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Longitudinal analysis of serology and neutralizing antibody levels in COVID19 convalescents

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40-word-or-less summary:

Analysis of COVID19 convalescents reveals that neutralizing antibody levels decline rapidly early after infection. Some clinical serological assay platforms give quantitative outputs that predict neutralizing antibody titer, but some have diagnostic sensitivity that deteriorates with time post infection.

Footnote page

Competing interest statement

All authors declare: no support from any organization or financial relationships with any organizations that might have an interest in the submitted work and no other relationships or activities that could appear to have influenced the submitted work.

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Abstract

Background – Understanding the longitudinal trajectory of SARS-CoV-2 antibodies is crucial for diagnosis of prior infection and predicting future immunity.

Methods We conducted a longitudinal analysis of COVID19 convalescents, using neutralizing antibody assays and SARS-CoV-2 serologic assay platforms employing SARS-CoV-2 spike (S) or nucleocapsid (N) antigens.

Results Sensitivities of serologic assays to diagnose prior SARS-CoV-2 infection changed with time. One widely used commercial platform that had an initial sensitivity of >95% declined to 71% at 81-100 days post diagnosis. The trajectories of median binding antibody titers measured over ~3 to 4 months were not dependent on the use of SARS-CoV-2 N or S proteins as antigen. The median neutralization titer decreased by ~45% per month. Each serological assay gave quantitative antibody titers that correlated with SARS-CoV-2 neutralization titers, but S-based serological assay measurements better predicted neutralization potency. Correlation between S-binding and neutralization titers deteriorated with time and decreases in neutralization titers were not predicted by changes in S-binding antibody titers.

Conclusions – Different SARS-CoV-2 serologic assays are more or less well suited for surveillance versus prediction of serum neutralization potency. Extended follow up should facilitate the establishment of appropriate serologic correlates of protection against SARS-CoV-2 reinfection.

Key Words: SARS-CoV-2, COVID19, Serology, Neutralizing antibodies

Introduction

The emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent of COVID-19, has resulted in a devastating global pandemic. Diagnosis of SARS-CoV-2 infection is principally dependent on RT-PCR using nasal and throat swabs, which is not ideally suited to mass population testing. RT-PCR-diagnosed case numbers have therefore underestimated the prevalence of SARS-CoV-2 infection, and serology assays must be deployed to determine the true number of infections using a surveillance approach. Serology assays also have a critical role in screening volunteers for vaccine trials and convalescent plasma donation, as well as predicting immunity. Although several commercial SARS-CoV-2 immunoassays are in common use, evaluation of their sensitivity has often used samples from hospitalized patients soon after infection. Knowledge of the long-term kinetics of antibody titers and the corresponding effectiveness of commercial assays is sparse(1-3).

Serology assays for SARS-CoV-2 employ viral nucleocapsid (N) or spike protein (S) antigens. Because S binds target cells through its receptor binding domain (RBD), it is the target of neutralizing antibodies. Therefore, S-based assays may be preferable to N-based assays for the assessment future re-infection risk(4). This premise is based on the assumptions (1) that neutralizing antibodies constitute a major mechanism of protective immunity, and (2) that S-based serology assays accurately predict neutralizing activity.

Outstanding questions about the use of SARS-CoV-2 serology assays include (1) how circulating antibody levels change with time following natural infection and (2) which serological assays best predict protective immunity. The prognostic value of antibody measurements with respect to reinfection has yet to be demonstrated, and it is important to understand post infection serology to enable correlates of protection to be established. We present a longitudinal study of mildly symptomatic, non-hospitalised COVID19 positive patients during the first few months of convalescence. We compare the ability of four high-

throughput automated serology assays to diagnose prior SARS-CoV-2 infection and predict serum neutralizing activity.

Methods

Participants

Participants with prior RT-PCR-diagnosed COVID-19 were recruited. Recruits were surveyed to determine the date of the positive PCR test, the date of onset of symptoms, and if their symptoms required hospitalization. Serum samples were taken at a baseline visit (~3.5 to ~8.5 weeks post PCR test), and 2 weeks (visit 2), 4 weeks (visit 3) and 8 weeks later (visit 4). In total, 97 participants, who were not hospitalized during the course of their illness completed at least 3 visits. The mean age of the participants was 44.2 years (21 – 65 y), with 70 female (72% of cohort) participants. At visit 1 (baseline), the average number of days between PCR test and visit 1 (baseline) was 40.8 days (24 – 61 days); at visit 2 (2 weeks post-baseline), the average number of days post-PCR test was 55.1 days (40 – 79 days); at visit 3 (4 weeks post-baseline), the average number of days post-PCR test was 69.8 days (55 – 95 days); at visit 4 (8 weeks post-baseline), the average number of days post-PCR test was 98.4 days (85 – 110 days). Ethical approval was obtained for this study to be carried out through the NHS Lothian BioResource. All recruits gave written and informed consent for serial blood sample collection. De-identified sample were shipped to the Rockefeller University whose IRB reviewed and approved the study.

