



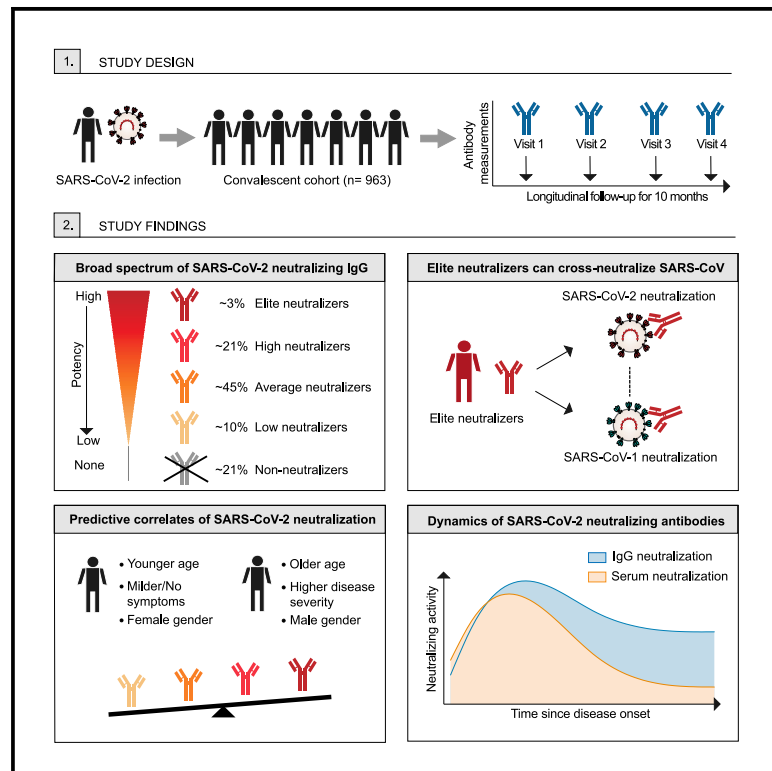
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Kinetics and correlates of the neutralizing antibody response to SARS-CoV-2 infection in humans

Graphical abstract



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In brief

Vanshylla et al. report longitudinal antibody kinetics in a mainly mild COVID-19 convalescent cohort of 963 individuals. There is broad variation in the initial response with older age and disease severity predicting higher SARS-CoV-2 neutralizing activity. Neutralizing IgG antibodies are detectable for up to 10 months in the majority of individuals.

Highlights

- Broad variation in neutralizing antibodies in SARS-CoV-2-convalescent individuals
- ~3% of individuals showed a potent antibody response with SARS-CoV-1 cross-reactivity
- Older age, symptoms, and severe disease predict higher SARS-CoV-2 neutralization
- Serum and IgG neutralization half-lives were 14.7 and 31.4 weeks, respectively



Article

Kinetics and correlates of the neutralizing antibody response to SARS-CoV-2 infection in humans

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SUMMARY

Understanding antibody-based SARS-CoV-2 immunity is critical for overcoming the COVID-19 pandemic and informing vaccination strategies. We evaluated SARS-CoV-2 antibody dynamics over 10 months in 963 individuals who predominantly experienced mild COVID-19. Investigating 2,146 samples, we initially detected SARS-CoV-2 antibodies in 94.4% of individuals, with 82% and 79% exhibiting serum and IgG neutralization, respectively. Approximately 3% of individuals demonstrated exceptional SARS-CoV-2 neutralization, with these “elite neutralizers” also possessing SARS-CoV-1 cross-neutralizing IgG. Multivariate statistical modeling revealed age, symptomatic infection, disease severity, and gender as key factors predicting SARS-CoV-2-neutralizing activity. A loss of reactivity to the virus spike protein was observed in 13% of individuals 10 months after infection. Neutralizing activity had half-lives of 14.7 weeks in serum versus 31.4 weeks in purified IgG, indicating a rather long-term IgG antibody response. Our results demonstrate a broad spectrum in the initial SARS-CoV-2-neutralizing antibody response, with sustained antibodies in most individuals for 10 months after mild COVID-19.

INTRODUCTION

Coronavirus disease 2019 (COVID-19) is caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which was first identified in December 2019 (Zhou et al., 2020; Zhu et al., 2020). Since then, the virus has rapidly spread across the globe and caused more than 140 million proven infections and over 3 million deaths (Dong et al., 2020). Disease severity can range from asymptomatic infection to symptoms like cough, fever, muscle pain, and diarrhea, to severe course of infection including pneumonia with severe respiratory distress and a high mortality risk (Huang et al., 2020b; Mizrahi et al., 2020; Rothe et al.,

2020). While the majority of infected individuals experience a mild course of disease, elderly individuals or those with pre-existing conditions are at a higher risk for severe course of COVID-19 (Williamson et al., 2020). In symptomatic non-hospitalized cases, the acute course of disease typically spans 7–14 days (He et al., 2020; Wölfel et al., 2020). However, a significant fraction of COVID-19 patients suffer long-lasting symptoms post-recovery, the so called “post-COVID syndrome” (Augustin et al., 2021; Cirulli et al., 2020; Galván-Tejada et al., 2020; Huang et al., 2021).

SARS-CoV-2 infects human cells by using the virus spike (S) protein (Walls et al., 2020) for targeting the angiotensin-converting enzyme-2 (ACE-2) receptor (Hoffmann et al., 2020). The



S-protein carries immunodominant epitopes against which humoral B and T cell responses are generated upon natural infection and vaccination (Ni et al., 2020; Piccoli et al., 2020; Rydzynski Moderbacher et al., 2020; Sahin et al., 2020; Widge et al., 2020). S-specific IgM, IgA, and IgG antibodies are detected early after infection (Long et al., 2020; Seow et al., 2020) and IgG antibody levels and IgG memory B cells can persist post-infection (Dan et al., 2021; Wajnberg et al., 2020).

Neutralizing antibodies (NAbs) are powerful molecules that target viruses and block infection. Moreover, they can eliminate circulating viruses and infected cells by antibody-mediated effector functions (Zohar and Alter, 2020). As a result, NAbs are crucial to overcome infectious diseases and are an important correlate of protection (Corti and Lanzavecchia, 2013). For SARS-CoV-2, vaccine-induced NAbs as well as purified IgG from convalescent animals have been shown to protect non-human primates (NHPs) from infection in a SARS-CoV-2 challenge model (McMahan et al., 2021; Mercado et al., 2020). Moreover, highly potent monoclonal NAbs have been isolated (Kreer et al., 2020; Robbiani et al., 2020; Zost et al., 2020) and are being used for treatment of COVID-19 in humans (Chen et al., 2020a; Weinreich et al., 2020).

Given the short time SARS-CoV-2 has been studied, information on long-term antibody dynamics are limited. Recent studies show that serum neutralizing activity is detectable within a week after onset of symptoms (Ng et al., 2020; Wu et al., 2020) and can persist in the first months after infection (Dan et al., 2021; Iyer et al., 2020; Wajnberg et al., 2020). Moreover, studies with symptomatic and hospitalized individuals have shown that more severe courses of disease result in a stronger SARS-CoV-2 neutralizing antibody response (Chen et al., 2020b; Piccoli et al., 2020; Zeng et al., 2020). While these studies provide important insights, a precise quantification of SARS-CoV-2 neutralizing activity and dynamics, as well as clinical correlates of developing a protective antibody response, are less known.

In this study, we set out to provide a deeper understanding of the neutralizing antibody response to SARS-CoV-2. To this end, we determined anti-S antibody levels and neutralizing serum and IgG activity of 2,146 samples from a longitudinally monitored cohort of 963 individuals, together with detailed information on the course of disease and past medical history. We combined statistical modeling to infer antibody decay rates after SARS-CoV-2 infection and built a prediction model for evaluating how clinical or disease features correlate with NAb titers. Finally, we performed longitudinal analyses to study anti-S antibody levels as well as NAb titers for a time period of up to 10 months post-SARS-CoV-2 infection. Our results inform on the kinetics, longevity, and features affecting the antibody response to SARS-CoV-2. They are critical to understand SARS-CoV-2 immunity and to aid non-pharmacological interventions and vaccination strategies to overcome COVID-19 (Lanzavecchia et al., 2016).

RESULTS

Establishing a cohort for investigating SARS-CoV-2 immunity

To investigate the development of SARS-CoV-2 immunity, we established a cohort of COVID-19 patients who recently recov-

ered from SARS-CoV-2 infection. Time since disease onset was derived from self-reported symptom onset or date of positive naso-/oro-pharyngeal swab. In addition, each participant reported details on the course of infection, symptoms, and past medical history (Table S1). Participants enrolled ranged from 18 to 79 years of age (median: 44 years) with a balanced distribution of males (46.1%) and females (53.9%). Disease severity included asymptomatic (4.6%), mildly symptomatic (91.7%), and hospitalized individuals (2.9%; Figure 1; Table S1). 20.6% of participants reported pre-existing conditions that have been described to influence COVID-19 outcomes (Williamson et al., 2020).

