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

Persistent symptoms among some individuals who develop COVID-19 have led to the hypothesis that SARS-CoV-2 might, in some form or location, persist for long periods following acute infection.[1,2] Studies on SARS-CoV-2 persistence to date, however, have been limited by small and non-representative study populations, short durations since acute infection, unclear documentation of vaccination and reinfection histories, and the absence of a true negative comparator group to assess assay specificity (appendix p 2). To address these limitations, we evaluated the presence of SARS-CoV-2 antigens in once-thawed plasma from a well characterised group of 171 adults (appendix pp 3, 9) at several timepoints in the 14 months following RNA-confirmed SARS-CoV-2 infection, most of whom were studied before vaccination or reinfection (so-called pandemic-era participants).[3] To understand the specificity of our findings, we compared them to 250 adults (appendix pp 3, 9) whose plasma was collected before 2020, who, by definition, were not infected with SARS-CoV-2 (pre-pandemic era). We used the Simoa (Quanterix) single molecule array detection platform to measure SARS-CoV-2 spike, S1, and nucleocapsid antigens (appendix p 4).[4,5]

Of 660 pandemic-era specimens tested, 61 (9·2%) specimens from 42 participants (25% of the group), had one or more detectable SARS-CoV-2 antigens (figure A). The most commonly detected antigen was spike (n=33, 5·0%), followed by S1 (n=15, 2·3%) and N (n=15, 2·3%). Compared with the pre-pandemic era participants who had 2% assay positivity, detection of any SARS-CoV-2 antigen was significantly more frequent among the pandemic-era participants at all three timepoints in the post-acute phase of infection (figure B–E). The absolute difference in SARS-CoV-2 plasma antigen prevalence was +10·6% (95% CI +5·0 to +16·2) at 3·0–6·0 months post-onset of COVID-19; +8·7% (+3·1 to +14·3) at 6·1–10·0 months; and +5·4% (+0·42 to +10·3) at 10·1–14·1 months (appendix p 11).

Compared with those not hospitalised, participants who required hospitalisation for acute COVID-19 were nearly twice as likely to have SARS-CoV-2 antigens detected (prevalence ratio 1·97, 95% CI 1·11 to 3·48),

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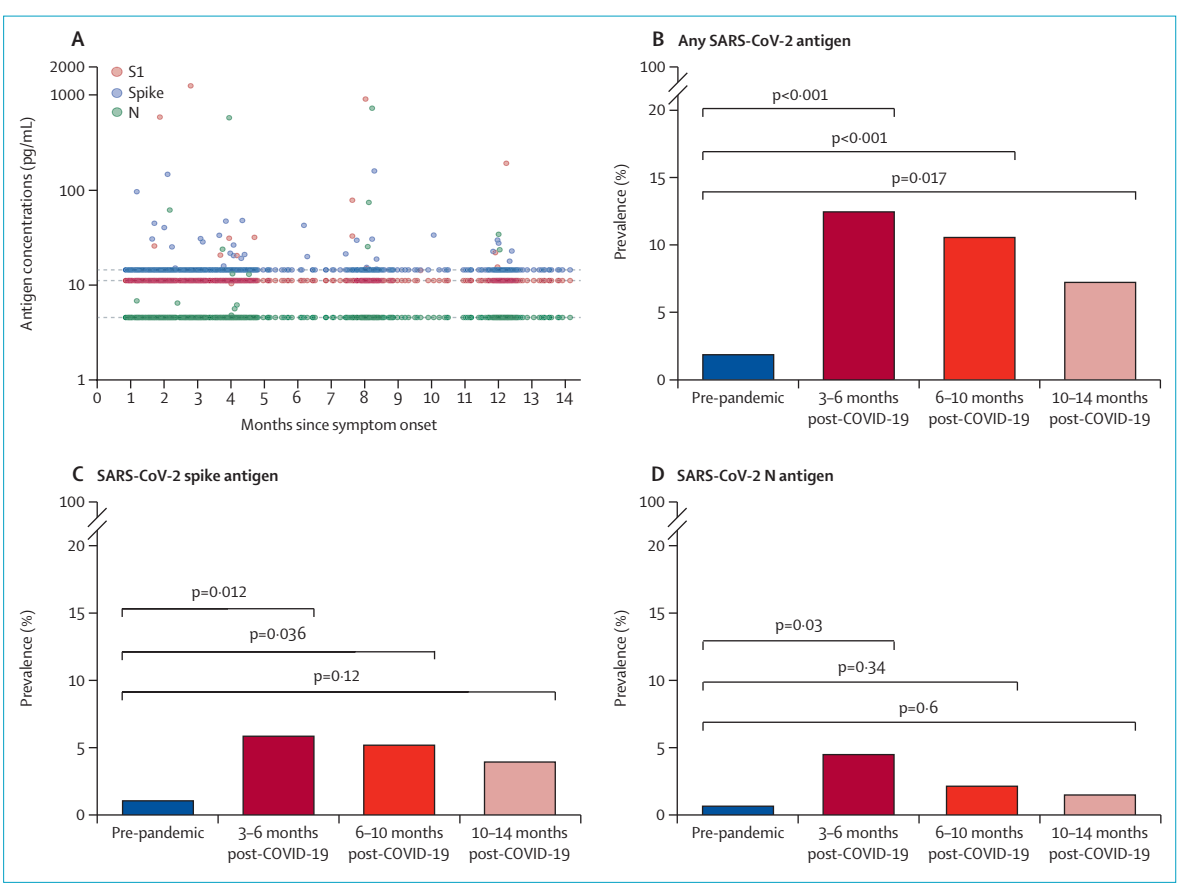

