

Persistence of residual SARS-CoV-2 viral antigen and RNA in tissues of patients with long COVID-19

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Short Report

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Abstract

The World Health Organization has defined long COVID-19 (LC) as a condition where patients exhibit persistent symptoms over time after its acute phase, which cannot be explained by alternative diagnosis. Since we have previously reported residual viral antigens in tissues of convalescent patients, we now aim to assess the presence of such antigens in post-convalescent tissues. Here, we established the presence of residual virus within the appendix and breast tissue of 2 patients who exhibited LC symptoms, 175 to 462 days upon positive diagnosis, using immunohistological techniques. We observed positive staining for viral nucleocapsid protein (NP) in the appendix, and tumour-adjacent region of the breast, but not within the tumour via multiplex immunohistochemistry. Notably, with RNAscope, both positive-sense and negative-sense (replicative intermediate) viral RNA were detected. As a single-stranded virus, SARS-CoV-2, have to produce a replicative intermediate as a template to synthesize new genomic RNAs. Thus, the detection of negative-sense viral RNA suggests ongoing viral replication. While viral RNA and antigen from gastrointestinal and stool samples of convalescent patients has been extensively reported, we believe this is the first study to detect viable virus. Furthermore, our positive finding in the breast tissue also corroborated with recent reports that immunocompromised patients had also experienced LC symptoms and persistent viral replication. Overall, our findings, along with emerging LC studies, raises the possibility of the gastrointestinal tract functioning as a reservoir.

Full Text

We read with great interest the article by Neurath *et al*, which discusses how local infection of intestinal epithelial cells by SARS-CoV-2 may affect the gut microbiome,[1] and in a separate study by Chen *et al*, which associates gut dysbiosis with COVID-19 recovery process.[2] These are consistent with the growing evidence of a residual viral reservoir, as well as with emerging studies on long COVID-19 (LC). LC can be defined as a condition in which patients exhibit persistent symptoms, such as chronic fatigue, brain fog, and shortness of breath, over time after the acute phase of COVID-19 that cannot be explained by an alternative diagnosis.[2, 3, 4, 5, 6, 7] Previously, we have also reported the persistence of residual viral antigens for up to 180 days in gastrointestinal and hepatic tissues of COVID-19 convalescent patients.[5] Here, we aimed to assess the presence of such antigens in tissues from LC patients.

We obtained samples of the appendix, skin, and breast in two patients who exhibited LC symptoms 175-426 days after a positive diagnosis of COVID-19 (Supplementary Materials and Methods, Supplementary Table 1). Using multiplex immunohistochemistry, we detected SARS-CoV-2 nucleocapsid protein (NP) in the appendix (Figure 1A-B) and breast (Figure 1C-D), supporting the persistence of residual viral particles in these tissues for more than a year after infection. Interestingly, in the breast, viral NP was present only in the tumour-adjacent area, but not in the tumour itself (Figure 1D). Negative staining in the skin was observed, possibly due to a high skin cell turnover rate (Figure not shown). To rule out non-specific staining and to validate our findings, SARS-CoV-2 NP antibody was tested on gastrointestinal tissues that had been previously obtained before 2019 from a separate cohort.[5]

Having established the presence of residual virus within the tissues of our LC patients, we then aimed to assess its functional significance. Persistent shedding of viral RNA for an extended period after the onset of acute symptoms has been reported,[5, 8, 9] but none of a viable virus. By utilizing RNAscope, we were able to detect both positive-sense (genomic) and negative-sense (replicative intermediate) viral RNA in the appendix and breast tissue (Figure 2). Interestingly, the detection of negative-sense viral RNA is suggestive of ongoing viral replication.[10] While the presence of viral RNA and/or antigen in gastrointestinal samples of convalescent patients has been widely reported,[5, 8] we believe this study is the first to detect viral RNA and/or antigen in the tissues of patients with LC, up to 426 days after the onset of COVID-19 symptoms.

The gastrointestinal tract is a major viral shedding route, with high ACE2 expression and residual viral RNA and/or antigen detectable throughout its tissues and bodily fluids, despite negative SARS-CoV-2 test results.[5, 8, 9] Thus, based on the emerging studies on LC, including our own, and the growing evidence of the residual virus,[2, 3, 4, 5] we suggest that the gastrointestinal tract might serve as a SARS-CoV-2 reservoir among convalescent and post-convalescent patients. Moreover, the presence of residual viral antigen in breast tissue is also corroborated by recent studies that reported that immunocompromised cancer patients had LC symptoms and persistent viral replication over a long time period.[11, 12] Conversely, the absence of residual viral antigen in the skin tissue calls for further investigation on viral distribution across different organs in patients with LC. Overall, we report on persistent SARS-CoV-2 lingering within tissues of patients with LC. Although suggestive, these antigens were potentially in the state of active replication. To validate our findings, future studies should recruit a larger cohort of patients from different populations and geographical regions.

Declarations

DG, JCTL, and SBF are joint first authors.

Contributions: JYPS conceived and supervised the study. DG, JCTL, JNL, CRJ, and ZWN performed immunohistochemistry procedures and imaging. JYPS, DG, SBF, SG and MCL collated, analysed, and interpreted the data. DG drafted the manuscript and obtained the final approval of all authors.

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Patient and public involvement: Patients and/or the public were not involved in the design, conduct, reporting, or dissemination of this research.

Ethics approval: This study was approved by Agency of Science, Technology and Research (A*STAR), IRB: 2021-161.

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Figures

Figure 1

Multiplex immunohistochemical staining of SARS-CoV-2 nucleocapsid protein in tissues obtained from two patients with LC

(A, C) Representative images of (A) appendix and (C) breast tissues stained for Hematoxylin and Eosin, with nuclear component (hematoxylin) and cytoplasmic components (eosin) differentially stained. **(B, D)** Representative images of (B) appendix and (D) breast tissues stained for DAPI (blue), CK/EpCAM (red), CD45 (cyan), SARS-CoV-2 nucleocapsid protein (COVID NP; green), and CD68 (yellow). Scale bar, 100 μm .

Figure 2

RNAscope staining suggests a potential ongoing viral replication

(a-D) Representative images of (A-B) Appendix and (C-D) breast tissues subjected to RNAscope in situ hybridisation, with nuclear component (hematoxylin) counterstained. (A, C) SARS-CoV2 positive-sense SARS-CoV-2 spike RNA was labelled in green dots. (B, D) SARS-CoV2 negative-sense orf1ab genomic RNA was labelled in red dots (arrows), potentially representing active replication of the positive-sense RNA virus. (A-D) Scale bar, 20 μm .

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