Blood samples were collected from 963 individuals at study visit 1 (median 7.3 weeks post-disease onset) with follow-up analyses at study visit 2 for 616 participants (median 18.8 weeks post-disease onset), study visit 3 for 430 participants (median 30.1 weeks post-disease onset), and study visit 4 for 137 participants (median 37.9 weeks post-disease onset; Figure 1). Other participants were lost in follow-up or did not reach the respective study visit at the time of our analysis. Anti-S IgG was quantified by ELISA and chemiluminescent immunoassays (CLIA), and the NAb response to SARS-CoV-2 was analyzed using serum dilutions as well as purified IgG to precisely quantify neutralizing activity (Figures S1A–S1D). In total, 4,516 measurements were collected for visit 1 with another 2,275 subsequent measurements for visits 2 to 4 to determine the SARS-CoV-2 antibody response for 10 months following infection.

Broad spectrum of the initial SARS-CoV-2 neutralizing antibody response

NAb levels were quantified by testing serum and purified IgG from plasma/serum against pseudovirus particles expressing the Wuhan01 S protein (EPI_ISL_406716). Serum neutralization at study visit 1 was categorized based on titer into non- ($ID_{50} < 10$), low ($ID_{50} = 10–25$), average ($ID_{50} = 25–250$), high ($ID_{50} = 250–2500$), and elite neutralizers ($ID_{50} > 2500$; Figure 2A). Mean serum ID_{50} titer was 111.3 with 17.7% of individuals that did not reach 50% neutralization at the lowest serum dilution of 1:10. In addition, all samples were purified for IgG and the neutralizing response was determined and categorized based on IC_{50} values into no ($IC_{50} > 750 \mu\text{g/mL}$), low ($IC_{50} = 500–750 \mu\text{g/mL}$), average ($IC_{50} = 100–500 \mu\text{g/mL}$), high ($IC_{50} = 20–100 \mu\text{g/mL}$), and elite neutralization ($IC_{50} < 20 \mu\text{g/mL}$; Figure 2B). At study visit 1, out of 963 participants, 10%, 44.8%, and 20.9% demonstrated low, average, and high neutralization, respectively. 21% did not mount an IgG neutralizing response of an IC_{50} below $750 \mu\text{g/mL}$. 3.3% of individuals were classified as elite neutralizers with IC_{50} values as low as $0.7 \mu\text{g/mL}$ detected in one individual at 8.6 weeks post-disease onset. Combining serum and IgG measurements, 87.3% individuals showed detectable NAb activity at a median of 7.3 weeks after SARS-CoV-2 infection (Figure 2C). The serum and IgG neutralization potency categorization matched for most individuals with a high correlation between serum ID_{50} titers and IgG IC_{50} values (Spearman $r = -0.72$, $p < 0.0001$; Figure 2C). Moreover, only 8.5% of samples had only serum and no IgG neutralization, indicating that IgG antibodies form the dominant NAb isotype in serum. To further determine the predictive value of IgG binding to the S protein for SARS-CoV-2 neutralization, we performed

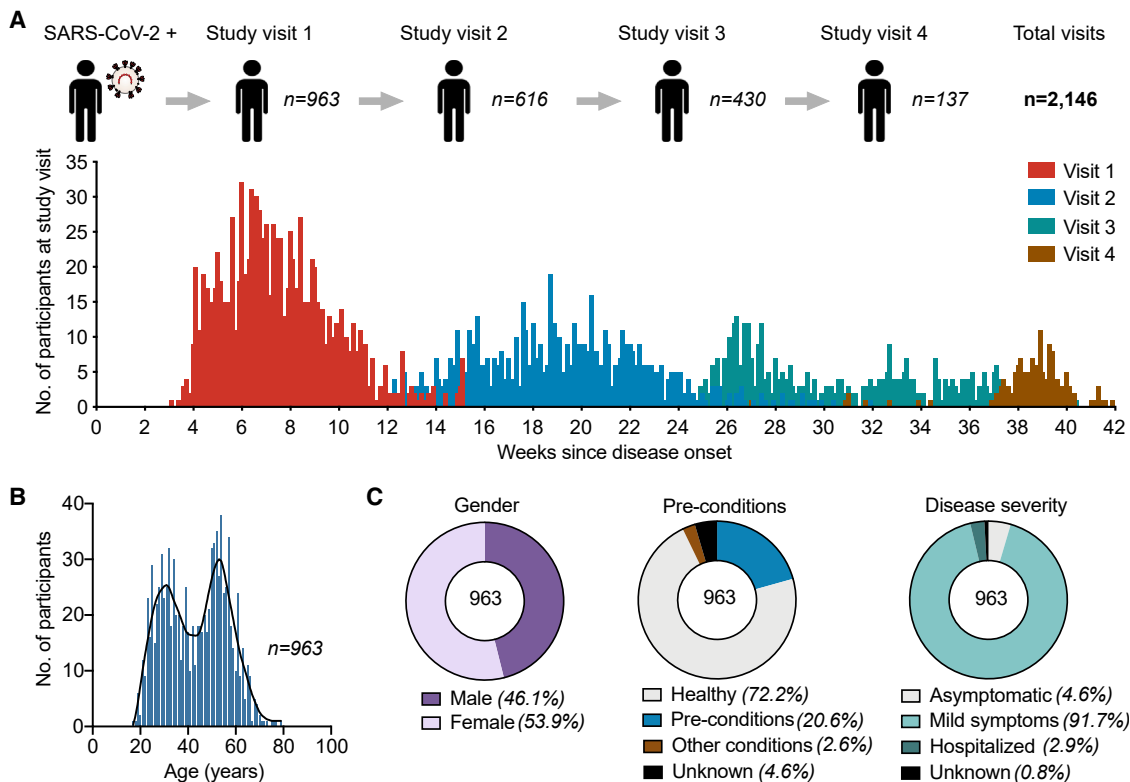


Figure 1. SARS-CoV-2-recovered cohort and study design

(A) Illustration depicting study timeline and number of individuals analyzed at each study visit. Graph represents sample collection time for participants in weeks since disease onset (symptom onset date or positive PCR date).

(B) Age distribution of the cohort.

(C) Gender distribution, presence of pre-conditions, and disease severity.

an S1-reactive ELISA (Euroimmun) on all samples of visit 1. 82.8% and 70.2% of individuals possessed S-reactive IgG (Figures 2D and 2E) and IgA Abs, respectively (Figure S1E). Of note, the serum-only neutralizers had very low serum neutralization levels with geometric mean (GeoMean) ID_{50} of 24 and 47.6% of this fraction demonstrating IgA reactivity. Anti-S IgG levels were directly proportional to IgG NAb IC_{50} values (Spearman $r = -0.62$, $p < 0.0001$; Figure 2F) and IgG Ab levels better correlated with serum neutralization than IgA Ab levels (Figures S1F–S1I).

Finally, we determined the fraction of individuals lacking any detectable antibody response. To this end, we combined the results of different IgG and IgA assays detecting binding to SARS-CoV-2 S1, S1/S2, and N, as well as three neutralization assays (Figure 2G). Out of the 166 anti-S1-IgG negative (12.7%) or equivocal (4.6%) individuals, we found binding antibodies in 62.0% in at least one of four assays and neutralizing activity in 54.2% in at least one of three assays (Figures 2G and 2H). Combining these results and accounting for assay specificity (see STAR Methods), we show that only 5.6%–7.3% of individuals do not mount a detectable antibody response against SARS-CoV-2. Notably, while only 3.6% (1 of 28) of hospitalized patients and 4.9% (43 of 877) of individuals with mild symptoms lacked anti-SARS-CoV-2 antibodies, 22.7% (10 of 44) of asymptomatic individuals were negative for a detectable antibody

response in at visit 1 (Figure 2I). We conclude that 92.7%–94.4% of individuals naturally infected with SARS-CoV-2 mount an antibody response against the virus within the first 12 weeks. Among those, we detected a broad variation in neutralizing activity with approximately 3% generating a highly potent serum and IgG NAb response.

