Blinded 2-Year Longitudinal Evaluation of SARS-CoV-2 Antigenemia in Long COVID

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L Mateu[1,2,3], L L Hansen[4], J P Carmezim[5], G Kijak[6], CTebe[5], R Paredes[1,7,8], D Walt[4], M Massanella[7]

[1] Department of Infectious Diseases, Hospital Germans Trias i Pujol, Badalona, Catalonia, Spain. [2]Universitat Autònoma de Barcelona, Bellaterra, Catalonia, Spain. [3]Universitat de Vic - Universitat Central de Catalunya, Vic, Catalonia, Spain. [4] Department of Pathology, Brigham & Women's Hospital, Harvard Medical School, Boston, MA, USA. [5] Biostatistics Support and Research Unit, Germans Trias i Pujol Research Institute and Hospital (IGTP), Catalonia, Spain. [6] AstraZeneca, Gaithersburg, MD, United States. [7] IrsiCaixa, Badalona, Catalonia, Spain. [8]Universitat Politècnica de Catalunya – BarcelonaTech, Terrassa, Catalonia, Spain

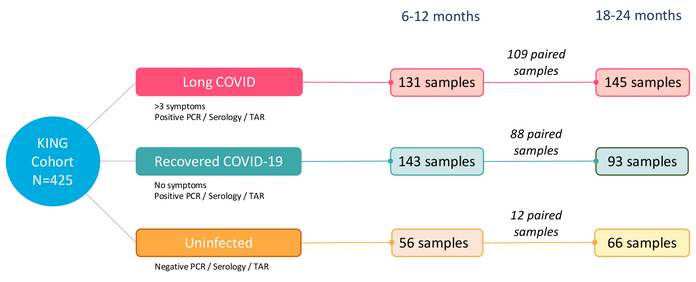
BACKGROUND

Long COVID (LC) affects at least 5% of individuals following acute SARS-CoV-2 infection, translating to more than 400 million cases globally. Among the proposed mechanisms underlying LC pathogenesis, viral persistence has garnered increasing attention. Persistent detection of SARS-CoV-2 antigens in plasma has been reported up to 12 months post-infection, suggesting ongoing viral activity or antigenic stimulation. However, the long-term temporal dynamics and longitudinal evolution of antigenemia over several years following acute infection remain poorly characterized. Determining if plasma SARS-CoV-2 antigenemia can identify Long COVID is crucial for guiding monitoring, treatment decisions, and advancing our understanding of Long COVID immunopathogenesis.

METHODS

We conducted a 2-year prospective, blinded, longitudinal study involving 425 adults: 167 with LC, 148 fully recovered from acute COVID-19 (R), and 110 uninfected (U) controls. Plasma samples were collected at 6–12 months (n=330; Visit 1) and 18–24 months post-infection (n=304; Visit 2). SARS-CoV-2 spike, S1 subunit, and nucleocapsid antigens were quantified using the ultra-sensitive Simoa® platform blinded for clinical features. Serological profiling included in-house ELISA-based detection of anti-S2+RBD antibodies, as well as isotype-specific responses (IgG, IgA, IgM) and neutralizing antibody titers against WH1. All samples classified as uninfected were seronegative for anti-N antibodies (in-house ELISA).

Figure 1: Flowchart of participant stratification and plasma sample distribution across follow-up visits



SARS-CoV-2 antigenemia is detectable in about one-third of subjects one year after SARS-CoV-2 infection and rarely by 2 years, regardless of whether they develop Long COVID or fully recover

Figure 2: Longitudinal evolution of antigenemia in participants with paired samples
(Long Covid n=109, Recovered n=88, Uninfected n=12).