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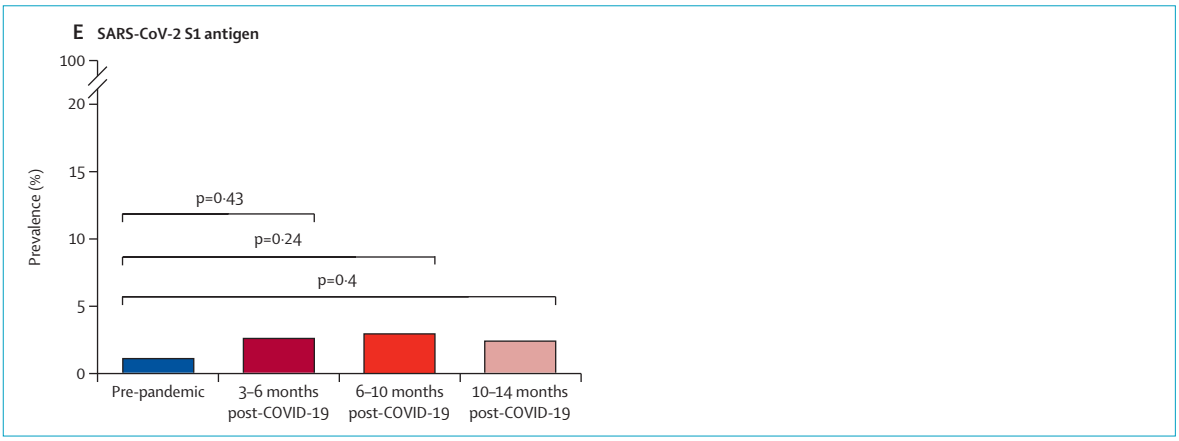

Figure 1: **SARS-CoV-2 antigen positivity in plasma among participants in the post-acute phase of COVID-19 in comparison with pre-pandemic participants**
(A) Quantitative measurement of concentrations of SARS-CoV-2 spike, S1, and nucleocapsid antigens in plasma among all pandemic-era participants during the post-acute phase of SARS-CoV-2 infection. Dashed lines indicate limit of quantification for each antigen. (B–E) Prevalence of SARS-CoV-2 antigen positivity in plasma among participants in the post-acute phase of COVID-19 compared with pre-pandemic participants. P-values are derived from Fisher's two-sided exact tests. (B) Prevalence of any SARS-CoV-2 antigen positivity (spike, S1, or nucleocapsid). (C) Prevalence of spike antigen presence. (D) Prevalence of nucleocapsid antigen presence. (E) Prevalence of S1 antigen presence.