Age, symptoms, and disease severity predict SARS-CoV-2 neutralization

Next, we analyzed how age, disease severity, gender, and the presence of pre-existing conditions correlate with the anti-S antibody and SARS-CoV-2 neutralizing responses (Figures 3A and S2). The IgG NAb response was significantly higher in older individuals ($p < 0.0001$), with participants >60 years comprising 7.7% of elite and 42.8% of high neutralizers (Figure 3A). Hospitalized patients had significantly higher NAb activity compared to individuals with symptoms ($p = 0.0008$) and asymptomatic individuals ($p = 0.0003$), and 43.2% (25 of 44) of the asymptomatic lacked detectable IgG NAb (Figure 3A). Males showed higher SARS-CoV-2 neutralization than females (GeoMean IC_{50} 136.3 $\mu\text{g}/\text{mL}$ versus 188.4 $\mu\text{g}/\text{mL}$; $p < 0.0001$). Individuals with pre-existing conditions had only slightly higher NAb activity compared to those without them (GeoMean IC_{50} 161.9 $\mu\text{g}/\text{mL}$ versus 174.6 $\mu\text{g}/\text{mL}$, $p = 0.022$; Figure 3A). Similar to IgG NAb activity, serum neutralizing activity and anti-S antibodies were also

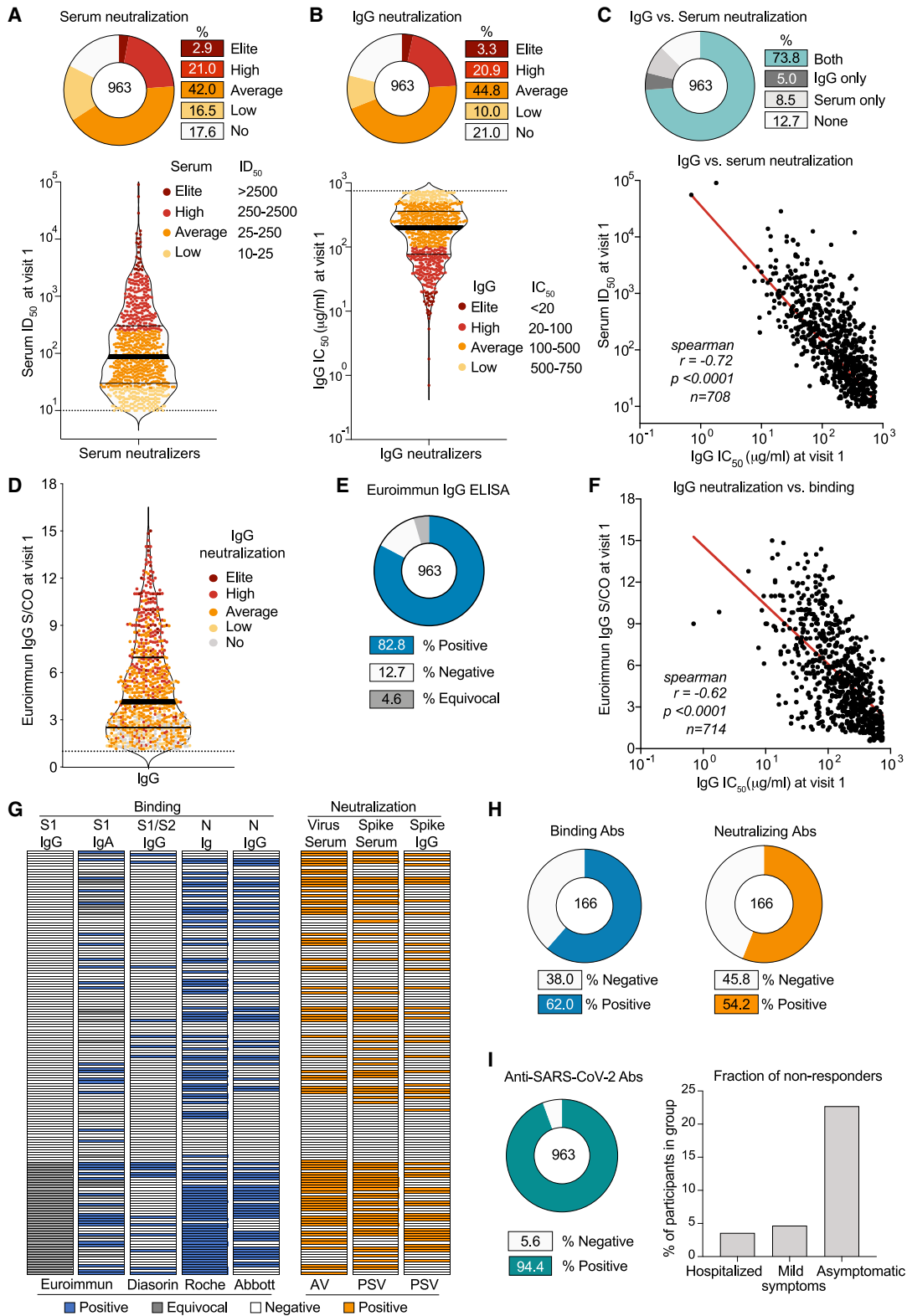


Figure 2. Neutralizing antibody response after recovery from SARS-CoV-2 infection

(A) Pie chart illustrating fraction of serum neutralizers against Wu01 pseudovirus at study visit 1. Violin plot depicts serum ID₅₀ values for the neutralizers (n = 793), categorized based on serum ID₅₀ titers. Dotted line represents the LOD (10-fold dilution) of the assay.

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higher in older individuals, patients with a more severe course of disease, and males (Figures S2A–S2C). Next, we performed a multivariate statistical analysis to determine the interplay between clinical features and the NAb response and to rule out confounders. Features included gender, age, disease severity, presence of pre-existing conditions, disease symptoms (Table S1), and weeks since disease onset. We applied a stepwise regression that adds new features only if they significantly improved the model according to a likelihood ratio test. The resulting IC₅₀ prediction model (adjusted R² = 0.177) revealed that age is most predictive for SARS-CoV-2 neutralizing activity ($p = 1.3^{-278}$), followed by fever during infection ($p = 2.9 \times 10^{-10}$), disease severity ($p = 6.3 \times 10^{-5}$), diarrhea during infection ($p = 0.003$), time since disease onset ($p = 0.003$), and male gender ($p = 0.004$; Figures 3B and 3C). Pre-existing conditions were not a significant feature of neutralizing activity in the multivariate analysis. Similar to the results for IgG neutralization, age, time since disease onset, fever during acute infection, male gender, disease severity, and diarrhea during infection were highly predictive of serum ID₅₀ (Figure S3A). Additionally, we built a Bayesian network model to determine the feature dependencies and how they predict the SARS-CoV-2 IgG (Figure 3D) and serum neutralization response (Figure S3B). The Bayesian network revealed that features like age and fever are directly predictive of neutralization, while other features are indirectly predictive through their correlation with other features. In addition, we investigated the possible effect of viral load obtained from naso-/oro-pharyngeal swabs at the time of diagnosis on the antibody response at study visit 1, but no correlation was found (Figures S3C and S3D). In summary, older age, fever or diarrhea during acute infection, disease severity, shorter time since infection, and male gender are highly predictive of the development of SARS-CoV-2 neutralizing activity.

Elite SARS-CoV-2 neutralizers exhibit SARS-CoV-1 cross-neutralization

Individuals mounting a highly potent neutralizing antibody response are often considered “elite neutralizers” (Simek et al., 2009). These individuals are of particular interest (1) to identify factors associated with the development of effective humoral immunity, (2) to guide vaccine design, and (3) to isolate highly potent neutralizing monoclonal antibodies (Walker and Burton, 2018). In order to characterize the small fraction of SARS-CoV-2 elite neutralizers in our cohort (3%, IC₅₀ < 20 μg/mL; Figure 2B), we selected 15 individuals of each group of non-, low, average, high, and elite neutralizers, testing them against authentic SARS-CoV-2 as well as SARS-CoV-1 pseudo-

virus (Figure 4A). Neutralization of SARS-CoV-2 pseudovirus correlated well with authentic virus neutralization (Spearman $r = 0.79$; Figure 4B). SARS-CoV-1 neutralization was not observed in non- and low neutralizers and only in 1 out of 15 average neutralizers. However, in the high and elite neutralizing groups, 8/15 and 15/15 samples carried SARS-CoV-1 cross-neutralizing activity, respectively, with potencies (IC₅₀) as low as 5.1 μg/mL IgG (Figure 4A). Of note, while all SARS-CoV-2 elite neutralizers demonstrated SARS-CoV-1 cross-neutralization, variation in potency ranged from 12.1 to 634.9 μg/mL and an overall low correlation (Spearman $r = 0.3745$; Figure 4C) was observed. Next, we studied the neutralizing potency of the elite neutralizers against six different SARS-CoV-2 strains carrying several mutations that became prominent at a global level (Figure 4D; Hadfield et al., 2018). IgG from elite neutralizers was potent against all tested SARS-CoV-2 strains, including both S1 and S2 mutants, as well as variants (BAVP1 and DRC94) carrying the D614G mutation (Figure 4E). Intriguingly, testing the neutralizer groups against recent SARS-CoV-2 variants of concern, B.1.1.7 and B.1.351 (Figure 4F), revealed no significant effect on neutralizing activity against the B.1.1.7 variant for all five groups (Figure 4F). On the other hand, the B.1.351 variant caused almost a 20-fold drop in GeoMean IgG IC₅₀ in elite neutralizers, with many average/low neutralizers losing neutralizing capacity against this variant (Figure 4F). We conclude that individuals mounting a potent SARS-CoV-2 NAb response possess cross-reactive antibodies against SARS-CoV-1 without any known prior exposure and can neutralize most of the prevalent SARS-CoV-2 strains. In addition, while the B.1.1.7 variant did not alter neutralization profile of IgG from convalescent individuals, neutralization capacity against the B.1.351 variant was markedly reduced.