High throughput automated serology assays

Four commercial assays, that employ either S or N protein antigens and are designed for high throughput in healthcare settings were used. All the assays generate a qualitative positive/negative result based on assay-dependent signal thresholds. The Abbott SARS-CoV-2 IgG assay detects anti-N IgG using a two-step chemiluminescent microparticle immunoassay (CMIA) method with an acridinium-labelled anti-human IgG. The DiaSorin SARS-CoV-2 IgG assay is also a two-step CMIA method targeting undisclosed epitopes in

the SARS-CoV-2 S protein and employs an isoluminol conjugated anti-human IgG. The Roche Anti-SARS-CoV total antibody assay is a two-step bridging electrochemiluminesent immunoassay (ECLIA) using ruthenium-labelled and biotin conjugated N protein. The Siemens SARS-CoV-2 total antibody assay is a one-step bridging CLIA method that detects antibodies against the RBD, using acridinium and biotinylated S1 RBD. Assays were performed on the Abbott Architect and DiaSorin Liason platforms (NHS Lothian), and the Roche Elecsys (NHS Lanarkshire) and Siemens Atellica (NHS Tayside) platforms. Serum, collected and stored according to the manufacturer's recommendations, was used in all cases.

SARS-CoV-2 Neutralization assays

To measure neutralizing activity, sera, beginning with a 1:12.5 dilution, were five-fold serially diluted in 96-well plates over four dilutions. Thereafter, approximately $5x10^3$ infectious units of an HIV-1_{NL}ΔEnv-NanoLuc/SARS-CoV-2 pseudotype virus were mixed with the serum dilutions at a 1:1 ratio and incubated for 1 hour at 37 degrees. The mixture was then added to 293T/ACE2cl.22 target cells(5) plated at $1x10^4$ cells/well in 100 µl medium in 96-well plates the previous day. Thus, the final starting serum dilution was 1:50. Cells were cultured for 48h and harvested for NanoLuc luciferase assays, as previously described(5).

Results

The cohort consisted of participants who were not hospitalised during the course of their illness and were therefore relatively mildly symptomatic. Approximately 70% of people reported at least one of the 3 main WHO -identified symptoms, namely fever, cough and anosmia. The most common of symptom was anosmia and the majority of participants reported the presence of 2 of these 3 symptoms (Table 1). Serum samples were collected from 97 participants at ~4 weeks (visit 1), 6 weeks (visit 2) and 8 weeks (visit 3) post diagnosis (by RT-PCR). Additionally, serum was collected from a subset (28 of the 97 participants) at ~12 weeks post diagnosis (visit 4).

We compared the diagnostic sensitivity of 4 high throughput SARS-CoV-2 serology assays that are in routine use in hospital settings. Each assay gives a qualitative positive or negative result, based on assay specific thresholds, and sensitivities were calculated for each assay using these thresholds. Inter and intra-assay analytical precision for each assay is detailed in Supplementary Table 1. To account for the differences in time post PCR diagnosis that participants made their first visit, sensitivity across a 20 day rolling time window was calculated. The Abbott, Roche and Siemens assays all had sensitivities of 95 to 100% at 21-40 days post PCR-positive test, while the DiaSorin assay had a lower sensitivity of 85% (Figure 1A). However, the relative sensitivities of the assays changed with time. Specifically, the sensitivity of the Abbott assay declined to 85% in the 61-80 day window, and 71% at >81 days post diagnosis (Figure 1A). Conversely, the sensitivities of the other assays were maintained or increased over time (Figure 1A). In terms of intra-individual change, 14/91 participants that were positive on the Abbott assay at visit 1 were negative by visit 3 or 4, whereas none of the participants with a positive result at visit 1 on the other assays became negative at visit 3 or 4. For the DiaSorin assay, 2 participants that were negative at visit 1 were positive at visit 3 (both participants had an equivocal result at visit 1, and showed a small increase above the assay threshold at visit 3). In the Siemens assay, 3 participants were consistently negative, and in the Roche assay only a single participant was negative at each visit.

The serological assays give a quantitative assessment of antibody titer as well as a threshold-based positive/negative result. We next analysed changes in the quantitative results over time for each platform (Figure 1 B, C). Mean antibody titers decreased in the Abbott assay at visits 2 and 3 compared to visit 1 (Figure 1B) but increased in the DiaSorin and particularly the Roche assays and remained approximately constant in the Siemens assay (Figure 1 B). Notably, 79 out of 97 (81%) of participants showed a decrease in antibody titer on the Abbott platform, while 82/97 (85%) showed an increase on the Roche assay, despite the fact that both assays detect N-specific antibodies (Figure 1 B, C).

