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Supplementary appendix

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Plasma-based antigen persistence in the post-acute phase of COVID-19

Peluso MJ, Swank ZN, Goldberg SA, et al.

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Table S1. Prior studies of SARS-CoV-2 persistence in tissue and blood in the post-acute phase of infection.

First Author	N Infected	N Uninfected	Days post infection	Anatomic Site
Tissue				
Appleman[1]	46	Not specified*	93-665†	Muscle
Cheung[2]	5	Not specified*	9-180	Appendiceal, ileal, colonic, hepatic, gallbladder, lymph node
deMelo[3]	4	0	110-196	Olfactory
Gaebler[4]	14	10	84-166	Duodenal, ileal
Goh[5]	2	0	163-426	Appendiceal, skin, breast tissue
Hany[6]	80	0	274-380	Gastric, gallbladder
Miura[7]	Unknown‡	Unknown‡	Unknown‡	Tonsillar
Peluso[8]	5	1*	158-676	Rectosigmoid
Rendiero[9]	12	7	Not specified-359	Lung (autopsy)
Roden[10]	43	Not specified	28-252	Lung (autopsy)
Stein[11]	44	60§	31-230	Multiple (autopsy)
Xu[12]	24	86	25-303	Tonsillar
Yao[13]	16	107	42-441	Tongue papillae
Zollner[14]	46	4	94-257	Duodenal, ileal, colonic
Blood				
Craddock[15]	47	15	28-500	Blood plasma
Kanberg[16]	31	17	104-268†	Blood plasma
Menezes[17]	60	0	440-1005†	Whole blood
Peluso[18]	46	12	35-84	Blood plasma
Rodriguez[19]	150	Not specified¶	1-900 (approx.)	Blood plasma
Schultheiß[20]	39	2	28-517	Blood plasma
Swank[21]	63	0	28-365	Blood plasma
Tejerina[22]	29	0	39-67†	Blood plasma

*Number of uninfected participants not stated but at least one "representative image" provided. †Interquartile range reported rather than absolute range. ‡Recruitment was not based on known infection status; 48 total children who were not known to have prior SARS-CoV-2 infection were included. §Reference is made to tissues rather than individual participants. ¶Uninfected participants included in some analysis but not specified whether these were included in measures of SARS-CoV-2 persistence.

Supplemental Methods

Overall Design

In cross-sectional analyses, we compared participants in the post-acute phase of SARS-CoV-2 infection to persons studied prior to the COVID-19 pandemic for the presence of three SARS-CoV-2 antigens in plasma. Among persons in the post-acute phase of SARS-CoV-2 infection, we also evaluated several sociodemographic characteristics and clinical factors related to acute COVID-19 for their influence on SARS-CoV-2 antigen detection in the post-acute phase.

Participants

We studied two groups of participants. The first (hereafter known as pandemic-era) were participants in the University of California, San-Francisco (UCSF)-based Long-term Impact of Infection with Novel Coronavirus (LIINC) study (NCT04362150). Selection of participants has been described previously.[23] Briefly, using facility- and community-based advertising (through the internet and word-of-mouth), we enrolled (beginning in April 2020) consecutive adult volunteers who had earlier experienced their first episode of acute SARS-CoV-2 infection (confirmed by detection of SARS-CoV-2 RNA or antigen) and who were at least two weeks removed from their onset of symptoms. These volunteers were responding to advertising that described the study's interest in a variety of long-term biochemical and clinical outcomes of COVID-19. Participants were examined at an initial study visit and every four months thereafter. For the present analysis, we sampled participants who had the greatest number of completed study visits (with stored plasma specimens) in the first 1.25 years following COVID-19 onset. The second group of participants (hereafter known as pre-pandemic-era) were from the UCSF-based Study of the Consequences of the Protease Inhibitor Era (SCOPE), a cohort study begun in 2001 originally focused on pathogenesis of HIV infection. It contains participants at variety of stages of HIV infection as well as ambulatory HIV-uninfected comparators, all of whom were volunteers from the community. For the present analysis, we randomly selected, among SCOPE participants with stored plasma specimens prior to December 2019, four HIV-uninfected participants to every one HIV-infected participant, attempting to match the age and race/ethnicity distribution of the pandemic-era group. All participants provided written informed consent.