Long-term persistence of IgG NABs after SARS-CoV-2 infection

In order to study antibody kinetics, we first investigated the development of SARS-CoV-2-directed antibodies in the first 4 weeks after disease onset. To this end, we evaluated 251 samples obtained from an additional 110 individuals. In this subgroup, 45% and 55% were male and female, respectively, and 41.8% had been hospitalized (Figure S4A). Anti-S IgG and IgA could be detected in some people within the first week after disease onset, with IgA levels starting to decline by week 4 (Figure S4B). Out of eight individuals that were closely monitored, most individuals sero-converted between 2 and 3 weeks post-disease onset (Figure S4C).

In order to assess longevity of humoral immunity following SARS-CoV-2 infection, we applied a linear regression mixed

- (B) Pie chart depicting the fraction of IgG neutralization against Wu01 pseudovirus at study visit 1. Violin plot depicts IgG IC₅₀ values for the neutralizers ($n = 760$), categorized based on IgG IC₅₀. Dotted line represents the LOD (750 μg/mL) of the assay.
- (C) Pie chart comparing fraction of samples with neutralization at serum and/or IgG level. Spearman correlation plot between serum ID₅₀ and IgG IC₅₀ values at study visit 1.
- (D) Violin plot of Euroimmun ELISA signal over cut-off (S/CO) ratios for anti-spike IgG. Dotted line represents LOD (S/CO = 1.1) of the assay.
- (E) Pie charts illustrating fraction of anti-spike IgG reactive individuals in the Euroimmun ELISA.
- (F) Spearman correlation between Euroimmun IgG S/CO and IgG IC₅₀ at study visit 1.
- (G) Plot depicting binding against spike, nucleocapsid (N), and neutralizing response against authentic virus (AV) and Wu01 pseudovirus (PSV) of the IgG negative fraction ($n = 166$) with each row representing 1 individual.
- (H) Pie charts showing total fraction of individuals with binding or neutralizing activity in the IgG fraction from (G).
- (I) Pie chart representing total combined binding and NAb response in the cohort ($n = 963$) and bar graph of the Ab-negative individuals based on disease severity. Bars in violin plots in A, B, and D depict median and interquartile range. LOD, limit of detection.

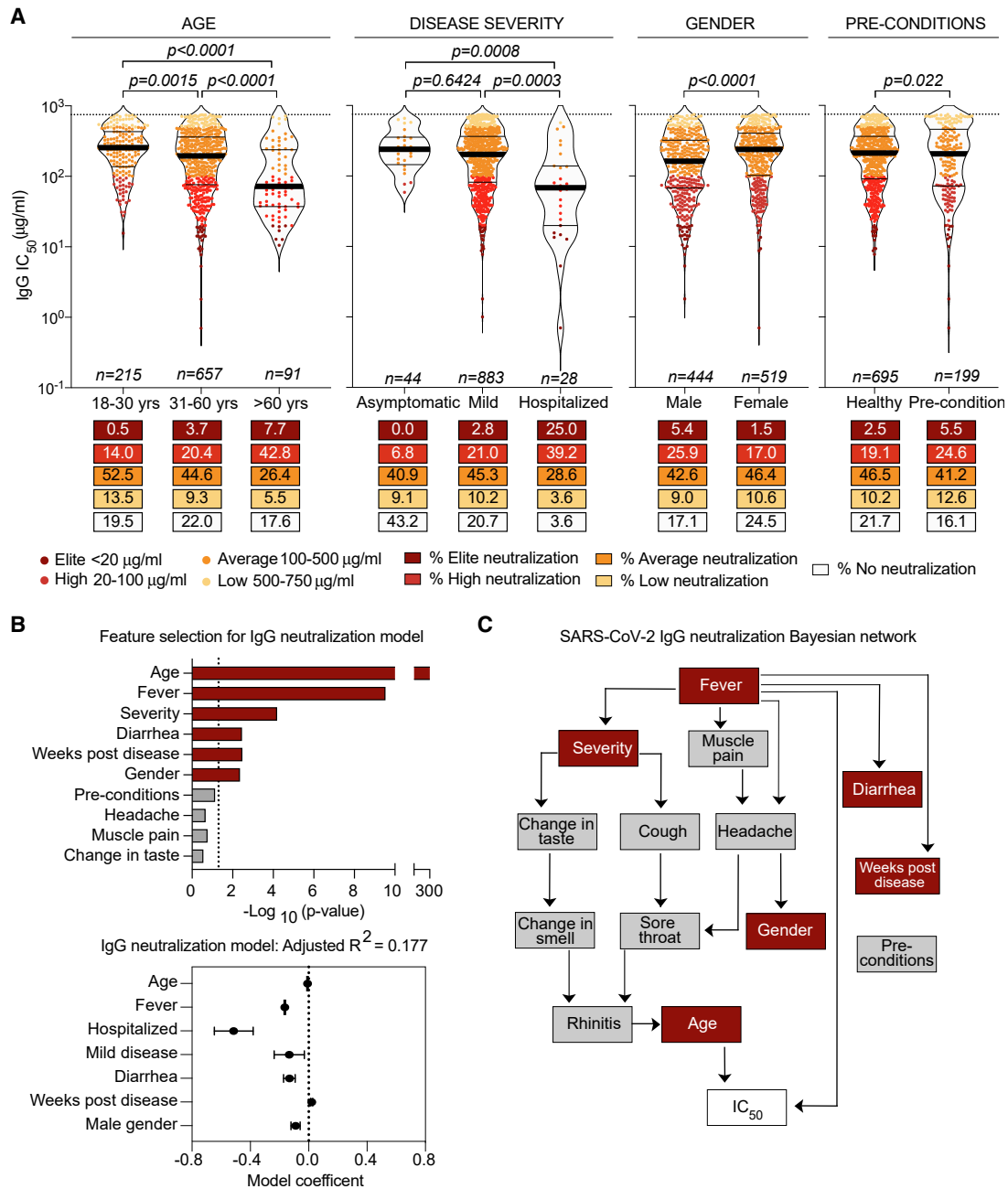


Figure 3. Correlates of SARS-CoV-2 neutralizing activity

(A) Violin plots depicting IgG neutralization IC₅₀ values at study visit 1 against Wu01 pseudovirus, subdivided based on age, disease severity, gender, and pre-conditions. Dotted line represents the limit of detection (750 μg/mL) of the assay. Statistical analysis was performed Kruskal-Wallis and Mann-Whitney tests. Bars represent median and interquartile range.

(B) Multiple linear regression model for predicting IgG IC₅₀ using the features of gender, age, disease severity, pre-conditions, weeks since infection, and the nine reported symptoms. Plot below depicts model coefficients to study the relationship of features and response in the final IC₅₀ prediction model.

(C) Bayesian network of the features predicting IgG IC₅₀ are plotted using the bnlearn R package. The graph connects the features, which are predictive of each other with IgG IC₅₀ as sink.

Features highlighted in red in (C) had p values <0.05 in the multiple regression model in (B).

effects model to antibody measurements obtained between 3.1 to 41.9 weeks post-infection. The half-life of anti-S IgG was estimated to be 34.9 weeks (Figure 5A). For systematic tracking of the antibody response within individuals, we analyzed anti-S

antibodies in 131 individuals at four study visits (range 3.1 to 38.7 weeks post-disease onset; Figures 5B and 5C). The data show that IgG levels decrease between the first and second study visits (GeoMean S/C0 = 4.6 versus GeoMean