an absolute difference of +18·4% (95% CI +0·3 to +36·5). Among participants not hospitalised, those with worse self-reported health during acute COVID-19 had greater post-acute antigen detection (appendix pp 7, 10). These findings suggest the influence of the acute phase of infection in establishing a persistent SARS-CoV-2 reservoir. Coupled with a 2024 study of replication-competent virus in blood during acute infection,[6] our findings suggest that SARS-CoV-2 might seed distal sites through the bloodstream and establish protected reservoirs in some sites. Alternatively, more severe acute infection could be a marker of higher inoculum in sites of primary infection, which then have a greater chance of evading immune clearance.

Because our measurement of SARS-CoV-2 is via immunoreactivity, it cannot be assumed that every signal is specific for SARS-CoV-2 antigen. Antigens from related pathogens or the host can theoretically cross-react, differing from the detection of nucleic acid for which specificity is typically complete and direct analyte evaluation by sequencing is possible. Therefore, understanding the specificity of any immune-based assay purporting to detect SARS-CoV-2 antigens is crucial and requires a large true negative group to precisely estimate false positivity. The 98% specificity that we documented for our Simoa assay is high but imperfect, so caution is needed if interpreting individual-level results.

To mitigate concerns that vaccination against SARS-CoV-2 or recent reinfections could affect interpretation of positive results,[1] we studied specimens largely collected before these occurrences. Most samples were collected before the emergence of the Delta and Omicron SARS-CoV-2 variants, when reinfections became common.[7]

Although not without limitations (appendix p 14), our data provide strong evidence that SARS-CoV-2, in some form or location, persists for up to 14 months following acute SARS-CoV-2 infection (appendix p 13). This persistence is influenced by the events of acute infection. These findings motivate an urgent research agenda regarding the clinical manifestations of SARS-CoV-2 persistence, specifically whether it is causally related to either post-acute chronic symptoms (eg, fatigue, pain, and cognitive difficulty) or discrete incident complications (eg, cardiovascular events).

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1 Proal AD, VanElzakker MB, Aleman S, et al. SARS-CoV-2 reservoir in post-acute sequelae of COVID-19 (PASC). *Nat Immunol* 2023; **24:** 1616–27.

2 Davis HE, McCorkell L, Vogel JM, Topol EJ. Long COVID: major findings, mechanisms and recommendations. *Nat Rev Microbiol* 2023; **21:** 133–46.

3 Peluso MJ, Kelly JD, Lu S, et al. Persistence, magnitude, and patterns of postacute symptoms and quality of life following onset of SARS-CoV-2 infection: cohort description and approaches for measurement. *Open Forum Infect Dis* 2021; **9:** ofab640.

4 Wu D, Milutinovic MD, Walt DR. Single molecule array (Simoa) assay with optimal antibody pairs for cytokine detection in human serum samples. *Analyst* 2015; **140:** 6277–82.

5 Swank Z, Senussi Y, Manickas-Hill Z, et al. Persistent circulating severe acute respiratory syndrome coronavirus 2 spike is associated with post-acute coronavirus disease 2019 sequelae. *Clin Infect Dis* 2023; **76:** e487–90.

6 Platt A, Singh M, Stein S, Soherwardi S, de Wit E, Chertow DS. Replication-competent virus detected in blood of a fatal COVID-19 case. *Ann Intern Med* 2024; **177:** 113–15.

7 Ma KC, Dorabawila V, León TM, et al. Trends in laboratory-confirmed SARS-CoV-2 reinfections and associated hospitalizations and deaths among adults aged ≥18 years – 18 US jurisdictions, September 2021–December 2022. *MMWR Morb Mortal Wkly Rep* 2023; **72:** 683–89.