Negative or positive change was approximately equally likely in the S-based assays; specifically, 57% and 47% of intra-individual changes were negative for the DiaSorin and Siemens assays respectively (Figure 1 B, C).

We measured neutralizing activity in serum samples from the first 3 visits for 80 of the 97 participants using a SARS-CoV-2 pseudotyped virus neutralization assay that is amenable to high throughput and does not require a BSL-3 facility. This assay employs HIV-1-based virions carrying a nanoluc luciferase reporter, pseudotyped with the SARS-CoV-2 spike protein. Neutralization titers obtained using these pseudotyped particles correlate well with titers obtained using neutralization of authentic SARS-CoV-2(5). Moreover, this assay has been successfully applied for analysis of convalescent plasma samples and in a campaign to identify potent human monoclonal antibodies(6, 7). Consistent with our analyses of other cohorts(6, 7), a broad range of neutralizing titers were evident in sera collected from 80 participants at three timepoints (Figure 2A). In samples collected at visit 1, the neutralizing activity, as determined by half-maximal neutralizing titer (NT_{50}), ranged from <30 to 4300, with a geometric mean of 234 (arithmetic mean was 411) (Figure 2A, red symbols). Consistent with other cohorts (6, 7) 34/80 (42%) had NT_{50} of less than 250 while only in 11/80 participants (14%) had NT_{50} values higher than 1000.

NT_{50} values measured at each timepoint for individual participants correlated with each other, although there was divergence in NT_{50} values over time (Figure 2 A inset). Notably, neutralizing activity decreased at each time point for the majority of participants (Figure 2 A, blue and green symbols). Overall, the decrease in median NT_{50} was ~25% per two-week sampling interval, resulting in a ~45% reduction in NT_{50} over the 4 weeks between visit 1 and visit 3 (Figure 2B). As a result, distribution of NT_{50} values the cohort differed between visits (Figure 2C). The relative decline in NT_{50} between visits 1 and 2 versus visits 2 and 3 did not differ significantly, and the majority of participants exhibited a similar relative decrease in neutralizing activity over time, regardless of their initial NT_{50} values or the number of days post PCR at visit 1, suggesting exponential decay (Figure 2D).

NT_{50} values at each sampling timepoint were poorly correlated with age (Supplementary Figure 1A), and no correlation was observed between age and NT_{50} decay with time. As has been previously reported, there was a trend toward lower NT_{50} values in females than in males(6, 7), but there was no difference between sexes in NT_{50} decay with time (Supplementary Figure 1B). Individual clinical parameters such as GI symptoms, fever or recovery time, did not predict NT_{50}, serological values or decay parameters for any antibody measurement.

Next, we compared neutralizing activity in serum with quantitative results obtained from the serological assays. Analysis of combined results from the three visits by 80 participants revealed a significant correlation between any combination of two serological assays (Supplementary Figure 2). However, stronger correlations were observed between the two S-based assays, Siemens and DiaSorin ($r=0.92$, $p<0.0001$) and between the two N-based assays Abbott and Roche ($r=0.81$, $p<0.0001$), The S-based assays correlated less well, but significantly ($p<0.0001$), with the N-based assays (Supplementary Figure 2).

All the serological assays gave quantitative values that correlated with NT_{50} measurements, but as expected, the S-based assay measurements correlated more closely with NT_{50} measurements (Figure 3A-D). The S1/S2-based DiaSorin assay was the best predictor of NT_{50} ($r=0.84$, $p<0.0001$, Figure 3A), followed by the RBD-based Siemens assay ($r=0.74$, $p<0.001$, Figure 3B), the N-based Abbott assay ($r=0.69$, $p<0.0001$, Figure 3C) and, lastly, the Roche assay ($r=0.56$, $p=0.0001$, Fig3D).

The correlation between NT_{50} and the individual serological assays was best at the first visit and deteriorated to some extent thereafter (Figure 3A-D, see color-coded r-values in individual graphs, $p<0.0001$ for all correlations), The decrease in the strength of correlation might, in part, be attributable to the fact that later sampling timepoints have more samples with lower NT_{50} values, which may reduce measurement precision. The magnitude of the deterioration in the predictive value differed between serological assays, with the S-based assays exhibiting larger decreases in correlation coefficients ($r=0.89$ and 0.83 at visit 1, versus $r=0.83$ and 0.71 at visit 3 for DiaSorin and Siemens assays respectively Figure 3A-

D), Despite the increasing disparity over time, the DiaSorin assay was clearly superior at predicting NT_{50} at all visits (Figure 3A-D).