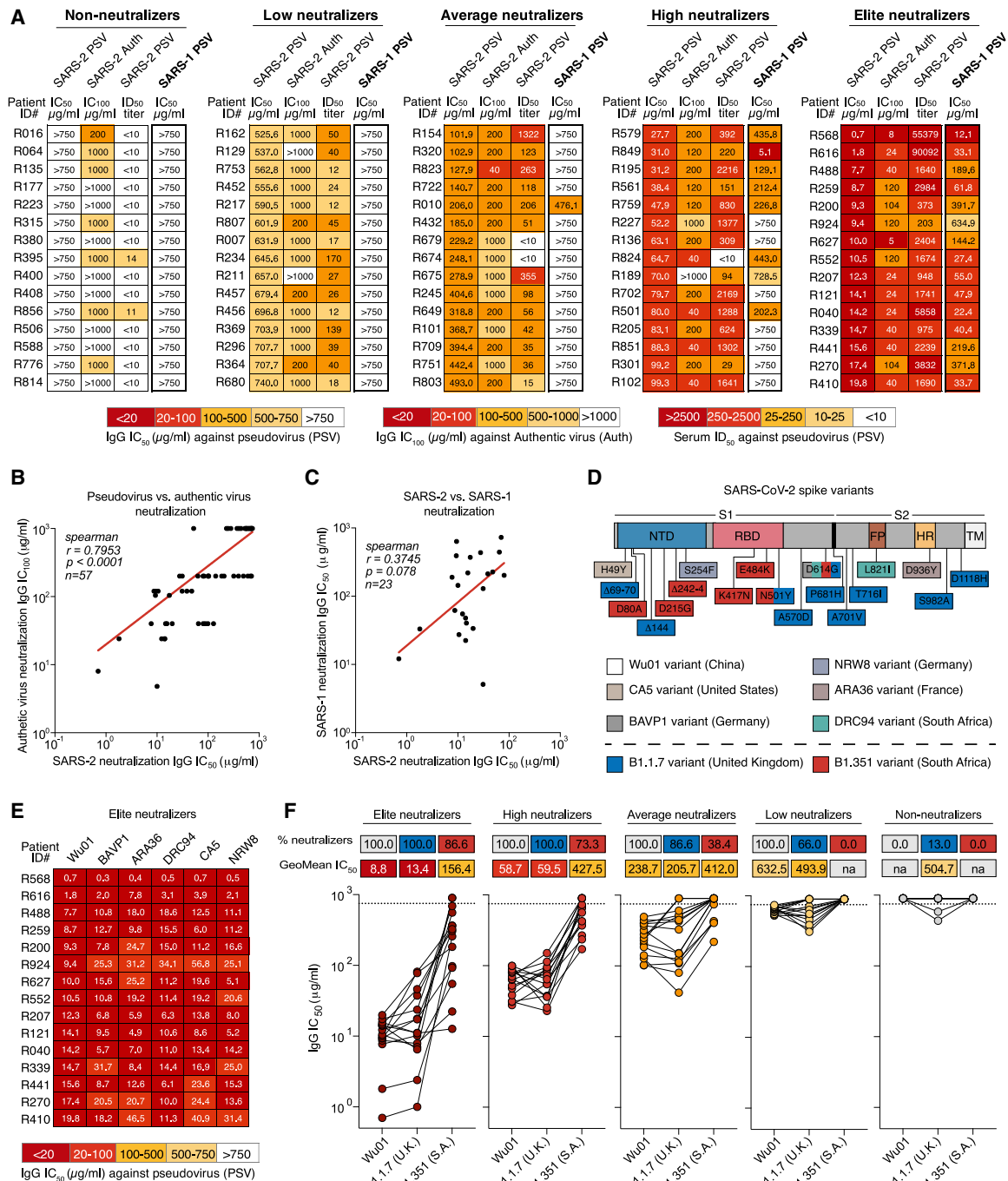


Figure 4. Cross-neutralization by SARS-CoV-2 elite neutralizers

(A) Heatmaps visualizing the neutralizing activity of 15 individuals from each neutralization category: elite, high, average, low, and non-neutralizers (total n = 75) against SARS-CoV-2-S (SARS-2) pseudovirus, SARS-CoV-2 authentic virus, and SARS-CoV-1 (SARS-1) pseudovirus.
 (B) Spearman correlation of lgG IC₅₀ against SARS-CoV-2-S and SARS-CoV-1-S pseudovirus.
 (C) Spearman correlation of lgG IC₅₀ against SARS-CoV-2-S pseudovirus and SARS-2 authentic virus.
 (D) Schematic of the SARS-CoV-2 spike domains and the mutations present in global strains of SARS-CoV-2 generated and used in this study. NTD: N-terminal domain; RBD: receptor binding domain; FP: fusion peptide; HR: heptad repeat; TM: trans-membrane.
 (E) Heatmap visualizing the IC₅₀ values of 15 elite neutralizers against the SARS-CoV-2 global spike variants.
 (F) Dot plots depicting lgG neutralizing activity of 15 individuals from each neutralization category: elite, high, average, low, and non-neutralizers (total n = 75) against SARS-2-S variants Wu01, B.1.1.7, and B.1.351.

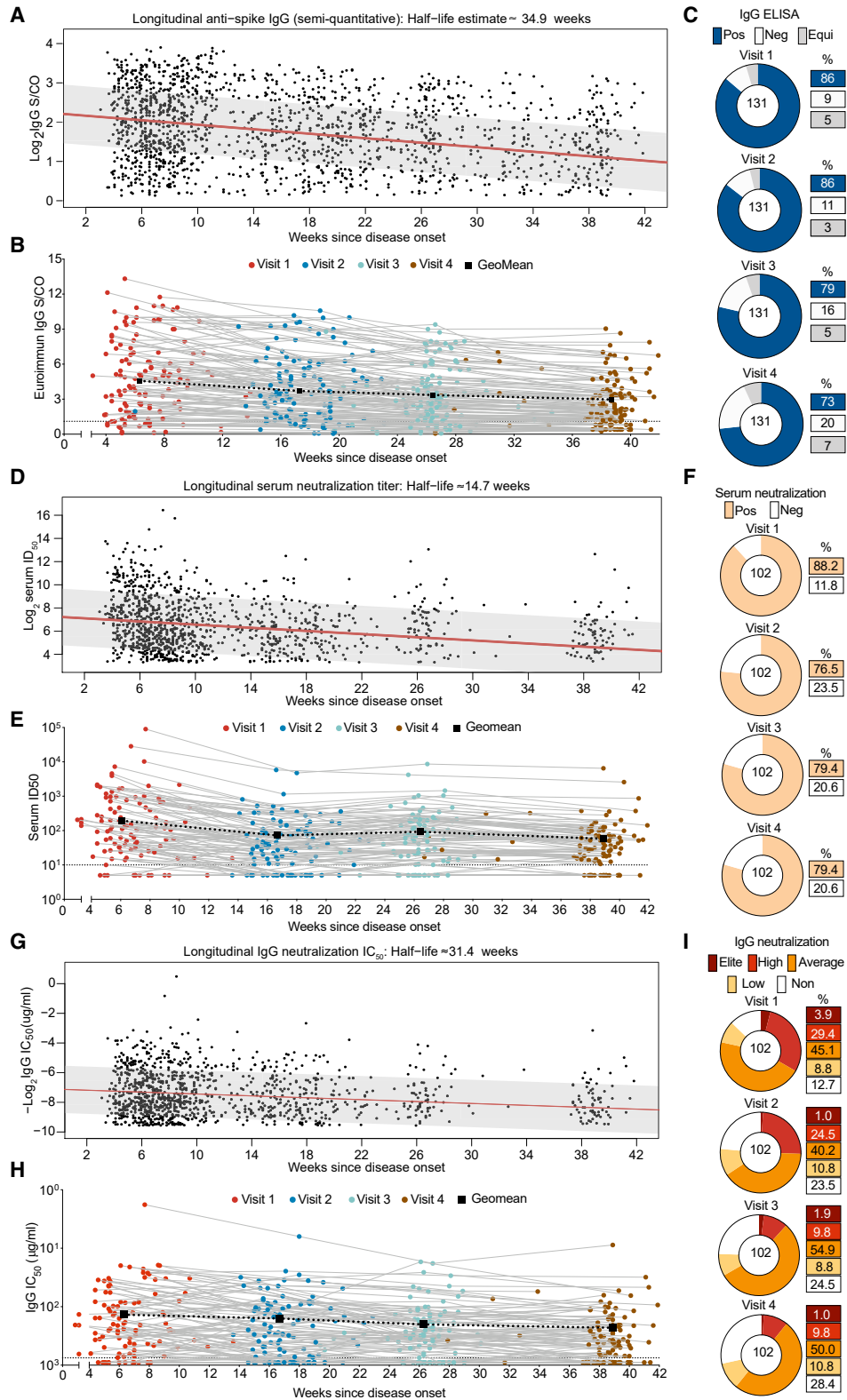


Figure 5. Longitudinal kinetics of anti-SARS-CoV-2 antibody titers

(A, D, and G) Semiquantitative IgG ELISA ratios (n = 1,669) (A), serum ID₅₀ titers (n = 1,196) (D), and IgG IC₅₀ values (n = 1,167) (G) plotted against weeks since infection for half-life estimate using a linear mixed-effects model.

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S/CO = 3.7), followed by relatively constant IgG levels for 10 months after infection (GeoMean S/CO = 3.0; [Figure 5B](#); [Table S1](#)). While the detection of S1 reactivity stays equal at the first and second visits (86%), the fraction of individuals that are positive for S1-reactive antibodies decays to 79% (7% drop from visit 1) at the third visit and to 73% (13% drop from visit 1) at visit 4 (9–10 months post-disease onset).