Measurements

Questionnaire-based. In both groups, interviewer-administered questionnaires collected data on sociodemographic and economic characteristics. In the pandemic-era group, we also inquired about details concerning the acute phase (first 3 weeks) of SARS-CoV-2 infection, including symptoms experienced, self-reported worst perception of overall health on a 0 to 100 scale, and whether hospitalization for COVID-19 occurred. The pandemic-era group also had all SARS-CoV-2 vaccinations recorded as well as any additional SARS-Co-V-2 re-infections since the initial infection. Additional details of our approach to measurement in this study have been reported previously.[23]

Laboratory-based. Peripheral blood was collected in EDTA-coated tubes and plasma stored at -80° C using similar procedures in both the pre-pandemic era and pandemic-era groups. Using once-thawed plasma, we employed the Simoa® (Quanterix) single molecule array detection platform to measure SARS-CoV-2 antigens from spike, S1, and nucleocapsid (N) proteins; detailed methods have been described elsewhere.[21,24] Briefly, plasma samples were centrifuged at 2000 x g for 10 minutes at 4° C and treated with 5 mM dithiothreitol (Pierce™ No-Weigh™ Format, Thermo Fisher Scientific) and protease inhibitors (Halt™ Protease Inhibitor Cocktail, Thermo Fisher Scientific) for 15 minutes at 37° C. Each plasma sample was diluted 8-fold in a 96-well plate with Sample Diluent Buffer (Quanterix) and analyzed automatically with a three-step format on a HD-X Analyzer (Quanterix). In the first step, the plasma samples are incubated with antibody-coated magnetic beads. Assays for S1, spike, and N were performed separately, using antibodies against S1 (40150-D006, Sino Biological), S2 (MA5-35946, Invitrogen), and N (40143-R004, Sino Biological) conjugated to carboxylated magnetic beads (Quanterix). In the second step, the beads are resuspended in a solution of biotinylated detector antibodies. The same detector antibody against S1 is used for the S1 and spike assays (LT-1900, Leinco) and another antibody against N is used for the N assay (40143-R040, Sino Biological). In the third step, the beads are incubated in a solution of streptavidin conjugated β-galactosidase and lastly resuspended in a solution of resorufin β-D-galactopyranoside and loaded into a microwell array. The array is then sealed with oil and imaged. Average enzyme per bead (AEB) values are calculated by the HD-X Analyzer software thereafter and converted to concentration values based on a calibration curve fit with a four-parameter logistic regression. Separately, the limit of detection (LOD) is calculated as the background AEB plus three times the

standard deviation and converted to a concentration. The LOD was determined to be 14.47 pg/mL for the spike assay, 11.16 pg/mL for S1, and 4.55 pg/mL for N.

Statistical analysis

When comparing antigen prevalence in the pandemic-era group to the pre-pandemic era group, we defined three time periods for the pandemic-era group: 3.0-6.0 months, 6.1-10.0 months, and 10.1-14.1 months post-onset of COVID-19 symptoms. If there was more than one time point per person in a given time period, we chose the timepoint closest to the period's midpoint. In each plasma specimen tested, antigen detection was defined in four ways: a) presence or absence on each of three individual antigen assays; and b) presence of at least one of the three antigens (vs absence on all three). Comparison between groups were expressed with prevalence ratios and differences. All analyses were performed using Stata version 17.0 (StataCorp, College Station, Texas).

Supplemental Results

Study participants

We studied 171 pandemic-era participants, who contributed 660 plasma specimens obtained between 0.9 and 14.1 months following initial SARS-CoV-2 symptom onset, and 250 pre-pandemic-era participants who each contributed one plasma specimen between 2003 and 2019 (Table S1). The groups were similar in age, but the pandemic-era group had more women, more Latino and White participants, and higher measures of socioeconomic status. Both groups originated from underlying research studies that were deliberatively enriched for people with HIV (PWH), and, as a result, the prevalence of HIV infection was similar in the two groups but much higher than the general population. All but four participants in pandemic-era group developed COVID-19 prior to SARS-CoV-2 vaccination and prior to the Omicron era. Of the 660 pandemic-era plasma specimens included, 93% were collected prior to July 1, 2021.