Interestingly, comparison of the extent of change in neutralization activity over the 4-week observation interval with the concomitant change in values obtained using serological assays, revealed only minimal correlation (Figure 4A-D, supplementary Figure 3). Notably, in most participants, the decline in serum neutralizing activity was clearly greater than the decline in antibody titer measured using any serological assay (Figure 4A-D supplementary Figure 3). Even for the Diasorin assay, which gave the best prediction of neutralizing activity at each time point (Figure 4A), declines in neutralizing activity were not well predicted by declines in Diasorin assay measurements (Figure 4A, supplementary Figure 3). While both the Abbott assay and the NT_{50} measurements exhibited declining antibody titers with time, the magnitudes of these declines did not correlate with each other (Figure 4D, supplementary Figure 3).

Discussion

Serological assays for infectious agents have two major and distinct uses, namely (1) to diagnose chronic infections (e.g. HIV-1) and (2) to determine past infection or immunisation status (e.g. measles, VZV) which may be able to predict immunity from future infection. The use of SARS-CoV-2 serological assays requires understanding of how these assays perform in populations over time. During the current SARS-CoV-2 pandemic it has become clear that the magnitude of serologic immune responses is highly variable(6, 7). Nevertheless, the vast majority of individuals with a PCR-confirmed SARS-CoV-2 infection generate antibodies at a sufficient level for diagnosis of recent infection(8). A number of high throughput commercial assays have been deployed for SARS-CoV-2 antibody testing, and evaluated mostly using hospitalized participants(9, 10). Non-hospitalised patients with mild disease typically have lower levels of antibodies than hospitalized patients with severe illness(11-15), Differences in antibody titers between individuals may be driven in part by differences in antigen exposure. However, several variables, variable viral load trajectories,

variable time of diagnosis and sampling relative to infection, variable sampling efficiency using swabs, and variable relationship between nasal viral load and systemic antigen exposure would make relationships between viral load and antibody responses difficult to establish.

Using our cohort of non-hospitalized participants with mild disease, all four assays evaluated herein had sensitivities at visit 1 (an average of 40.8 days after PCR testing) that were comparable those reported using hospitalised patients(16). This would therefore make all four assays suitable for the detection of COVID-19 antibodies shortly after infection as a confirmatory test for diagnostic purposes, when used in conjunction with RT-PCR assays and clinical history. However, differences in assay diagnostic sensitivity become apparent at later time points. Specifically, the sensitivity of the widely used Abbott assay declined with time, to ~70% at >81 days post PCR. Consequently, this assay is not appropriate for seroprevalence studies, for identification of SARS-CoV-2 naive vaccine trial participants, or for investigation of individuals presenting with long term chronic symptoms. Altering the positive/negative threshold, may mitigate this issue(17), but would not ultimately alter the downward trend in assay signal over time. Notably our study is one of the few that would capture this information, as most other studies have examined seroconversion at early time points(14, 18-20). Reasons for the differences in assay performance over time are unclear but cannot be attributed solely to the choice of antigen. Although other studies have reported an inherent difference in the dynamics of S versus N antibodies(21) our findings do not support this contention, during the first ~100 days of convalescence. Both Abbott and Roche assays employ the N-proteins as an antigen, but Abbott assay titers decline while those in the Roche assay increase during this time period. One possible explanation for this difference is the use of an antigen bridging approach in the Roche assay, where declines in the total amount of antibody might be compensated by increases in affinity or avidity as antibodies mature through somatic hypermutation. Alternatively, it is possible that the range of N epitopes recognized by sera might change with time. Whatever the explanation, it is clear that that the trajectories of antibody titers measured using assays based on recognition

of the same or related antigens can differ(22-25). Overall, given their superior sensitivity at each of the time points investigated thus far, the Siemens and Roche assays appear most appropriate for diagnosis of recent SARS-CoV-2 infection, and would report a higher population prevalence than Abbott or DiaSorin assays in the 1 to 4 month post infection period.

Serological assays that serve a diagnostic function are likely optimized for sensitivity/specificity rather than for dynamic range, Thus, a quantitative signal in a high throughput serological assay might not correspond linearly with antibody titer. Neverthless, convalescent plasma is being used therapeutically, with unit selection based on titers measured using serological assays (26). Additionally, because vaccine or infection elicited neutralizing antibodies will likely confer protection against SARS-CoV-2 infection, it was of interest to evaluate the potential ability of serological assays to predict neutralizing antibody titer. While the Roche assay exhibited the best diagnostic sensitivity during this time period, it had the lowest ability to predict neutralizing antibody titer. This finding might be expected, as neutralizing antibodies are directed to the S protein while N-specific antibodies are not expected to be neutralizing. The DiaSorin assay best predicted neutralizing titers, and marginally outperformed the Siemens assay in this regard, perhaps because the dominant neutralizing and/or S-binding activity in some sera is provided by antibodies that recognize epitopes outside the RBD(27, 28). It is important to recognize however, that many S-binding antibodies are not neutralizing – measurements of S-binding antibodies remain correlates of, and not direct measures of, neutralizing antibodies(7).