NAb activity was longitudinally monitored for 102 individuals from visit 1 (median 6.4 weeks post-infection) up to visit 4 (median 37.9 weeks post-infection; [Figures 5D–5H](#)). Regression modeling showed that serum neutralizing titers had a short half-life of 14.7 weeks ([Figure 5D](#)) compared to a much longer 31.4-week half-life for IgG NAb levels ([Figure 5G](#)). Out of 102 individuals, 88.2% had serum neutralizing activity at visit 1, whereas only 79.4% had NAb activity remaining at visit 4 ([Figure 5F](#)). The overall fraction of IgG neutralizers changed from 87.3% to 71.6% between visits 1 and 4. The most dramatic change from GeoMean IC₅₀ of 40.14 to 166.6 μg/mL was detected in elite/high neutralizers from visits 1 to 4, 76% of whom lost their elite or high status. 35% of average/low neutralizers at visit 1 became negative at visit 2 ([Figure 5I](#)). Approximately 9% of these individuals did not develop any NAb and remained serum and IgG neutralization negative throughout the observation period. Overall, only 3% of individuals lost detectable antibody responses against SARS-CoV-2 between 1.5 and 10 months post-infection ([Figures S4D and S4E](#)). Among the SARS-CoV-1 cross neutralizers, there was an almost 4-fold drop in IgG neutralizing activity against SARS-CoV-2 between 7.4 and 19.4 weeks post-disease onset. However, reactivity to SARS-CoV-1 did not change in most individuals ([Figure S4F](#)). Finally, we also performed regression modeling to estimate half-life based on age, gender, pre-conditions, and disease severity ([Figure S5](#)). We found that females had more stable antibody levels than males and that younger individuals had more sustained antibody levels over time. The presence of pre-conditions did not significantly affect the decay rate of antibodies. Finally, hospitalized individuals with a more potent initial response exhibited a more rapid decay of anti-S antibodies than asymptomatic individuals or those with mild symptoms ([Figures S5A–S5D](#)).

In summary, these results show that in most individuals, anti-S IgG antibody levels are maintained for 10 months with a half-life estimate of 8.7 months. Moreover, even though there is a rapid decline in serum NAb activity, IgG NAb function remains relatively constant with an estimated half-life of 7.9 months. We conclude that although there is a decay of antibody titers in serum, the humoral IgG response persists for as long as 10 months after SARS-CoV-2 infection.

DISCUSSION

In order to end the COVID-19 pandemic, widespread SARS-CoV-2 protective immunity will be required. Antibodies are critical for effective clearance of pathogens and for prevention of viral infections ([Murin et al., 2019](#)). In this study, we examined the neutralizing antibody response in 963 individuals who had recently recovered from SARS-CoV-2 infection. The cohort consisted primarily (91.7%) of patients with mild COVID-19, therefore representing the predominant clinical course of this disease ([Williamson et al., 2020](#)). Since higher disease severity was shown to correlate with higher antibody responses ([Garcia-Beltran et al., 2021](#); [Piccoli et al., 2020](#)), cohorts mainly composed of hospitalized individuals have limited applicability on the majority of COVID-19 cases ([Röltgen et al., 2020](#); [Seow et al., 2020](#); [Suthar et al., 2020](#); [Zeng et al., 2020](#)). Moreover, to our knowledge, this is the most comprehensive study (n = 963) in which neutralizing antibody activity has been reported to date.

Upon recovery from COVID-19, we detected the development of a broad spectrum of IgG neutralizing activity ranging from no neutralization (threshold IC₅₀ < 750 μg/mL, 21%) to low (IC₅₀ = 50–750 μg/mL, 10%), average (IC₅₀ = 100–500 μg/mL, 44.8%), high (IC₅₀ = 20–100 μg/mL, 20.9%), and elite SARS-CoV-2 neutralization (IC₅₀ < 20 μg/mL, 3.3%). 94.4% of individuals were found to possess S- or N-reactive antibodies or neutralizing activity at the serum or IgG level. Thus, while most individuals develop a detectable antibody response upon natural infection, the extent of SARS-CoV-2 neutralizing activity is highly variable, with the fraction of non-responders being highest for asymptomatic individuals (23%).

The broad spectrum of neutralizing activity observed in COVID-19-recovered individuals may impact the level of protective immunity. For instance, asymptomatic infection is estimated to account for up to 40% of all infections ([Oran and Topol, 2020](#)). In these individuals and in other patients with weak antibody responses, lower IgG titers may contribute to a higher susceptibility to re-infection ([Lumley et al., 2021](#)). Recently, mutated virus strains were reported ([Andreano et al., 2020](#); [Tegally et al., 2020](#)), some of which possess mutations causing partial resistance to convalescent plasma ([Tegally et al., 2020](#)) or SARS-CoV-2 monoclonal antibodies ([Thomson et al., 2020](#)). A weak antibody response could make these individuals carriers and help propagate escape variants, thereby complicating effective measures to combat the COVID-19 pandemic.

To aid prevention strategies, it is critical to understand the development of antibody responses upon natural infection. The NAb response presented here is comparable to recent S-based mRNA vaccine studies in age group 18–55, where geometric mean neutralizing titers were in the range of 100–300 ID₅₀ (depending on dose) 1.5 months post-vaccination

(B) Longitudinal mapping of IgG levels in 131 individuals from visits 1–4. Dot plots illustrate antibody titer against the weeks since infection to study visit 1 (red), study visit 2 (blue), study visit 3 (turquoise), and study visit 4 (brown). Geometric mean change shown in black. Dotted lines represent limit of detection (S/CO = 1.1 for IgG ELISA).

(C) Pie charts illustrate the change in the fraction of IgG ELISA positive (Pos), negative (Neg), and equivocal (Equi) samples (n = 131) between the study visits. (E and H) Longitudinal mapping of serum neutralization (E) and IgG neutralization (H) in 102 individuals at study visits 1–4. Serum and IgG non-neutralizers were assigned values of ID₅₀ = 5 and IC₅₀ = 900 for plotting. Dotted lines represent limit of detection (ID₅₀ of 10 and IC₅₀ of 750 μg/mL for serum and IgG neutralization assays).

(F and I) Pie charts illustrate the change in the fraction of serum neutralizers (F) and IgG neutralizers (I) in the samples (n = 102) between the study visits.

(Jackson et al., 2020; Walsh et al., 2020) versus 111.3 ID₅₀ in this study. Recent studies have reported that age, gender, and disease severity^{14,36,44} can impact SARS-CoV-2 NAb titers (Chen et al., 2020b; Garcia-Beltran et al., 2021; Luchsinger et al., 2020; Piccoli et al., 2020; Röttgen et al., 2020). However, a comprehensive analysis on a large cohort was missing. Using multivariate statistical analysis on the clinical data, we found that older age, symptomatic infection, and a severe course of COVID-19 were good predictors of higher NAb titers. Based on our data on natural infection, the >60 age group had the highest level of neutralizing IgG antibodies (Geomean IC₅₀ = 84.8 μg/mL, Geomean ID₅₀ serum titer = 276.6). This is notable in comparison to vaccine studies where older individuals mount a less potent immune response to SARS-CoV-2 vaccination (Walsh et al., 2020).

In some individuals, we detected very high levels of SARS-CoV-2 neutralizing activity (IC₅₀ < 20 μg/mL, ID₅₀ serum titer > 2,500) ranking them as elite neutralizers. While cross-reactivity against SARS-CoV-1 and other Beta-CoVs has been shown for some SARS-CoV-2-recovered individuals (Lv et al., 2020; Prévost et al., 2020; Rogers et al., 2020), we found that all elite neutralizers have readily detectable cross-reactive IgG NABs against SARS-CoV-1. This suggests that a potent antibody response against SARS-CoV-2 is accompanied by diversification toward more broadly reactive antibodies. Moreover, IgG from elite neutralizers could efficiently block infection of various SARS-CoV-2 strains. Of these, BavP1 and DRC94, containing the D614G mutation in the S protein (Korber et al., 2020), and the B1.1.7 variant are associated with higher infectivity (Davies et al., 2021; Weissman et al., 2020). Given the eminent risk of novel emerging CoVs and monoclonal antibody-resistant SARS-CoV-2 variants, developing antibodies with greater neutralization breadth will be critical. Further evaluation of the antibody response in such elite neutralizers at the single B cell level will be required to understand the details of such potent NAb responses and could yield the identification of new highly potent cross-reactive monoclonal antibodies.

Effective neutralization and clearance of pathogens is mainly mediated by IgG antibodies, which are typically formed within 1–3 weeks post-infection and often provide long-term immunity that can last decades (Amanna et al., 2007). Protective immunity to seasonal coronaviruses like NL63, 229E, OC43, and HKU1 is known to be short lived, and re-infection is common (Etridge et al., 2020). In addition, the antibody response to SARS-CoV-1 and Middle East respiratory syndrome (MERS)-CoV was shown to wane over time (Huang et al., 2020a). Upon SARS-CoV-1 infection, serum IgG and NABs were shown to decline 3 years after infection (Cao et al., 2007). This suggests that immunity to CoVs is rather short lived compared to some other viruses such as measles virus, for which life-long antibody immunity is observed (Amanna et al., 2007). In our study, we not only measured serum neutralization, but also quantified SARS-CoV-2 IgG neutralizing activity. While serum neutralization waned quickly (half-life of 3.6 months), levels of purified IgG rather persisted with a longer half-life of 7.8 months. The sharp drop in serum neutralization could be partially attributed to a decline in anti-S IgA and IgM titers (Iyer et al., 2020), which, along with IgG, cumulatively contribute to serum NAB activity (Klingler et al., 2020; Wang et al., 2021). Finally, SARS-CoV-2 S-based mRNA vaccines (Sahin et al., 2020) were shown to induce NAB titers in different age groups for a time

span of at least 4.25 months (Widge et al., 2020). In this study, we found that although SARS-CoV-2-neutralizing IgG levels decline by 16% within the first 4 months after infection, anti-S neutralizing IgG can be persistently detected in the majority of COVID-19 cases for up to 10 months post-infection.