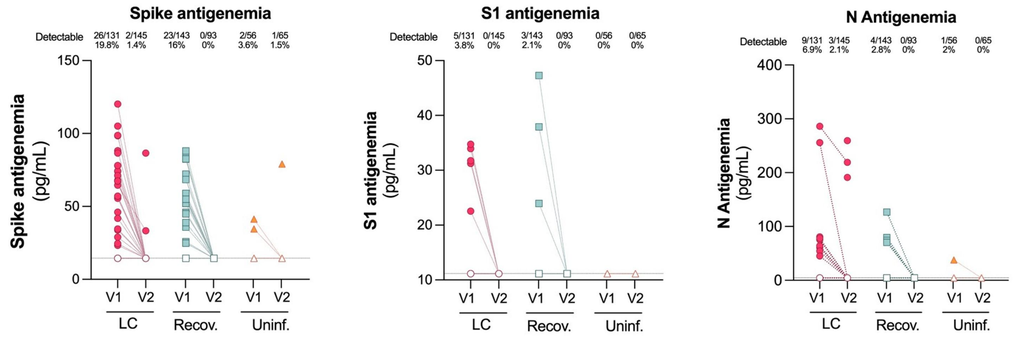


Table 1: Baseline characteristics

	Long COVID N=167	Recovered N=148	Uninfected N=110
Age, median [IQR]	49 [40-57]	52 [36.5-62]	46 [36-58]
Female sex at birth, n (%)	120 (72)	61 (41)	70 (64)
Comorbidities, n (%)	150 (90)	112 (77)	60 (64)
Obesity	52 (35)	25 (23)	1 (1.7)
Allergy	52 (35)	31 (28)	16 (27)
Dyslipidemia	40 (27)	45 (40)	13 (27)
High blood pressure	37 (25)	38 (34)	9 (15)
Pulmonary Disease	29 (20)	21 (19)	4 (6.7)
Headaches	26 (17)	6 (5.3)	6 (10)
Autoimmune Disease	20 (13)	8 (7.1)	2 (3.3)
Intestinal Disease	18 (12)	9 (8.0)	2 (3.3)
Solid Neoplasia	14 (9.5)	12 (11)	6 (10)
Active	3 (1.8)	2 (1.4)	1 (0.9)
Past	11 (6.6)	10 (6.8)	5 (4.5)
SARS-CoV-2 infection variant, n (%)			
Ancestral (12 Mar 2020 – 7 Feb 2021)	136 (81)	128 (86)	-
Alpha (08 Feb 2021–27 Jun 2021)	17 (10)	17 (11)	-
Delta (28 Jun 2021–19 Dec 2021)	9 (5.4)	2 (1.4)	-
Omicron (20 Dec 2021–present)	5 (3)	1 (0.7)	-
Hospitalization during acute COVID-19, n (%)	84 (50)	53 (36)	-
COVID-19 severity, n (%)			
High Flow Oxygen	21 (13)	13 (8.8)	-
Low Flow Oxygen	44 (26)	38 (26)	-

RESULTS

At 6–12 months, SARS-CoV-2 antigenemia (any antigen) was detected in 31% of individuals with LC, in 20% of those fully recovered and in 1 negative control (Fisher exact test, p<0.001). Spike was the most frequent antigen detected (20%, 16% and 4%, in LC, R and U, respectively), whereas S1 and N were rarely observed (for S1: 4%, 2% and 2% and for N: 7%, 3% and 2%, in LC, R and U, respectively, Figure 2). By 18–24 months, positivity of any antigen declined to 3%, 0% and 0%, for LC, R and U, respectively (Fisher exact test, p=0.19). Antigenemia was not associated with number or type of persistent symptoms, antibody titers, including neutralizing capacity, or vaccination

CONCLUSIONS

SARS-CoV-2 antigenemia can be detected between 6 and 12 months after the acute Covid-19 episode in roughly one third of subjects with LC but also at similar frequency in individuals who recover from acute Covid-19. However, by two years post-infection, only a very small number of individuals remain positive. SARS-CoV-2 antigenemia cannot be considered a reliable sole biomarker for clinical monitoring or guiding treatment decisions in most cases of Long COVID.

Table 2: Clinical and immunological characteristics

	Long Covid		Recovered		Uninfected	
	Visit 1 N = 131	Visit 2 N = 145	Visit 1 N = 143	Visit 2 N = 93	Visit 1 N = 56	Visit 2 N = 66
Time after 1st infection, months	10 [8-12]	20 [18-24]	10 [7-12]	22 [19-24]	-	-
Individuals reinfected, n (%)	9 (6.9)	27 (19)	0 (0)	0 (0)	-	-
One reinfection	9 (6.9)	23 (16)				
Two reinfections	0 (0)	4 (3)				
Time after last re-infection, months	1 [1-5]	5 [3-12]	-	-	-	-
SARS-CoV-2 Vaccination, n (%)	40 (31)	133 (92)	63 (44)	90 (97)	0 (0)	66 (100)
Time post-Vaccination, months	5 [1-8]	10 [8-13]	3 [2-7]		-	7 [4-8]
anti-S2+RBD IgG	43 [18-573]	586 [242-1064]	57 [22-592]	775 [259-1598]	-	52 [20-110]
anti-S2+RBD IgM	3.5 [2.1-6.7]	4.5 [3.2-8.5]	3.9 [2.5-5.7]	6.7 [3.8-9.8]	-	2.9 [1.9-5.1]
anti-S2+RBD IgA	18 [8.8-69]	48 [27- 97]	20 [8.8-49]	54 [25-126]	-	10 [8.5-28]
Neutralization titers against WH1, reciprocal dilution	649 [273-2631]	2222 [1051-4640]	1211 [370-4662]	1966 [931-4876]	-	602 [342-1645]

** values are median [IQR] unless otherwise noted, IgG values are in AU=arbitrary units*

PLAIN LANGUAGE SUMMARY

About one third of people with Long COVID can still have detectable SARS-CoV-2 protein in their blood one year after infection, but almost no one has it by two years. This shows that blood antigen tests alone cannot reliably identify or track Long COVID

Author contact: lmateu@lluita.org

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