Differences in the mechanism of detection likely affects the relationship between antibody titers and assay signal output. Changes in the abundance of different antibody classes over time could also differentially affect readout in serological assays (e.g. IgM is polyvalent and short-lived and might give greater signals in bridging assays). Nevertheless, changes in antibody class composition do not easily explain the trends that we observe, for instance IgG is a longer lived antibody response and the Abbott assay is IgG specific, but Abbot assay titers were the least stable of those evaluated. Neutralization is not specific for

any antibody isotype/subclass and neutralizing titers will reflect the combined activity of all neutralizing antibodies in a sample, Again the comparative stability of the IgG response and the dominant role of IgG in neutralizing plasma (29) does not comport with the idea that changing antibody class abundance could explain declining neutralizing titers.

Very recent reports have similarly indicated that SARS-CoV-2 neutralizing antibody titers decline with time(23, 24), while another study reported that neutralization titers remained stable for at least 3 months post infection(30). However, in the latter case neutralization titers were inferred based on a serologic ELISA measurement that was calibrated using a neutralization assay performed on a small subset of samples. As shown herein, neutralizing antibody levels indeed decline in most patients, even when S-binding or RBD-binding antibody titers measured in serology assays are maintained. Thus, the trajectory of neutralizing antibody levels cannot necessarily be accurately deduced from serologic measurements.

Key future questions include to what degree the titers of neutralizing antibodies, or antibodies that simply bind to S or N correlate with protection from reinfection or severe disease. Many adults possess circulating antibodies to seasonal hCoVs OC43 and 229E(31), and children seroconvert to NL63 and 229E before ~3.5 years of age(32). These baseline levels increased upon infection, returning to baseline within one year. High levels of circulating neutralizing antibody correlated with protection from re-infection (33, 34). However, hCoV re-infections occur(34, 35) often within 12 months(36), but with more mild illness. Thus, these data suggest that seasonal hCoV immunity wanes with time. For SARS-CoV and MERS-CoV, antibody responses also decline in the majority of infected individuals(37) Indeed, analyses of the decay of SARS-CoV antibodies indicate kinetics consistent with those reported herein (38). Moreover, recent studies have documented SARS-CoV-2 reinfection (39-41), in one case in the context of waning neutralizing antibodies (42). Overall, it seems possible that SARS-CoV-2 reinfection might be common. Importantly however, the magnitude and stability of antibody responses to SARS-CoV-2 vaccines might be quite different to those following natural infection. Specifically, if the great variability in

post infection SARS-CoV-2 antibody levels are indeed a consequence of variable antigen exposure, then differences might be mitigated in the context of vaccination.

If neutralizing antibodies constitute a major protective mechanism against SARS-CoV-2 infection, then serological assays that use S-based antigens and best predict neutralizing titer are the most appropriate for prognostication of immunity. Conversely, if other immune mechanisms, such as long-lived memory T-cells play a dominant role in protection (43-46), then the optimal choice of antigen for serology assays might differ. Because detailed analyses of T-cell responses are not currently feasible in a high throughput clinical setting, future work should examine the frequency of reinfection and clinical outcomes with detailed longitudinal analyses of serum antibodies to both N and S antigens to determine the prognostic value of such measurements.

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Author contributions

HW, SJ, TH and PDB conceived and designed the study. HW, BB, MS, ES, CR, JM, SC, EF, NG, GH, KT and SJ acquired and analyzed data using the serological assay platforms. FM and JCCL performed the neutralization assays. FM and TH did additional data analysis. HM, BB, SJ FM and PDB wrote the first draft of the manuscript. HM, BB, FM, TH, SJ and PB critically reviewed and revised the draft. All authors approved the final version of the manuscript for submission. HW, BB and MS contributed equally. The corresponding author attests that all listed authors meet authorship criteria and that no others meeting the criteria have been omitted.

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Table 1 Percentage of participants per cohort displaying the three main WHO symptoms

	Fever	Cough	Anosmia	0 of 3 symptoms	1 of 3 symptoms	2 of 3 symptoms	All 3 symptoms	Self-reported recovered
Reported symptom	65	69	74	1	19	42	35	44
%	67%	71%	76%	1%	16%	43%	36%	49%

N = 97 for all reported symptoms apart from “self-reported recovered”, where only 90 individuals responded to this survey question

Figure legends

Fig. 1. Longitudinal analysis of COVID-19 participant sera. **(A)** Sensitivity of the Abbott, DiaSorin, Siemens and Roche serological assays (as indicated) measured in samples collected at four different timepoints, as indicated, post PCR test and 95% confidence interval. **(B)** Relative antibody titers for the DiaSorin, Siemens, Abbott and Roche, assays at visits 1-3, normalized to visit 1. Horizontal line indicates median value with 95% confidence interval. Statistical significance was assessed with the Wilcoxon test. (C) Values for DiaSorin, Siemens, Abbott and Roche serological assays for each participant plotted over time (each line represents one participant). Assay thresholds are indicated by a dotted horizontal line.