In summary, the data presented in this study provide insight into the features that shape the SARS-CoV-2 NAB response in COVID-19-recovered individuals. Longitudinal mapping of antibody responses reveals a relatively long-lived IgG antibody response lasting at least 10 months after SARS-CoV-2 infection in the majority of individuals. Since many SARS-CoV-2 vaccines are S protein based (Krammer, 2020), studying antibody dynamics against S informs us on longevity of natural immunity and may help to inform on vaccination strategies and outcomes in the population.

STAR★METHODS

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at <https://doi.org/10.1016/j.chom.2021.04.015>.

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AUTHOR CONTRIBUTIONS

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The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
HbnC3t1p1_C6	Kreer et al., 2020	N/A
Bacterial and virus strains		
SARS-CoV-2 CoV2-P3 authentic virus	This paper	N/A
SARS-CoV-2 Wu01 Pseudovirus	Crawford et al., 2020	N/A
SARS-CoV-2 BAVP1 Pseudovirus	This paper	N/A
SARS-CoV-2 ARA36 Pseudovirus	This paper	N/A
SARS-CoV-2 DRC94 Pseudovirus	This paper	N/A
SARS-CoV-2 CA5 Pseudovirus	This paper	N/A
SARS-CoV-2 NRW8 Pseudovirus	This paper	N/A
SARS-CoV-2 B.1.1.7 Pseudovirus	This paper	N/A
SARS-CoV-2 B.1.351 Pseudovirus	This paper	N/A
Biological samples		
Plasma/serum from convalescent individuals	This paper	N/A
Chemicals, peptides, and recombinant proteins		
FuGENE® 6 Transfection Reagent	Promega	Catalog# E2691
Adenosine 5'-triphosphate disodium salt hydrate ATP	Sigma-Aldrich	Catalog# A2383-10G
Coenzyme A sodium salt hydrate, cofactor for acyl transfer	Sigma-Aldrich	Catalog# C3144-500MG
Igepal® CA-630 for molecular biology	Sigma-Aldrich	Catalog# I8896-100ML
D-Luciferin, Sodium Salt	ZellBio	Catalog# LUCNA-1G
Protein G Sepharose® 4 Fast Flow	Sigma-Aldrich	Catalog# GE17-0618-05
Critical commercial assays		
Anti-SARS-CoV-2-ELISA IgG (spike S1 antigen)	Euroimmun	Catalog# EI2606-9601 G
Anti-SARS-CoV-2-ELISA IgA (spike S1 antigen)	Euroimmun	Catalog# EI2606-9601 A
LIAISON® SARS-CoV-2 ELISA (spike S1/S2 antigen)	Diasorin	Catalog# 311450
Elecsys®-Assay pan IgG (nucleocapsid antigen)	Roche	Catalog# 09 203 079 190
Alinity I (nucleocapsid antigen)	Abbott	Catalog# 6R9022
Q5® Site-Directed Mutagenesis Kit	NEB	Catalog# E0554
Experimental models: Cell lines		
HEK293T-ACE2 cells	Jesse Bloom lab; Crawford et al., 2020	BEI Resources Catalog# NR-52511
VeroE6 cells	ATCC	Catalog# CRL-1586
HEK293T	ATCC	Catalog# CRL-11268
Oligonucleotides		
Primers for generating spike variants, see Table S2	This paper	N/A
Recombinant DNA		
pHDM-IDTSpike-fix (expressing Wu01 spike)	Jesse Bloom lab; Crawford et al., 2020	N/A
pHDM-tat1b	Jesse Bloom lab; Crawford et al., 2020	RRID:Addgene_164442
pHDM-Hgpm2	Jesse Bloom lab; Crawford et al., 2020	RRID:Addgene_164441
pRC-CMV-Rev1b	Jesse Bloom lab; Crawford et al., 2020	RRID:Addgene_164443
pHAGE-CMV-Luc2-IRES-ZsGreen-W	Jesse Bloom lab; Crawford et al., 2020	RRID:Addgene_164432
SARS-CoV-2 Wu01 codon optimized spike	Stefan Pöhlmann lab; Hoffmann et al., 2020	N/A

(Continued on next page)

Continued		
REAGENT or RESOURCE	SOURCE	IDENTIFIER
Wu01 spike sequence source	State Key Laboratory of Virology, Wuhan University	EPI_ISL_406716
BAVP1 spike sequence source	Charité Universitätsmedizin Berlin, Institute of Virology	EPI_ISL_406862
ARA36 spike sequence source	Institut des Agents Infectieux (IAI), Hospices Civils de Lyon	EPI_ISL_418432
DRC94 spike sequence source	Viral Respiratory Lab, National Institute for Biomedical Research (INRB)	EPI_ISL_417947
CA5 spike sequence source	California Department of Health	EPI_ISL_408010
NRW8 spike sequence source	Center of Medical Microbiology, Virology, and Hospital Hygiene, University of Duesseldorf	EPI_ISL_414508
B.1.1.7 spike sequence source	Chand et al., 2020	N/A
B.1.351 spike sequence source	Tegally et al., 2020	N/A
pCDNA TM 3.1/V5-HisTOPO-SARS-CoV-2 Wu01 spike	This paper	N/A
pCDNA TM 3.1/V5-HisTOPO-SARS-CoV-2 expressing spike variants	This paper	N/A
Software and algorithms		
GraphPad Prism, Version 7 and 9	GraphPad Software, Inc	https://www.graphpad.com
Geneious R10v10.0.9	Biomatters	https://www.geneious.com
Illustrator® CC 2018	Adobe	https://www.adobe.com
BertholdTech TriStar2S ICE, Version 1.0.9.5	Berthold Technologies	https://www.berthold.com/en

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Florian Klein (florian.klein@uk-koeln.de).

Materials availability

SARS-CoV-2 spike variants and authentic virus used in this study will be made available by the lead contact with a Material Transfer Agreement.

Data and code availability

All data are provided in the manuscript or supplementary material.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Enrollment of human subjects and study design

Blood samples were collected from donors who gave their written consent under the protocols 20-1187 and 16-054, approved by the Institutional Review Board (IRB) of the University Hospital Cologne. All samples were handled according to the safety guidelines of the University Hospital Cologne. Individuals that met the inclusion criteria of i.) 18 years old and older, and ii.) history of SARS-CoV-2 positive polymerase chain reaction (PCR) from nasopharyngeal swab or collected sputum were enrolled in this study. Demographical data, COVID-19-related pre-existing conditions, and information on the clinical course were collected at study visit 1. The final cohort comprised 963 individuals with 46.1% males and 53.9% females. Blood samples were collected starting from study visit 1, for up to 4 follow up visits between the 6th of April and 17th of December 2020.

METHOD DETAILS

Processing of serum, plasma and whole blood samples

Blood samples were collected in Heparin syringes or EDTA monovette tubes (Becton Dickinson) and fractionated into plasma and peripheral blood mononuclear cell (PBMC) by density gradient centrifugation using Histopaque-1077 (Sigma). Plasma aliquots were stored at -80°C till use. Serum was collected from Serum-gel tubes (Sarstedt) by centrifugation and stored at -80°C till use.

Isolation of IgGs from serum and plasma samples

For the isolation of total IgG, 0.5–1 mL plasma or serum was heat inactivated at 56°C for 45 min and incubated overnight with Protein G Sepharose 4 Fast Flow beads (GE Healthcare) at 4°C. Next day, beads were washed on chromatography columns (BioRad) and Protein G bound IgG was eluted using 0.1M Glycine pH = 3 and instantly buffered in 1M Tris pH = 8. Buffer exchange to PBS (GIBCO) was performed using 30 kDa Amicon Ultra-15 columns (Millipore) and the purified IgG was stored at 4°C.

Cell lines

VeroE6 cells, HEK293T cells and 293T-ACE2 cells were maintained in DMEM (Gibco) containing 10% FBS, 1% Penicillin-Streptomycin, 1mM L-Glutamine and 1mM Sodium pyruvate. Cells were grown on tissue culture treated dishes in a T75 flask (Sarstedt) at 37°C and 5% CO₂. For passaging cells, the medium was removed, PBS (5 mL) was used to wash the cells and incubated at 37°C, 5% CO₂ for 1–2 min with trypsin (1–2 mL). The sample was diluted with medium (10 mL) and centrifuged at 400 g, 20°C for 4 min. The pellet was resuspended in an appropriate volume of medium and the culture continued.