SARS-CoV-2 antigen detection in pandemic era compared to pre-pandemic era

Positivity in at least one SARS-CoV-2 antigen assay was present in the plasma of 5 pre-pandemic participants (2%; 95% confidence interval (CI): 0.65% to 4.6%). Given the definitional absence of target in these specimens, these 5 instances are considered false positive, thus establishing any-antigen assay specificity to be 98% (95% CI: 95% to 99%). For the individual antigens, spike was detected in 3 (1.2%) participants, S1 in 3 (1.2%), and N in 2 (0.80%). The 5 participants were a 23-year-old White woman (S1 alone [34.67 pg/mL]), 52-year-old Asian man (spike alone [83.46 pg/mL]), 49-year-old White man (spike alone [609.96 pg/mL]), 36-year-old Asian man (S1 [285.31 pg/mL] and N [649.28 pg/mL]), and 44-year-old White woman (spike [646.02 pg/mL], S1 [115.89 pg/mL], and N [5716.32 pg/mL]). Of the 5, 2 (40%) were HIV-seropositive.

Of the 660 pandemic-era plasma specimens tested, 61 (9.2%) representing 42 unique participants (25% of the group) had one or more detectable SARS-CoV-2 antigens (Figure 1a). The most commonly detected antigen was spike (n=33, 5.0%), followed by S1 (n=15, 2.3%) and N (n=15, 2.3%). In most instances (59/61, 97%) only a single antigen was detected; one specimen was positive for S1 and N and a second was positive for spike and N. Of those with detectable antigen, the median (IQR) concentration was 27.7 pg/mL (IQR 20.5 to 33.7) for spike, 31.2 pg/mL (IQR 20.5 to 193.0) for S1, and 23.6 pg/mL (IQR 6.46 to 62.0) for N.

Compared to the pre-pandemic group, detection of any SARS-CoV-2 antigen was significantly more frequent among pandemic era participants at all three time periods that we evaluated in the post-acute phase of infection. The absolute difference in antigen prevalence was +11% (95% CI: +5.0% to +16%) at 3.0-6.0 months post-onset of COVID-19; +8.7% (95% CI: +3.1% to +14%) at 6.1 to 10.0 months; and +5.4% (95% CI: +0.42% to +10%) at 10.1-14 months (Figure 1b; Table S3). Regarding the individual antigens, significant differences between pandemic-era and pre-pandemic-era participants were observed for spike protein for up to 10 months and for N in the first 6 months after infection (Figure 1 b-e).

Profiles of SARS-CoV-2 antigen positivity over time within individual pandemic-era participants

Of 159 pandemic-era participants who had multiple timepoints studied, 29 (18%) had antigen detected at a single post-acute timepoint, 10 (6.3%) had antigen detected at two post-acute timepoints, and one (0.63%) had antigen detected at three, four, and five post-acute timepoints, respectively (Figure S1). Most timepoints at which antigen was detected (51/61, 84%) occurred before the participant had ever received a SARS-CoV-2 vaccine. There were five instances in which antigen was detected within three weeks of a SARS-CoV-2 vaccine dose (three for S1, one for Spike, and one for N).

Determinants of antigen positivity among pandemic-era participants

Among the pandemic-era participants, we found no strong evidence of an association between age, sex, race/ethnicity, HIV status, or body mass index (BMI) with SARS-CoV-2 antigen positivity at any point between 3 and 14 months in the post-acute period of infection (Table S2). In contrast, we found several markers of severity of the acute phase of SARS-CoV-2 infection to be related to subsequent SARS-CoV-2 antigen positivity in the post-acute phase. As compared to those not hospitalized, participants who required hospitalization for acute COVID-19 were nearly twice as likely to have antigen detected (prevalence ratio [PR] 1.97; 95% CI: 1.11 to 3.48), an absolute difference of +18% (95% CI: 0% to +37%). Among those not hospitalized for COVID-19, those who reported the worst overall health during the acute phase of COVID-19 (on a 0 to 100 scale) were over 2.5 times as likely to have antigen detected as compared to those with the

most benign self-report (PR 2.82; 95% CI: 0.66 to 12.1), an absolute difference of +23% (95% CI: -5.0% to +51%).

Table S2. Characteristics of pre-pandemic-era and pandemic-era participants who were examined for the presence of SARS-CoV-2 antigens in plasma.

