Residual SARS-CoV-2 viral antigens detected in GI and hepatic tissues from five recovered patients with COVID-19

We read with great interest the article published by Zuo et al, which highlighted the presence of SARS-CoV-2 RNA in stool samples during active and convalescence phases of COVID-19 infection. However, no study has reported the presence of viral antigens within GI and hepatic organs during the convalescent phase.

Using conventional immunohistochemistry, we detected SARS-CoV-2 nucleocapsid protein (NP) in the colon, appendix, ileum, haemorrhoid, liver, gallbladder and lymph nodes (figure 1A–K) from five patients who recovered from COVID-19, ranging from 9 to 180 days after testing negative for SARS-CoV-2 (online supplemental table 1). Notably, when multiple tissues were obtained from one patient (patients 1 and 4), all the tissues showed the presence of the viral antigen, suggesting widespread multiorgan involvement of the viral infection. Interestingly, for the colon, the viral antigen was only present in normal colonic crypts and polyps but not in the neoplastic tissues (figure 1Q). Similar negative staining in the hepatocellular carcinoma tumour region was also observed (figure 1R) albeit the positive staining in some of the scattered immune cells (figure 1D). Validating our findings, we detected SARS-CoV-2 spike protein (figure 1L–P) and RNA (figure 2B–F) in the above-mentioned tissues using conventional immunohistochemistry and RNAscope, respectively. However, we were unable to detect viral RNA in some patients’ tissues (online supplemental table 1), possibly because of higher RNA degradation rate as compared with protein and other patient-dependent factors such as disease severity, time since recovery and basal metabolic rate.

In addition, multiplex immunohistochemistry and RNAscope staining showed that some SARS-CoV-2-positive cells colocalised with ACE2 receptor and CD68 in the colon and liver (figure 2A,B). These cells were likely of monocyte lineage and liver-resident sinusoidal Kupffer cells, which therefore confirmed our earlier speculation that was based on cellular morphology. This suggests that SARS-CoV-2 might indeed infect these immune cells directly, as previously reported. Finally, on detection and validation of viral antigens in the tissues, we interrogated whether the tissues harboured an immune response to the virus. We performed ex vivo peptide stimulation assays whereby blood and tissues were incubated with a cocktail of the viral nucleocapsid, spike and membrane proteins, followed by flow cytometry analysis. Notably, SARS-CoV-2-specific CD38+Granzyme B+CD4+ T

Figure 1 Immunohistochemical staining of the SARS-CoV-2 nucleocapsid and spike proteins in intestinal and hepatic tissues. (A and B) Positive SARS-CoV-2 nucleocapsid protein (NP) staining in colonic crypts (A) and appendix (B), both with a granular supranuclear cytoplasmic pattern. (C) positive SARS-CoV-2 NP staining in scattered immune cells in haemorrhoid tissue (red arrows). (D) positive SARS-CoV-2 NP staining in sinusoidal Kupffer cells in the liver (red arrows). (E–H) Representative images of negative SARS-CoV-2 NP staining in colon, appendix, haemorrhoid and liver tissues taken from individuals with no history of COVID-19. (I) Positive SARS-CoV-2 NP staining in scattered immune cells within the lymph node. (J and K) Positive SARS-CoV-2 NP staining in ileum (J) and gallbladder (K), both with a granular supranuclear cytoplasmic pattern. (L) Positive SARS-CoV-2 spike protein staining in scattered immune cells within the lymph node. (M–P) Positive SARS-CoV-2 spike protein staining in the ileum (M), liver (N), colon (O) and appendix (P). (Q) Positive SARS-CoV-2 NP staining in normal colonic crypts (top right) but negative staining in the adjacent neoplastic glands (bottom left, red box). (R) Scattered SARS-CoV-2 NP-positive immune cells were seen along the invasive margin of hepatocellular carcinoma, as indicated by the red arrows. Hepatocellular carcinoma tumour region, as demarcated by the red box, was negative for SARS-CoV-2 NP. (A–R) Scale bar: 50 µm.
cells were isolated from the tissues in a comparable fashion with matched blood samples (figure 2G), suggesting that SARS-CoV-2-specific memory T cells may be maintained in both blood and tissue over a period of time. Nevertheless, further study is warranted to compare the tissue immune microenvironment before and after the infection and to confirm whether the immune cells in the proximity of viral antigen are indeed specific to SARS-CoV-2.