Cloning of SARS-CoV-2 spike variants

The codon optimized SARS-CoV-2 Wu01 spike (Hoffmann et al., 2020) (EPI_ISL_406716) was cloned into pCDNATM3.1/V5-HisTOPO vector (Invitrogen). SARS-2-S global strains (BavP1 EPI_ISL_406862; ARA36 EPI_ISL_418432; DRC94 EPI_ISL_417947; CA5 EPI_ISL_408010; NRW8 EPI_ISL_414508) (Elbe and Buckland-Merrett, 2017) were generated by introducing the corresponding amino acid mutations using the Q5® Site-Directed Mutagenesis Kit (NEB) and per manufacturer's protocol. SARS-2-S variants B.1.1.7 (Chand et al., 2020) and B.1.351 (Tegally et al., 2020) were generated by introducing the corresponding amino acid mutations in the Wu01 spike via PCR in the pCDNATM3.1/V5-HisTOPO backbone.

Production of SARS-CoV pseudovirus particles

Pseudovirus particles were generated by co-transfection of individual plasmids encoding HIV-1 Tat, HIV-1 Gag/Pol, HIV-1 Rev, luciferase followed by an IRES and ZsGreen, and the SARS-CoV-2 spike protein as previously described (Crawford et al., 2020). In brief, HEK293T cells were transfected with the pseudovirus encoding plasmids using FuGENE 6 Transfection Reagent (Promega). The virus culture supernatant was harvested at 48 h and 72 h post transfection and stored at –80°C until use. Each virus batch was titrated by infecting 293T-ACE2 and after a 48 h incubation period at 37°C and 5% CO₂, luciferase activity was determined after addition of luciferin/lysis buffer (10 mM MgCl₂, 0.3 mM ATP, 0.5 mM Coenzyme A, 17 mM IGEFAL (all Sigma-Aldrich), and 1 mM D-Luciferin (GoldBio) in Tris-HCL) using a microplate reader (Berthold). An RLU of approximately 1000-fold in infected cells versus non-infected cells was used for neutralization assays.

Pseudovirus assay to determine IgG/plasma/serum SARS-CoV-2 neutralizing activity

For testing SARS-CoV-2 neutralizing activity of IgG or serum/plasma samples, serial dilutions of IgG or serum/plasma (heat inactivated at 56°C for 45 min) were co-incubated with pseudovirus supernatants for 1 h at 37°C prior to addition of 293T cells engineered to express ACE2 (Crawford et al., 2020). Following a 48 h incubation at 37°C and 5% CO₂, luciferase activity was determined using the reagents described above. After subtracting background relative luminescence units (RLUs) of non-infected cells, 50% inhibitory concentrations (IC₅₀s) were determined as the IgG concentrations resulting in a 50% RLU reduction compared to untreated virus control wells. 50% Inhibitory dose (ID₅₀) was determined as the serum dilution resulting in a 50% reduction in RLU compared to the untreated virus control wells. Each IgG and serum sample were measured in two independent experiments on different days and the average IC₅₀ or ID₅₀ values have been reported. For each run, a SARS-CoV-2 neutralizing monoclonal antibody was used as control to ensure consistent reproducibility in experiments carried out on different days. Assay specificity calculated using pre-COVID-19 samples was found to be 100%. IC₅₀ and ID₅₀ values were calculated in GraphPad Prism 7.0 by plotting a dose response curve.

SARS-CoV-2 live virus isolation from nasopharyngeal swabs

For outgrowth cultures of authentic SARS-CoV-2 from nasopharyngeal swabs, 1x10⁶ VeroE6 cells were seeded onto a T25 flask (Sarstedt) on the previous day DMEM (GIBCO) containing 10% FBS, 1% PS, 1mM L-Glutamine and 1mM Sodium pyruvate. 0.2 mL swab in virus transport medium was diluted with 0.8 mL DMEM (GIBCO) containing 2% FBS, 1% PS, 1mM L-Glutamine and 1mM Sodium pyruvate. The swab dilution was added to VeroE6 cells and left for 1 h at 37°C, 5% CO₂ after which an additional 3 mL medium was added. The cultures were examined for the next days for cytopathic effects (CPE) and samples were sent for viral load analysis to track growth of virus by E-gene qPCR. Cell culture supernatant was harvested from positive cultures and stored at –150°C until use. Virus was titrated by adding serial dilutions of virus supernatant (8 replicates) on VeroE6 cells in DMEM (GIBCO) containing 2% FBS, 1% Penicillin-Streptomycin, 1mM L-Glutamine and 1mM Sodium pyruvate. After 4 days of incubation at 37°C, 5% CO₂, the presence or absence of CPE was noted in using a brightfield microscope. TCID₅₀ was calculated using the Spearman and Kaerber algorithm (Kärber, 1931; Spearman, 1908).

SARS-CoV-2 live virus neutralization assay

Live SARS-CoV-2 (termed CoV2-P3) was grown out from a swab in Cologne using VeroE6 cells as described above and then expanded in culture by superinfection of VeroE6 from the initial outgrowth culture. Whole genome sequencing of the isolated virus

was done isolating viral RNA using the QIAamp MinElute Virus Spine kit (QIAGEN) and performing Illumina sequencing. The virus spike amino acid sequence is identical to the Wu01 spike (EPI_ISL_406716) with the exception that it contains the D614G mutation. For the neutralization assay, dilutions of IgG were co-incubated with the virus (1000-2000 TCID₅₀) for 1 h at 37°C prior to addition of VeroE6 cells in DMEM (GIBCO) containing 2% FBS, 1% PS, 1mM L-Glutamine and 1mM Sodium pyruvate. After 4 days of incubation at 37°C, 5% CO₂, neutralization was analyzed by observing cytopathic effects (CPE) using a brightfield microscope and the highest dilution well with no CPE was noted to be the IC₁₀₀ for the antibody. Assay specificity calculated using pre-COVID-19 samples was found to be 100%. All samples were measured in two independent experiments on separate days and the average IC₁₀₀ from all measurements has been reported.

Detection of anti-SARS-CoV-2 spike IgG and IgA by ELISA

For assessing IgA and IgG antibody titers, the Euroimmun anti-SARS-CoV-2 ELISA using the S1 domain of the spike protein as antigen was used (Euroimmun Diagnostik, Lübeck, Germany). Serum or plasma samples were tested on the automated system Euroimmun Analyzer I according to manufacturer's recommendations. Signal-to-cut-off (S/CO) ratio was calculated as extinction value of patient sample/extinction value of calibrator. IgA and IgG S/CO values were interpreted as positive S/CO ≥ 1.1 , equivocal S/CO ≥ 0.8 - < 1.1 , and negative S/CO < 0.8 . Additional commercial kits used for antibody measurements were also used as per manufacturer's recommendations; Anti-S1/S2 IgG was measured using DiaSorin's LIAISON® SARS-CoV-2 ELISA kit with the following cut-off values: negative < 12.0 AU/mL, equivocal ≥ 12.0 - < 15.0 AU/mL and positive ≥ 15.0 AU/mL. Anti-N Pan-IgG were measured using Roche's Elecsys®-Assay with cut-off values: non-reactive $< 1,0$ COI and reactive $\geq 1,0$ COI. Anti-N IgG were measured with Abbott's Alinity i system with cut-off values: positive S/CO ≥ 1.4 and negative S/CO < 1.4 . Assay specificities calculated using pre-COVID-19 samples: Euroimmun IgG 100%; Euroimmun IgA 96%; Roche 98%; Diasorin 98%; Abbott 98%.

Measurement of SARS-CoV-2 RNA levels from nasopharyngeal swabs

Cycle threshold values for quantifying viral load in naso/oro-pharyngeal swabs was done by qPCR using LightMix® SarbecoV E-gene (Corman et al., 2020) plus EAV control (TIB Molbiol, Berlin, Germany) in combination with the N-gene (inhouse primer sets in multiplex PCR) on LightCycler® 480 (Roche Diagnostics).

QUANTIFICATION AND STATISTICAL ANALYSIS

Statistical modeling

To select features that are predictive for the log₁₀ response in a multivariate analysis (Figures 3 and S3), forward stepwise regression was applied, using the p value from a likelihood ratio test (R function `lmtest::lrtest`) as selection criterion in each step. The final multiple linear regression model (Figures 3 and S3) includes only features that show a significant model improvement ($\alpha = 0.05$) in the feature selection phase. To study the interplay of the different features regarding their relationship with the response (Figure 3D), a Bayesian network was learned by maximizing the BIC score for hybrid networks via hill-climbing (R function `bnlearn::hc`) (Scutari, 2010). To enforce it to be a sink in the network, all outgoing edges from the response variable were blacklisted prior to learning. For the longitudinal analyses (Figure 5), linear mixed effect models (R-function `nlme::lme`) were applied to all data points from both visits, where each patient has its own intercept. Since a binary transformation of the response was used, half-life estimates were computed as negative inverse of the common slope regression coefficient. Prediction intervals were computed using R-function `ggeffects::ggpredict` (Lüdtke, 2018).