Characteristic	Pandemic Era (n = 171)	Pre-Pandemic Era (n = 250)
Age, years[*]	46 (37-57)	48 (36-58)
Female Birth Sex	86 (50%)	56 (22%)
Sexual Orientation[†]		
Asexual	1 (0.58%)	0 (0.0%)
Bisexual	1 (0.58%)	24 (9.6%)
Gay/lesbian	34 (20%)	98 (39%)
Straight/heterosexual	110 (64%)	113 (45%)
Questioning/unsure	3 (1.8%)	0 (0.0%)
Other	1 (0.58%)	0 (0.0%)
Race/Ethnicity[†]		
Hispanic/Latino	47 (28%)	41 (16%)
White	92 (54%)	104 (42%)
Black/African American	8 (4.7%)	76 (30%)
Asian	17 (9.9%)	29 (12%)
Pacific Islander/Native Hawaiian	3 (1.8%)	0 (0.0%)
Education[†]		
Any high school or less	33 (19%)	79 (32%)
Any college	69 (40%)	117 (47%)
Any graduate school	69 (40%)	52 (21%)
Income[†]		
$30,000 or less	24 (14%)	115 (46%)
$30,001 to $70,000	24 (14%)	36 (14%)
More than $70,000	99 (58%)	40 (16%)
Body Mass Index[†]		
Less than 18.5 kg/m^2	2 (1.2%)	5 (2.0%)
18.5 kg/m^2 to 24.9 kg/m^2	58 (34%)	116 (46%)
25.0 kg/m^2 to 29.9 kg/m^2	49 (29%)	68 (27%)
More than 30.0 kg/m^2	61 (36%)	50 (20%)
HIV Seropositive	25 (15%)	50 (20%)
Hospitalized During Acute COVID-19 Infection[†]	33 (19%)	N/A
Symptom Count During Acute COVID-19 Infection[*]	9 (6-12)	N/A
Self-reported Health at Worst Point in Acute COVID-19[*‡]	45 (25-60)	N/A
Self-reported Current Health[†]		
Excellent	N/A	62 (25%)
Very Good	N/A	82 (33%)
Good	N/A	65 (26%)
Fair	N/A	26 (10%)
Poor	N/A	3 (1.2%)
Time from COVID-19 Symptom Onset to Enrollment, days[*]	56 (37-85)	N/A

*Median (interquartile range); [†]Missing and nonresponse. Sexual orientation: 35 missing, 1 prefer not to answer; Race/ethnicity: 4 missing; Education: 2 missing; Income: 3 missing, 80 prefer not to answer; BMI: 12 missing; Hospitalization: 1 missing; Self-reported health score at worst point in COVID illness: 82 missing; Self-reported current health: 12 missing. [‡]Response to "Using a scale from 0 to 100, we would like to know how good or bad your health was during the time you had COVID-19. A score of 100 means the best health you can imagine, and 0 means the worst health you can imagine."
N/A denotes not asked or not applicable.

Table S3. Association between sociodemographic and clinical characteristics and SARS-CoV-2 antigen positivity (detection of spike, S1, or nucleocapsid antigen at any timepoint 3 to 14.1 months post-COVID-19 onset) among pandemic-era participants.

