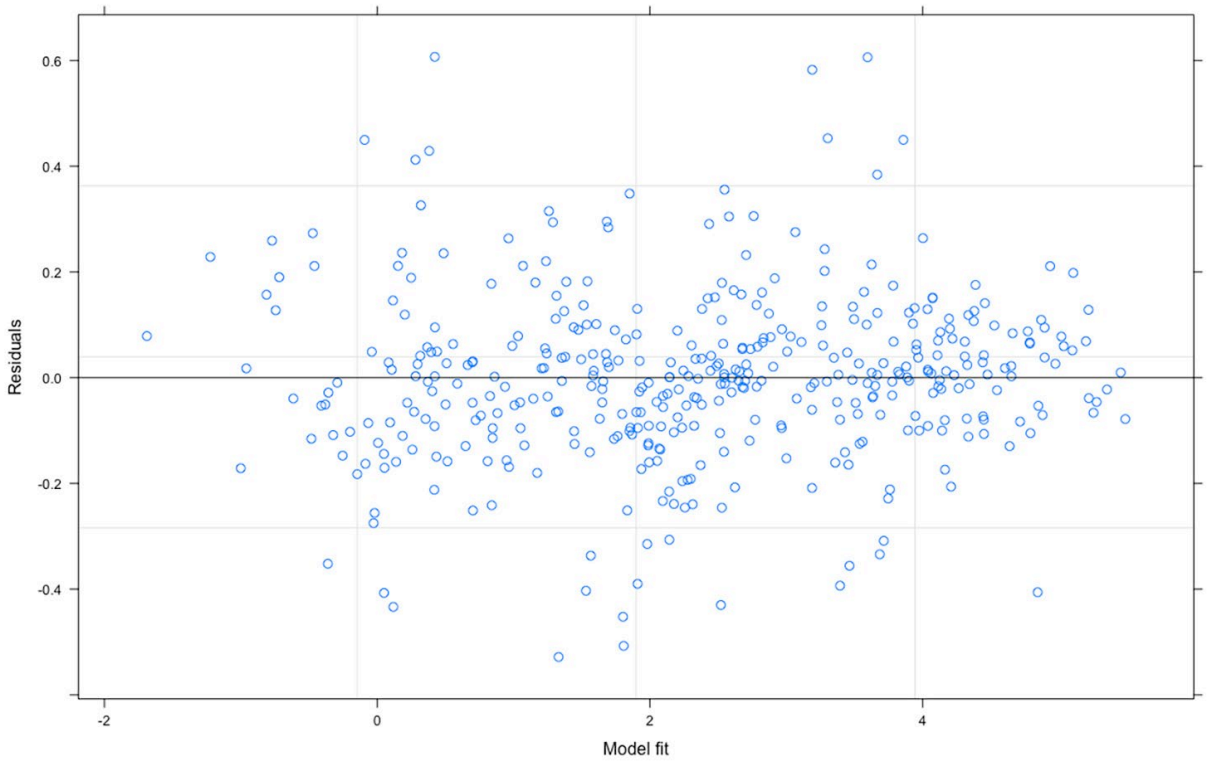
Appendix Table 1. Variables for study participants (n = 110)

Variable	No. participants (%)
Age, y	
20–29	43 (39)
30–49	45 (41)
≥50	22 (20)
Sex	
F	94 (85)
M	15 (14)
Not reported	1 (1)
Race	
Asian	3 (3)
Black	7 (6)
White	97 (88)
>1	1 (1)
Not reported	2 (2)
Ethnicity	
Hispanic	6 (5)
Non-Hispanic	100 (91)
Not reported	4 (4)
Body mass index, kg/mm^2	
18–24	65 (59)
25–29	27 (25)
≥30	13 (12)
Not reported	5 (5)
Concurrent conditions	
Diabetes	0 (0)
Asthma	11 (10)
High blood pressure	10 (9)
Coronary heart disease	1 (1)
Cerebral vascular accident (stroke)	0 (0)
Immunocompromised (HIV)	1 (1)

