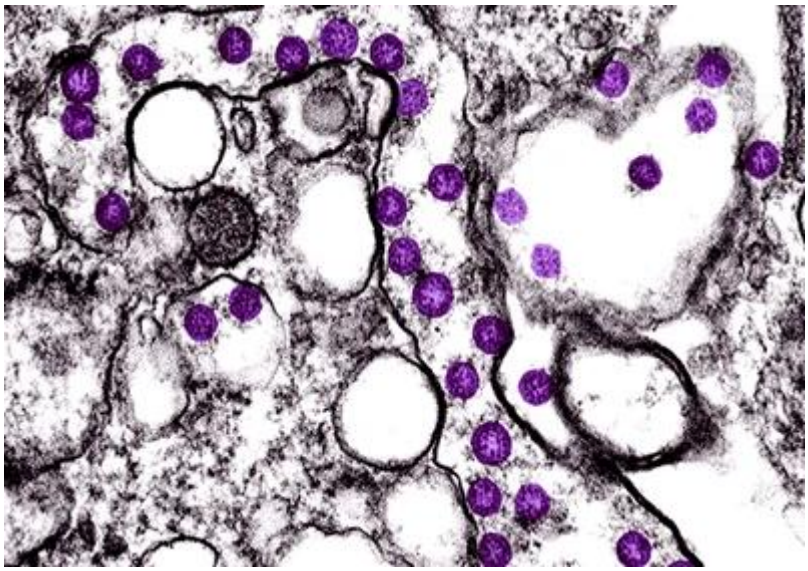
Infection with the severe acute respiratory syndrome coronavirus type-2 (SARS-CoV-2) can cause a new disease called long-COVID-19 or post-acute COVID-19 syndrome (PACS). This syndrome can occur in various populations, including children and young adults, as well as in those who had mild COVID-19. Several previous studies have proposed a pathophysiological model of long COVID based on the persistence of SARS-CoV-2, as an infection-associated chronic disease that affects every organ system and leads to multisystem injury in adults and children. The aim of this single-center, cross-sectional cohort study, conducted at China-Japan Friendship Hospital in Beijing, was to investigate the persistence of residual SARS-CoV-2 in different solid tissues at different time points after mild COVID-19, and the relationship between viral persistence and long COVID symptoms.

Several prior studies have discussed persistent multisystem injury in both adults and children as a consequence of a chronic disease associated with SARS-CoV-2 infection. Residual SARS-CoV-2 RNA or proteins have been detected in autopsy samples from various organs, and in diverse sample types from recovered patients. These samples include samples from the lung, breasts, skin, appendix, intestine, adenoid, tonsils, and olfactory neuroepithelium tissues, among others. The virus has also been detected in stool and plasma samples.

A *post-mortem* examination of the replication, persistence, and evolution of SARS-CoV-2 in infected human tissues revealed widespread distribution of SARS-CoV-2 RNA in 84 distinct anatomical locations up to 230 days after infection. The virus was not detected in plasma, however, high-sensitivity droplet digital PCR (ddPCR) detected viral persistence in multiple tissue samples from all decedents. Also, the detection of subgenomic RNA, a marker for recent viral replication, and the isolation of replication-competent viruses from respiratory and non-respiratory tissues indicated that viral replication may continue for several months after initial infection. Another study demonstrated that residual SARS-CoV-2 was detected in surgically resected intestinal specimens 6 months after COVID-19, despite the negative nasopharyngeal PCR results. Yang C et al. Association of SARS-CoV-2 infection and persistence with long COVID. Lancet Respir Med 2023. https://doi.org/10.1016/S2213-2600(23)00142-X Similarly, residual viral protein and RNA were detected in the appendix, skin, and breast tissue of two patients who developed long COVID symptoms 163 and 426 days after the onset of symptoms, respectively. https://discovermednews.com/sars-cov-2-rna-and-antigens-appendix-skin-breast-patients-long-covid/



About the study

The study comprised individuals diagnosed with mild COVID-19 during the Omicron (BA.5·2 and BF.7) wave of SARS-CoV-2, who were scheduled to undergo gastroscopy, surgery, chemotherapy, or immunotherapy, or hospitalized for other reasons. The participants were analyzed at different time points after recovery from mild COVID-19, at one month (ranging from 18 to 33 days), two months (ranging from 55 to 84 days), or four months (ranging from 115 to 134 days) after infection.

To detect viral RNA or proteins, the authors collected samples of gastric mucosa, blood, and residual surgical samples from 13 types of solid tissues, including stomach, lung, skin, intestine, blood vessel, kidney, breast, thyroid, liver, brain, pancreas, gallbladder, and appendix. They also collected 31 pairs of tumor and paratumor tissues and 198 paratumor-only tissues. For patients who underwent gastroscopy, both oropharyngeal swabs and gastric mucosa samples were collected to exclude potential contamination from the oral cavity.

Scientists also collected blood samples from nine patients with hematological malignancies, including two patients with diffuse large B-cell lymphoma, three patients with multiple myeloma, and one each with follicular lymphoma, non-Hodgkin lymphoma, idiopathic

thrombocytopenic purpura, and primary mediastinal large B-cell lymphoma, to investigate whether immunocompromised individuals with hematological malignancies might face challenges in clearing the virus at 2 months post-infection.

Other data included age, sex, BMI, history of cancer, current chemotherapy or immunotherapy, comorbidities such as hypertension, diabetes, anemia, hyperlipidemia, cardio/cerebrovascular disease, chronic respiratory diseases, thyroid dysfunction, and number of COVID-19 vaccinations.