Characteristic	No. of Participants	Prevalence	Prevalence Ratio (95% CI)	Prevalence Difference (95% CI)	P value
Age, years					
Less than 40	53	0.26	Ref	Ref	
41-65	96	0.19	0.71 (0.38 to 1.31)	-0.08 (-0.22 to +0.07)	0.28
Greater than 65	15	0.33	1.26 (0.54 to 2.95)	+0.07 (-0.20 to +0.34)	0.60
Sex at Birth					
Female	83	0.17	Ref	Ref	
Male	81	0.28	1.68 (0.93 to 3.04)	+0.12 (-0.01 to +0.24)	0.081
Race/ethnicity*					
White	90	0.21	Ref	Ref	
Hispanic/Latino	44	0.32	1.51 (0.83 to 2.72)	+0.11 (-0.05 to +0.27)	0.18
Black/African American	8	0.25	1.18 (0.33 to 4.21)	+0.04 (-0.27 to +0.35)	0.80
Asian	16	0.13	0.59 (0.15 to 2.31)	-0.09 (-0.27 to +0.10)	0.43
HIV Infection					
Absent	140	0.23	Ref	Ref	
Present	24	0.21	0.91 (0.39 to 2.11)	-0.02 (-0.20 to +0.16)	0.83
Autoimmune Disorder					
Absent	151	0.23	Ref	Ref	
Present	13	0.15	0.66 (0.18 to 2.46)	-0.08 (-0.29 to +0.13)	0.52
Cancer					
Absent	157	0.24	Ref	Ref	
Present	6	0.00	0.00 (Undefined)	-0.24 (-0.30 to -0.17)	0.34
Diabetes					
Absent	145	0.23	Ref	Ref	
Present	17	0.24	1.03 (0.42 to 2.57)	0.01 (-0.21 to +0.22)	0.94
Body Mass Index*					
18.6 kg/m^2 to 24.9 kg/m^2	57	0.18	Ref	Ref	
25.0 kg/m^2 to 29.9 kg/m^2	46	0.28	1.61 (0.78 to 3.34)	+0.11 (-0.06 to +0.27)	0.20
More than 30.0 kg/m^2	58	0.24	1.38 (0.67 to 2.85)	+0.07 (-0.08 to +0.21)	0.39
Hospitalized During Acute COVID-19					
No	131	0.19	Ref	Ref	
Yes	32	0.38	1.97 (1.11 to 3.48)	+0.18 (+0.00 to +0.37)	0.029
Symptom Count During Acute COVID-19†					
0-5 symptoms	29	0.21	Ref	Ref	
6-8 symptoms	34	0.21	1.00 (0.38 to 2.64)	+0.00 (-0.20 to +0.20)	0.99
9-11 symptoms	29	0.07	0.33 (0.07 to 1.53)	-0.14 (-0.31 to +0.04)	0.15
≥ 12 symptoms	40	0.25	1.21 (0.49 to 2.96)	+0.04 (-0.16 to +0.24)	0.68
Self-reported Health Score at Worst Point During Acute COVID-19†					
>60	16	0.12	Ref	Ref	
50-60	22	0.09	0.73 (0.11 to 4.69)	-0.03 (-0.24 to +0.17)	0.74
30-49	19	0.21	1.68 (0.35 to 8.11)	+0.09 (-0.16 to +0.33)	0.51
<30	17	0.35	2.82 (0.66 to 12.1)	+0.23 (-0.05 to +0.51)	0.14

*Individuals whose BMI was <18.5 kg/m^2 (N=2) and who are Pacific Islander/Native Hawaiian (N=2) were omitted from the analyses
†Analyses only conducted amongst participants who were not hospitalized during their acute COVID-19 infection

Table S4. Differences in prevalence of SARS-CoV-2 antigen positivity in plasma among participants in the post-acute phase of COVID-19 in comparison to pre-pandemic participants. P-values represent Fisher's 2-sided exact test.

Any SARS-CoV-2 Antigen

Group	**No. of participants**	**No. (%) antigen positive**	**Prevalence difference 95% confidence interval; p value**
Pre-pandemic	250	5 (2.0%)	Reference
Pandemic			
3 to 6.0 months	151	19 (13%)	+0.11 (+0.050 to +0.16); p < 0.001
6 to 10.0 months	131	14 (11%)	+0.087 (+0.031 to +0.14); p < 0.001
10 to 14 months	122	9 (7.4%)	+0.054 (+0.0042 to +0.10); p = 0.017

Spike

Group	**No. of participants**	**No. (%) antigen positive**	**Prevalence difference 95% confidence interval; p value**
Pre-pandemic	250	3 (1.2%)	Reference
Pandemic			
3 to 6.0 months	151	9 (6.0%)	+0.048 (+0.0075 to +0.088; p = 0.012)
6 to 10.0 months	131	7 (5.3%)	+0.041 (+0.0006 to +0.082; p = 0.036)
10 to 14 months	122	5 (4.1%)	+0.029 (-0.0087 to +0.067; p = 0.12)

Nucleocapsid

Group	**No. of participants**	**No. (%) antigen positive**	**Prevalence difference 95% confidence interval; p value**
Pre-pandemic	250	2 (0.80%)	Reference
Pandemic			
3 to 6.0 months	151	7 (4.6%)	+0.038 (+0.0031 to +0.074; p = 0.030)
6 to 10.0 months	131	3 (2.3%)	+0.015 (-0.013 to +0.043; p = 0.34)
10 to 14 months	122	2 (1.6%)	+0.0084 (-0.017 to +0.034; p = 0.60)

S1

Group	**No. of participants**	**No. (%) antigen positive**	**Prevalence difference 95% confidence interval; p value**
Pre-pandemic	250	3 (1.2%)	Reference
Pandemic			
3 to 6.0 months	151	4 (2.7%)	+0.015 (-0.015 to +0.043; p = 0.43)
6 to 10.0 months	131	4 (3.1%)	+0.019 (-0.014 to +0.051; p = 0.24)
10 to 14 months	122	3 (2.5%)	+0.013 (-0.018 to +0.043; p = 0.40)