Several groups have reported the phenomenon where patients who had recovered from mild or moderate COVID-19 later tested positive in nasopharyngeal swabs or sputum samples, raising concern for residual virus reservoirs and potential transmissibility in recovered individuals. Although respiratory transmission is responsible for most COVID-19 infections, there is increasing evidence of COVID-19 causing GI and hepatic manifestations, as studies reported the presence positive SARS-CoV-2 RNA in anal swabs and stool samples, despite nasopharyngeal or sputum specimens testing negative for the virus. These reports are in line with our findings of the intestinal tissues of patients with negative nasopharyngeal swab tests. Also, while SARS-CoV-2 viral antigen has been detected in the lung tissues of deceased patients despite negative nasopharyngeal swab tests, our findings constitute the first evidence of residual virus in extrapulmonary tissues during the convalescent phase, up to 6 months after recovery, in a non-postmortem setting. It seems that a negative swab result might not necessarily indicate complete viral clearance from the body. Yet unfortunately, we were unable to determine whether the viral antigens isolated from the tissues were infectious, as the virus was inevitably destroyed during tissue fixation. We also did not have access to anal swab, stool samples and viral isolation facility, which would have provided additional insight into the possibility of faecal–oral transmission. Regardless, based on these preliminary findings, we believe that further research in a larger cohort is warranted to explore the replication and infectivity of the virus in tissue specimens and to understand the GI and hepatic involvement in COVID-19.

Chun Chau Lawrence Cheung,1,2 Denise Goh,3 Xinru Lim,3 Tracy Zhijun Tien,3 Jeffrey Chun Tatt Lim,3 Bing Kiat Hon Lim1,2
1Department of Anatomical Pathology, Singapore General Hospital, Singapore
2Duke-NUS Medical School, Singapore
3Institute of Molecular and Cell Biology, Agency for Science, Technology and Research, Singapore
4Department of Microbiology, Singapore General Hospital, Singapore

Figure 2  Multiplex immunohistochemistry, RNAscope and flow cytometry analysis of tissues obtained from five patients with COVID-19. (A) Representative images of liver tissue stained using multiplex immunohistochemistry for DAPI (blue), SARS-CoV2 NP (red), ACE2 (green) and CD68 (magenta). #DP1: double positive cell 1; #TP1 and #TP2: triple positive cell 1 and 2, respectively. (B) Representative images of colon tissue stained using RNAscope for DAPI (blue), SARS-CoV2 S gene (red), ACE2 (green) and CD68 (magenta) RNA. (C–F) Representative RNAscope images of haemorrhoid (C and D) and ileum (E and F) stained for DAPI (blue), SARS-CoV2 S gene (red) and CD68 (green). Some SARS-CoV-2-positive cells were stained positive for CD68, as indicated by the white arrows (D and F). (G) SARS-CoV-2-specific CD4+ T cells were identified from the CD4+CD3+CD4+CD39+CD103+CD38+Granzyme B+ population, where CD39, CD103 and CD38 select for immune cells with a memory phenotype, and granzyme B selects for immune cells with a functional phenotype. Representative pseudo-colour plots of CD38+Granzyme B+CD4+ T cells following stimulation with SARS-CoV-2 peptides in tissue and blood samples of patient 1 against healthy donor blood. Similar results were obtained from patient 2. The numbers indicate the percentages in the drawn gates. The plots shown are representative of at least two independent experiments. (A–F) Scale bar: 100 µm.

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**Department of Colorectal Surgery, Singapore General Hospital, Singapore**

**Department of Infectious Diseases, Singapore General Hospital, Singapore**

**Yong Loo Lin School of Medicine, National University of Singapore, Singapore**

yeongps@nus.edu.sg and Dr Kiat Hon Lim, Dept of Anatomical Pathology, Singapore General Hospital, 20 College Road, Academic, Level 10, Diagnostics Tower, Singapore 169856, Singapore; lim.kiat.hon@ singhealth.com.sg

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**ORCID iDs** Chun Chau Lawrence Cheung http://orcid.org/0000-0002-6528-1064

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