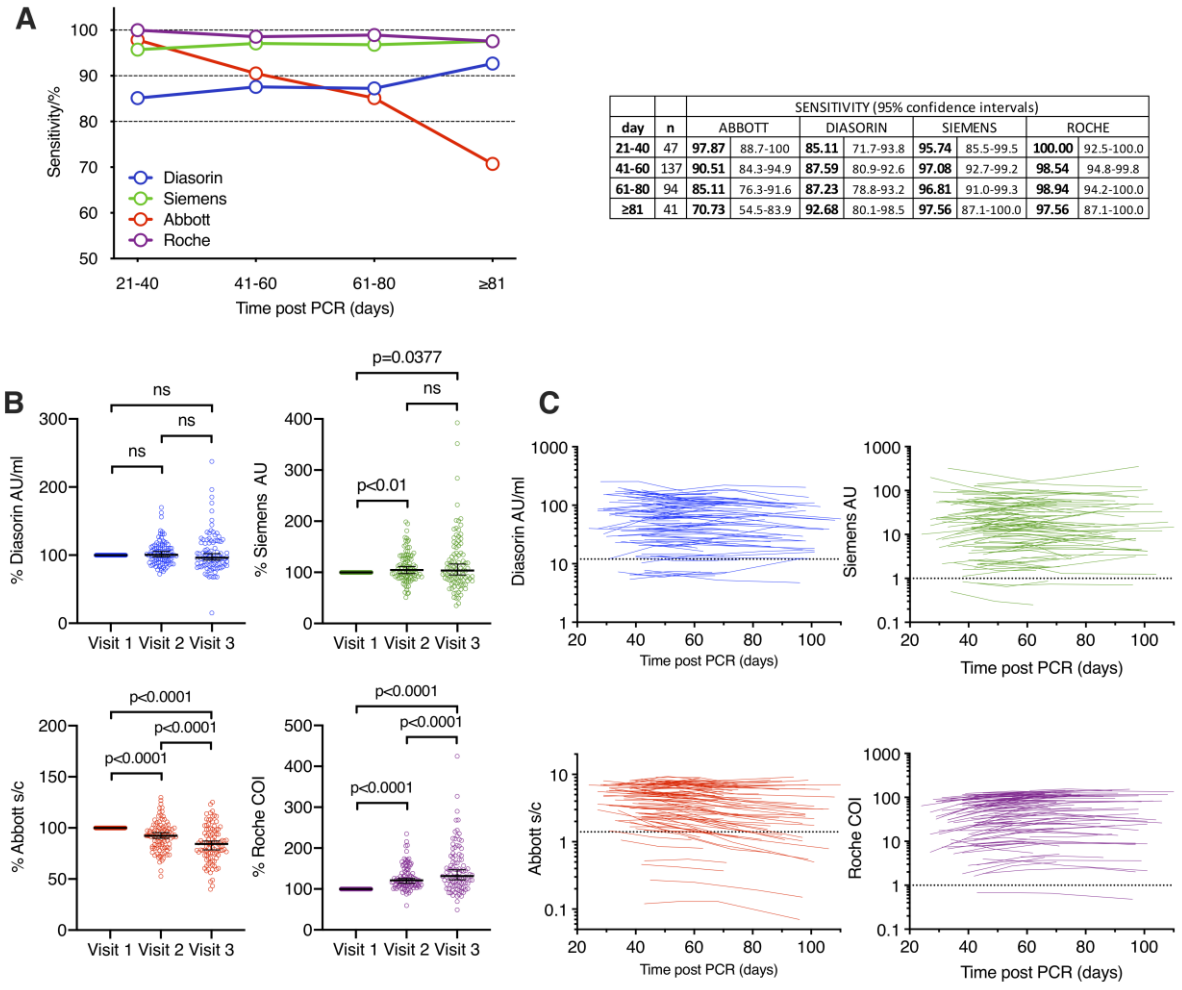
Fig. 2. Neutralization activity in COVID-19 participant sera. **(A)** Half-maximal neutralization titers (NT50s) for each individual participant measured in serum samples collected at three different visits, as indicated by color. Inserts show correlation of NT_{50} values for samples collected at each visit, the spearman r is indicated ($p<0.0001$). **(B)** Relative NT_{50} values in sera obtained at visit 1 to 3, normalized to visit 1. Horizontal line represents median with 95% confidence interval. Statistical significance was assessed with the Wilcoxon test. **(C)** Frequency of sera with NT_{50} values falling to various quantitative categories at each visit. **(D)** NT_{50} values for each participant plotted over time (each line represents one participant). The limit of detection (LOD) is indicated by a dotted horizontal line.