The researchers employed several techniques to detect SARS-CoV-2, such as digital droplet polymerase chain reaction (ddPCR), further confirmed by RNA *in-situ* hybridization, immunofluorescence, and immunohistochemistry. The expression of two host-cell factors that are important for SARS-CoV-2 entry into many cell types, angiotensin-converting enzyme 2 (ACE2) and transmembrane serine protease 2 (TMPRSS2) was evaluated in tumor or paratumor samples by quantitative real-time reverse-transcription PCR (qRT-PCR).

The association between the persistence of SARS-CoV-2 RNA and long COVID symptoms was assessed by follow-up telephone calls from trained physicians at around 4 months after infection.

Results

The study included 225 eligible patients. Most of them (95%, 213 patients) participated in the 4-month follow-up and were included in the analysis of the association between persistence of SARS-CoV-2 and long COVID symptoms. Five (7%) patients with long COVID symptoms and 21 (15%) patients without long COVID symptoms received chemotherapy or immunotherapy. Before infection, 78% of patients with long COVID symptoms and 86% of individuals without long COVID symptoms, had received three doses of the COVID-19 vaccines.

317 tissue samples were collected from 225 patients, including 201 residual surgical specimens, 59 gastroscopy samples, and 57 blood samples. At one month post-infection, 53 samples from 9 different tissues were collected from 38 patients. At two months post-infection, 198 samples from 13 different tissue types were collected from 138 patients, and at 4 months post-infection, 66 samples from 9 different tissue types were collected from 49 patients.

Viral RNA was detected in 30% of solid tissue samples at one month, 27% of solid tissue samples at two months, and 11% of solid tissue samples at four months. Viral RNA was

detected in 10 different types of solid tissues, including the liver, kidney, stomach, intestine, brain, blood vessels, lung, breast, skin, and thyroid. A decrease in the detection rate at 4 months after the infection indicates a slow but ultimately effective viral clearance mechanism within the human body. Immunohistochemistry, RNA in-situ hybridization, and immunofluorescence, used to validate the accuracy of ddPCR, showed consistency among these assays, and the highest sensitivity of ddPCR among these methods. Immunofluorescence identified the S protein in alveolar type I and type II epithelial cells and macrophages in lung samples.

SARS-CoV-2 N, ORF1ab, or subgenomic RNA positivity was further examined in tissue samples. During the entire period, viral N or ORF1ab RNA was found in the liver, kidney, stomach, intestine, brain, blood vessel, lung, breast, skin, and thyroid tissues. 44% of the samples positive for N1 or ORF1ab in the liver, stomach, lungs, intestine, breasts, kidneys, and blood vessels, were also positive for SARS-CoV-2 subgenomic RNA.

The authors also performed transcriptome sequencing in 24 lung tissue and 11 blood vessel samples. The genes involved in the innate and adaptive immune defense, such as *KLRD1*, *FYB1*, *VAV2*, *LILRB4*, *LILRB5*, *TICAM1*, *BTK*, *CD8A*, and *CD8B* were down-regulated in lung tissues. The genes related to the complement and coagulation cascades, such as *FGG*, *VTN*, *F12*, *FGB*, *SERPINA1*, *C5*, *C1QB*, *SERPINE2*, *SERPINA5*, and *VSIG4,* as well as the genes involved in cholesterol metabolism pathways, such as *APOC3*, *APOA1*, *APOH*, *APOA2*, *LIPG*, *APOC1*, *SCARB1*, *CD36*, and *PLTP* were dysregulated in the blood vessel samples positive for SARS-CoV-2. A significant down-regulation of zinc finger protein-related genes, which play a role in defense against SARS-CoV2, was found in the viral persistence group. These findings suggest that viral persistence may impact host cell functions.

Out of nine patients with hematological malignancies, plasma from three, granulocytes from one, and PBMCs from two patients were positive for viral RNA. None of these blood compartments were positive for viral RNA in ten immunocompetent patients. These findings suggest that a dysfunction in the host immune defense may contribute to poor virus clearance.

There was no association between SARS-CoV-2 detected by ddPCR in throat swabs and the gastric mucosa. This indicates that there was no contamination from nasal or oral sampling during gastroscopy. Also, there was no difference in the rate of viral nucleic acid detection between tumor tissues and paratumor tissues, as well as in the concentrations of the SARS-CoV-2 receptors ACE2 and TMPRSS2.

Of the 213 patients who completed the telephone questionnaire, 34% reported at least one

long COVID symptom. Fatigue (21%) was the most common symptom. Long COVID symptoms were significantly associated with viral persistence at one month and two months post-infection, but not at four months. Patients with higher viral copy numbers were more likely to develop long COVID symptoms. Long COVID symptoms were not associated with other variables.

Conclusion

This study showed the persistence of residual SARS-CoV-2 nucleic acid in solid tissue samples from various organs, including lung, liver, kidney, stomach, intestine, brain, breast, thyroid, blood vessels, and skin, in patients who had recovered from mild COVID-19 at one month, two months, and four months post-infection.

In addition, viral nucleic acids were detected in a proportion of plasma samples, granulocytes, and PBMCs from immunocompromised patients two months after SARS-CoV-2 infection, but not in immunocompetent individuals. This research also demonstrated an association between long COVID symptoms at four months after infection and the persistence of residual SARS-CoV-2 RNA.

Journal Reference

Zuo W, He D, Liang C e al. The persistence of SARS-CoV-2 in tissues and its association with long COVID symptoms: a cross-sectional cohort study in China. Lancet Infect Dis 2024; 24: 845–55. Open Access.
https://www.thelancet.com/journals/laninf/article/PIIS1473-3099(24)00171-3/fulltext