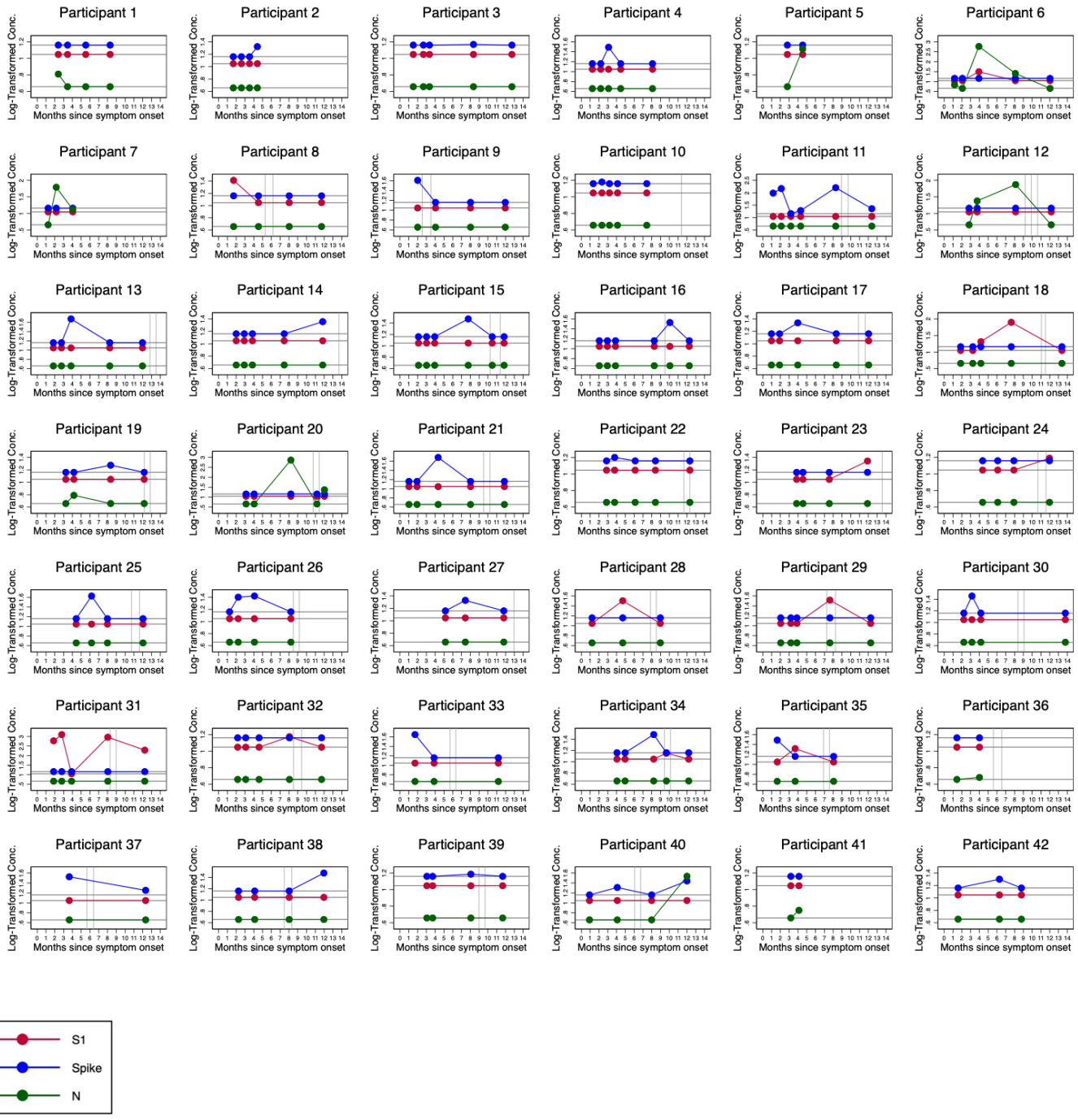

Figure S1. Individual participant-level profiles of quantitative measurement of SARS-CoV-2 spike, S1, and nucleocapsid antigens in plasma during the post-acute phase of SARS-CoV-2 infection, limited to participants with at least one positive antigen. Blue indicates spike, green nucleocapsid, and red S1. Horizontal dotted lines represent the assay limit of detection for each antigen. Vertical dotted lines indicate receipt of SARS-CoV-2 vaccine. Y-axis refers to log-transformed concentration of antigen in picograms per mL.