Fig. 3. Correlation of serology results with neutralization titers. (A-D) Serological assay values for the DiaSorin **(A)**, Siemens **(B)**, Abbott **(C)** and Roche **(D)** assays versus NT_{50} values. Samples collected at each visit are indicated by color and are plotted individually as well as on a composite graph. Spearman r for all visits (black) and individual visits are indicated ($p<0.0001$).

Fig. 4. Lack of correlation of changes serology results with changes in neutralization titers.

(A-D) Fold-change (visit 1 to visit 3) in serological assay values for the DiaSorin **(A)**, Siemens **(B)**, Abbott **(C)** and Roche **(D)** assay versus fold-change in NT_{50} values. Spearman r and p-value are indicated.

Figure 1



		SENSITIVITY (95% confidence intervals)							
		ABBOTT		DIASORIN		SIEMENS		ROCHE	
day	**n**								
21-40	47	**97.87**	88.7-100	**85.11**	71.7-93.8	**95.74**	85.5-99.5	**100.00**	92.5-100.0
41-60	137	**90.51**	84.3-94.9	**87.59**	80.9-92.6	**97.08**	92.7-99.2	**98.54**	94.8-99.8
61-80	94	**85.11**	76.3-91.6	**87.23**	78.8-93.2	**96.81**	91.0-99.3	**98.94**	94.2-100.0
≥81	41	**70.73**	54.5-83.9	**92.68**	80.1-98.5	**97.56**	87.1-100.0	**97.56**	87.1-100.0

