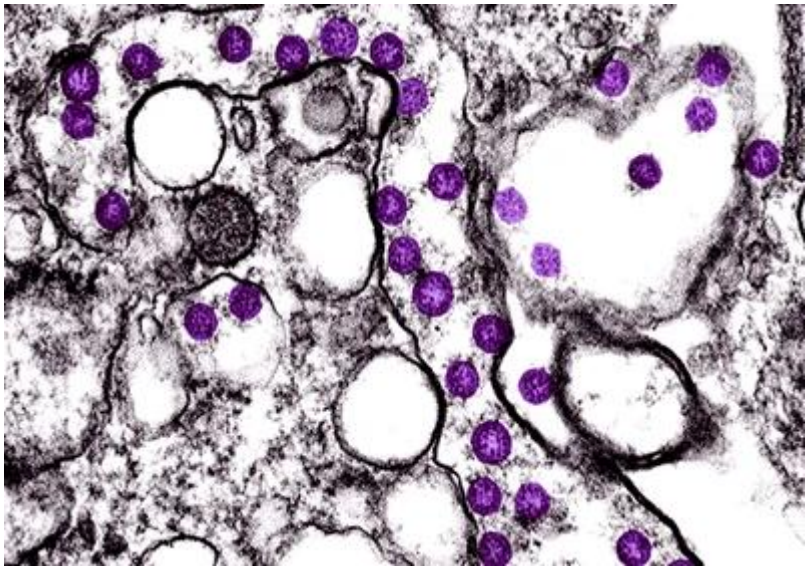
Residual SARS-CoV-2 RNA and proteins are persistent in solid tissue samples from various organs at one month, two months, and four months after mild COVID-19 | 1

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 have 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. This single-center, cross-sectional cohort study, conducted at China-Japan Friendship Hospital in Beijing, investigated 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, as well as in diverse sample types from recovered patients, including those from the lungs, breasts, skin, appendix, intestine, adenoid, tonsils, and olfactory neuroepithelium, among others. The virus has also been detected in stool and plasma samples.

The study, which examined the *post-mortem* 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. In all decedents, high-sensitivity droplet digital PCR (ddPCR) detected viral persistence in multiple tissue samples, but not in plasma. A detection of subgenomic RNA, a marker for recent viral replication, and the isolation of replication-competent viruses from respiratory and non-respiratory tissues suggested that viral replication may continue for several months after initial infection. In another study, residual SARS-CoV-2 was detected in surgically resected intestinal specimens 6 months after COVID-19, despite a negative nasopharyngeal PCR test. 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 In addition, in two patients with long COVID symptoms, residual viral protein and RNA were detected in the appendix, skin, and breast tissue 163 and 426 days after the onset of COVID-19. https://discovermednews.com/sars-cov-2-rna-and-antigens-appendix-skin-breast-patients-long-covid/



About the study

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

To detect SARS-CoV-2 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. In patients who underwent gastroscopy, both oropharyngeal swabs and gastric mucosa samples were collected to exclude potential contamination from the oral cavity.

To investigate whether immunocompromised individuals with hematological malignancies might face challenges in clearing the virus at 2 months post-infection, scientists 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.

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: 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).

Long COVID symptoms were assessed by follow-up telephone calls from trained physicians approximately 4 months after infection.

Results

The study included 225 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 and 21 (15%) patients without long COVID received chemotherapy or immunotherapy.

Before SARS-CoV-2 infection, 78% of patients with long COVID and 86% of individuals without long COVID 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. At 4 months post-infection, 66 samples from 9 different tissue types were collected from 49 patients.

At one month post-infection, SARS-CoV-2 RNA was detected in 30% of solid tissue samples, at two months in 27% of solid tissue samples, and at four months in 11% of solid tissue samples. 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 post-infection indicates a slow but ultimately effective viral clearance mechanism within the human body. Immunohistochemistry, 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.

During the entire period, RNA for SARS-CoV-2 nucleoprotein or ORF1ab was identified in samples of liver, kidney, stomach, intestine, brain, blood vessels, lungs, breast, skin, and thyroid tissue.

The authors also performed transcriptome sequencing in 24 lung 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 samples of blood vessels positive for SARS-CoV-2. These findings suggest that viral persistence may impact host cell functions.

Of nine patients with hematological malignancies, plasma from three patients, granulocytes from one patient, and PBMCs from two patients were positive for viral RNA. None of these blood compartments was 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 in throat swabs and the gastric mucosa, indicating that there was no contamination from nasal or oral sampling during gastroscopy.

Of the 213 patients who completed the telephone questionnaire, 34% reported at least one long COVID symptom. Fatigue (21%) was the most common symptom. Patients with higher viral copy numbers were more likely to develop long COVID symptoms.

Conclusion

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

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.

Journal Reference

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