Comparisons With Prior Literature

Our inferences are consistent with most but not all prior smaller studies investigating SARS-CoV-2 persistence three or more months following acute infection. In plasma, Swank et al. (n=40 SARS-CoV-2-infected participants versus n=45 uninfected),[21] Schultheiß et al. (n=29 versus n=2),[20] and Craddock et al. (n=47 versus n=15)[15] each found excess SARS-CoV-2 antigen prevalence in the post-acute period compared to uninfected persons, but Kanberg et al. (n=31 versus n=17) did not.[16] Studies of tissue have featured even smaller numbers of participants,[25] the exception being an investigation of 46 participants undergoing colonoscopy for inflammatory bowel disease in whom 70% had detectable SARS-CoV-2 RNA or antigen.[14] The proportion of antigen positivity in our study was lower than most of the prior reports,[15,20] including one using our assay.[21] This may be explained by other studies being enriched with highly symptomatic patients recruited from Long COVID clinics whereas our population was consecutive volunteers who were interested in a variety of long-term aspects related to COVID-19. Alternatively, our work and the cited studies have used three different never-compared antigen assays that may not be interchangeable. In addition to direct detection of SARS-CoV-2 components in the post-acute phase of infection, evolving B cell immunity over time also indirectly suggests that some aspect of SARS-CoV-2 is persistent.[4] Finally, regarding biologic plausibility, there is ample evidence of persistence of feline coronaviruses in their natural host.[26,27]

Several prior studies have demonstrated persistent infection (as identified by infectious viral shedding from the nasopharynx) and/or SARS-CoV-2 virus or protein persistence in immunocompromised populations.[28-30] Indeed, initial reports of this phenomenon stemmed from studies of persons with advanced HIV or those receiving immunosuppressive therapy for autoimmune conditions such as inflammatory bowel disease.[14,31] Since our goal is to enroll a cohort of adults derived from the general population, there are few individuals with significant immunocompromising conditions in our cohort. Nearly all our participants with HIV infection are on suppressive antiretroviral therapy with CD4+ lymphocyte counts in the normal range. Our analyses did not reveal associations between antigen persistence and chronic HIV infection, autoimmune disease (which in most cases in our cohort is reported as thyroiditis), diabetes, or cancer requiring systemic treatment in the preceding two years. Still, given the small numbers of individuals with these conditions in our study, it is

possible that antigen persistence would be observed to be more common among such individuals in larger analyses meant to compare immunocompetent versus immunocompromised individuals.

As in a prior report using our assay,[21] antigen detection was intermittent over time in individuals in whom it was ever present. To what extent this represents true biologic variability versus lack of assay reproducibility is unknown. Formally, if true biologic variability, this could stem from variability in antigen production, release from reservoirs into the bloodstream, or clearance. A more sensitive assay that could interrogate larger plasma volumes, such as one being developed by our team,[32] might provide substantial insights on these questions.

Limitations

Our work has several limitations. First, our pandemic era group was a convenience sample, enriched to some unknown degree by those experiencing symptoms. Thus, the approximately 5% to 10% excess prevalence of antigenemia that we observed cannot be taken as a population-based estimate for all individuals experiencing SARS-CoV-2 infection during the period of our study. However, we suspect identifying a more representative group, replete with available blood specimens, will be highly challenging. Second, our participants were mainly infected with ancestral SARS-CoV-2 strains without the benefit of prior vaccination or effective anti-viral agents. It is thus unclear how our findings will generalize to contemporary infections. Third, while our participants did not have known or clinically suspected reinfections prior to antigen detection, they were not systematically assessed for asymptomatic SARS-CoV-2 infection. It is therefore possible that some fraction of the antigenemia is from recent re-infection instead of the distant primary infection. Finally, our findings provide no direct evidence regarding the persistent presence of replication-competent or even transcriptionally active virus. Yet, finding of circulating antigen so far in time from acute infection at least suggests some source of antigen production to counteract clearance. Whatever the mechanism for establishing a reservoir, our findings suggest an explanation for the protective effect of SARS-CoV-2 vaccination and antiviral therapy during acute infection against the occurrence of post-acute sequelae of COVID-19 (PASC).[33-35]

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