Figure 2

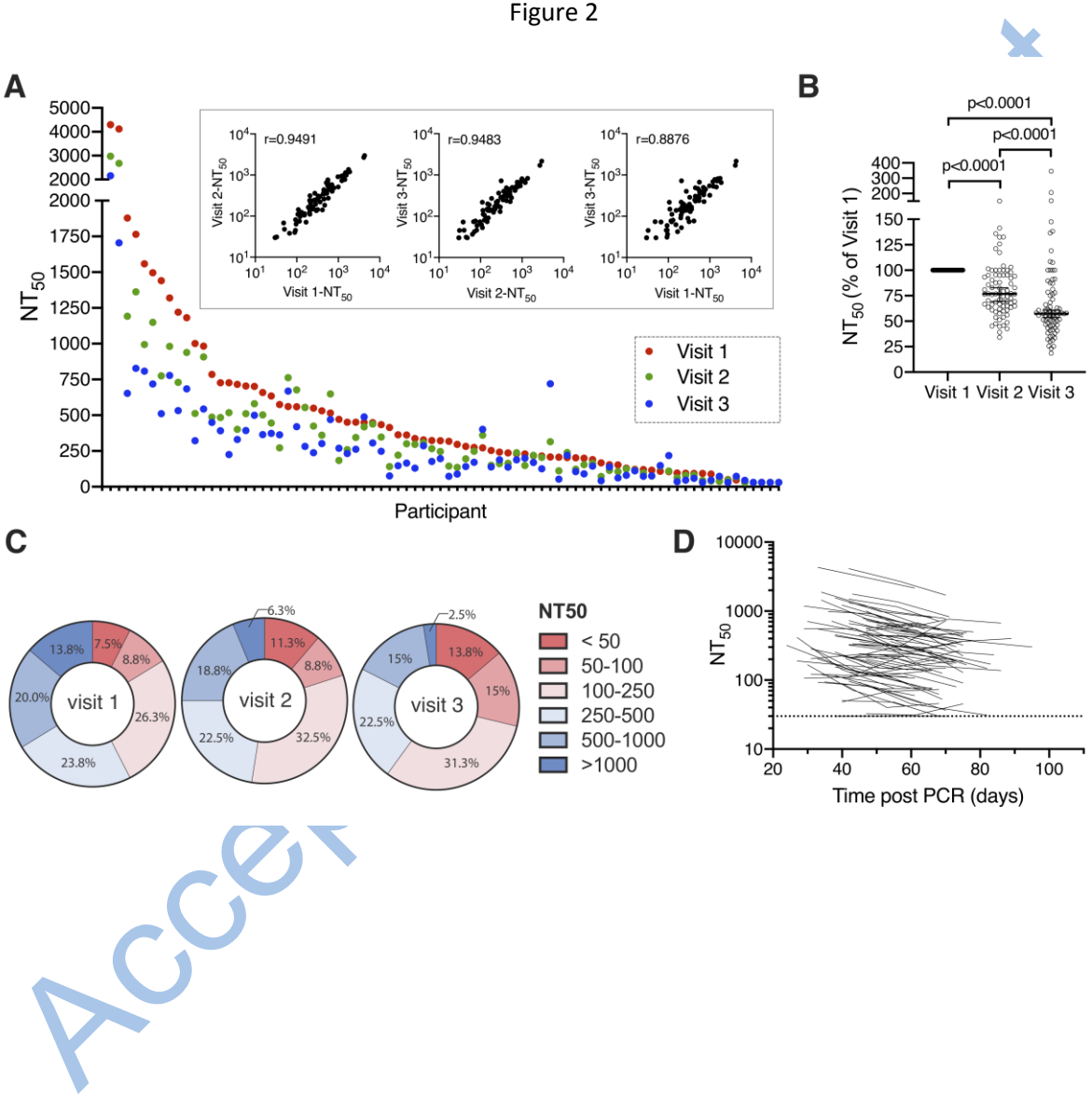


Figure 3

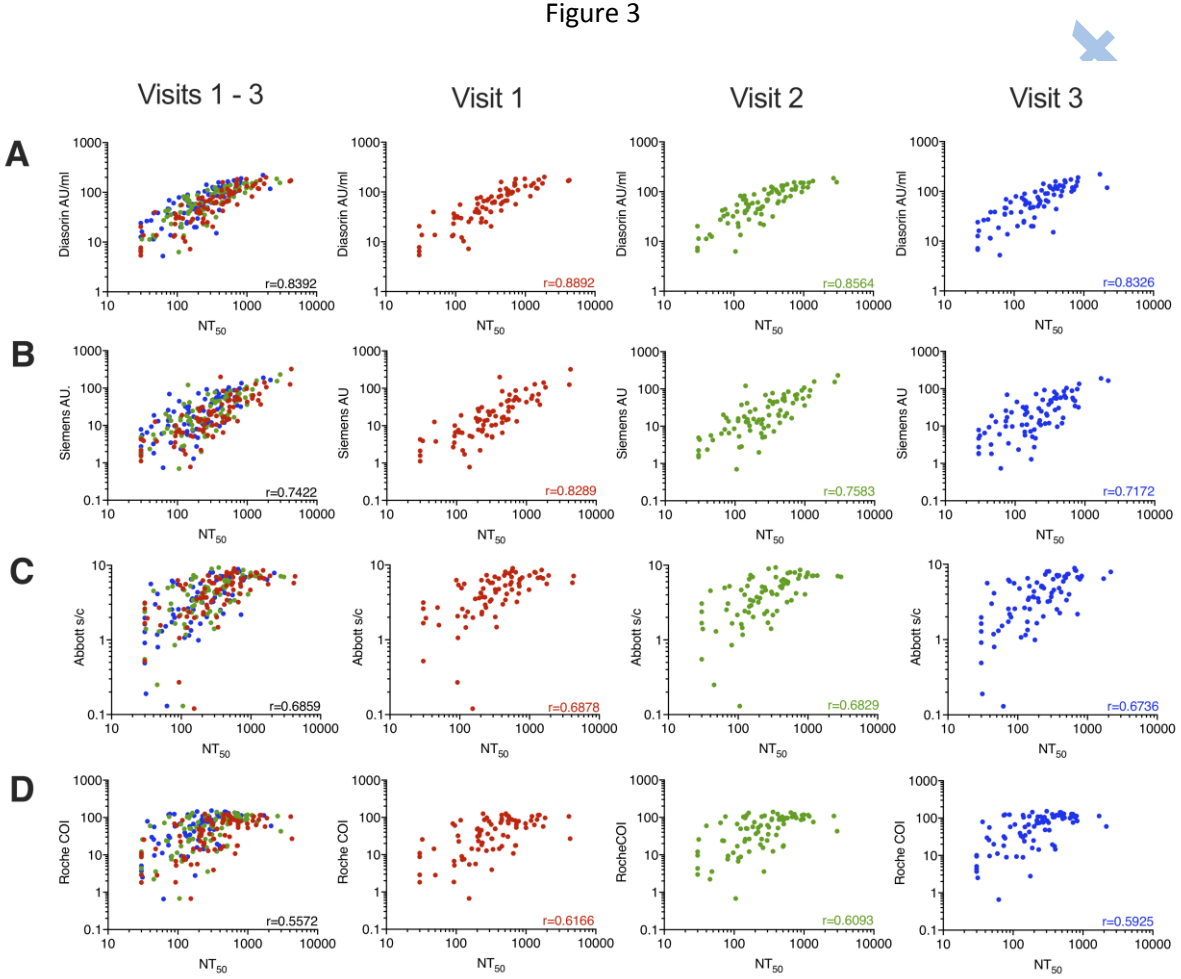


Figure 4