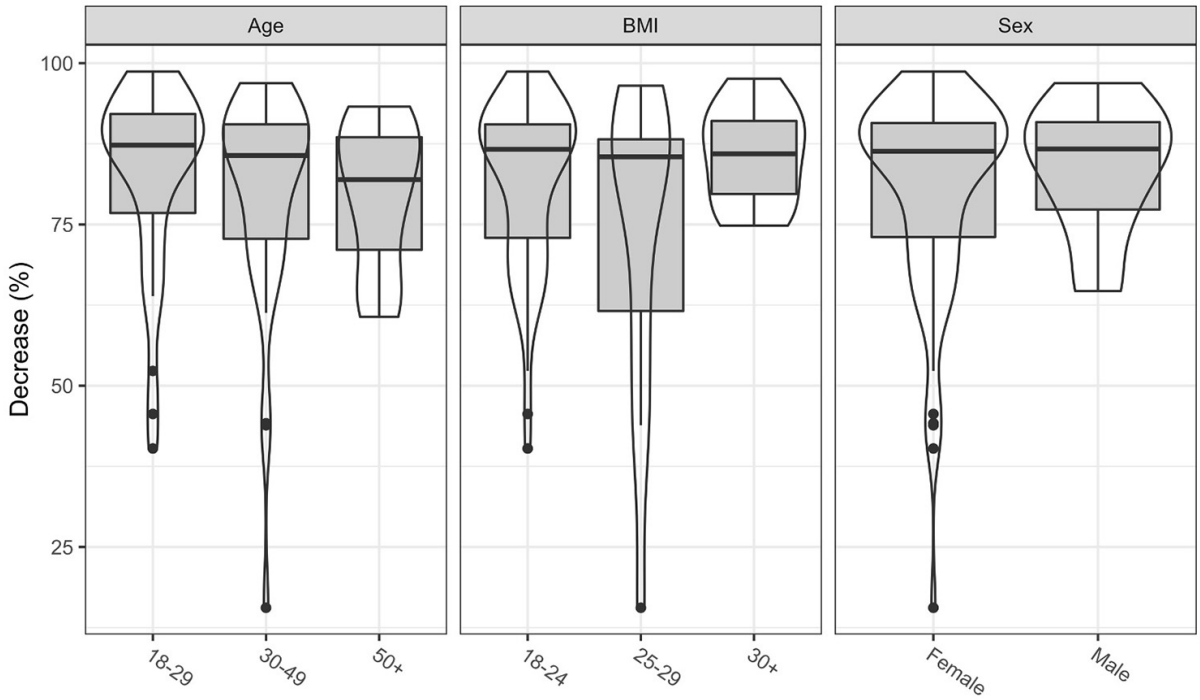
Appendix Table 2. Clinical features reported by SARS-CoV-2 PCR positive study participants (n = 70)*

Reported clinical feature	No. (%)
Measured fever (temperature ≥38.0°C)	9 (13)
Subjective fevers or chills	30 (43)
Cough	41 (59)
Shortness of breath	16 (23)
Sore throat	25 (36)
Nasal congestion or rhinorrhea	40 (57)
Nausea, vomiting, or diarrhea	12 (17)
Ageusia or anosmia	24 (34)
Chest pain or pressure	14 (20)
Myalgias or arthralgias	33 (47)
Fatigue	22 (31)
Any symptom	67 (96)

*Data were extracted from the Brigham Health electronic medical records system by using a standardized data extraction form. Symptoms reported within 14 d before or after the index PCR-positive date were included. Of 74 total participants with a registered PCR-positive SARS-CoV-2 test result, sufficient detail was available to assess symptoms for 70. Given timing of SARS-CoV-2 infection is not available for seropositive participants that did not register a positive PCR-test result, these study participants (n = 36) were excluded from assessment of clinical features.



Appendix Figure 1. Residuals plot of the linear mixed effect model used to estimate rate of nucleocapsid antibody contraction. Individual variation in rate of decay accounted for 29.6% of the variance.



Appendix Figure 2. Box plots depicting the distribution of individual level rate of decay for subjects reported as percent decrease from peak at 1 year stratified by demographic features. Horizonal black bar indicates median value, box plot limits indicate Q1–Q3, vertical line indicates minimum and maximum values without outliers, and dots indicate lower (Q1–1.5 IQR) and upper (Q3 + 1.5 IQR) outliers. Overlaid violin plots show distribution. Given the limited number of participants, statistical differences are not identified, but stepwise declines in percent decrease per year with increasing age suggest potential differences in durability of nucleocapsid antibodies by age might exist. BMI, body mass index; IQR, interquartile range.