Association of SARS-CoV-2 infection and persistence with long COVID

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Long COVID or post-COVID-19 condition can affect anyone exposed to SARS-CoV-2, regardless of age or severity of the original symptoms, characterised by long-term health problems persisting or appearing after the typical recovery period of COVID-19. WHO define long COVID as: "the continuation or development of new symptoms 3 months after the initial SARS-CoV-2 infection, with these symptoms lasting for at least 2 months with no other explanation". Although many studies on long COVID are underway, its pathogenesis remains unclear. As COVID-19 becomes increasingly prevalent in the coming years as the number of people who have been infected with SARS-CoV-2 increases, it is essential that we comprehensively understand the pathogenesis of long COVID to help patients, policymakers, and health-care systems develop and implement future strategies for prevention, treatment, and health-resource planning.

The longest bout of SARS-CoV-2 infection recorded to date was a patient in the UK who tested positive for COVID-19 for 505 days until her death.[1] 5 years after the end of the Ebola virus outbreak in 2016, the virus re-emerged in people who previously had Ebola virus disease in 2021.[2] Similarly, people who previously had West Nile virus infection with chronic symptoms still had positive urine RT-PCR tests 1·6–6·7 years after recovery from acute illness. Whether SARS-CoV-2 can also reactivate and retransmit after several years of dormancy is not yet known.

Researchers at the National Institutes of Health investigated the replication competence, persistence, and evolution of SARS-CoV-2 in human cells and looked for relevant histopathological features in infected tissues by performing autopsies on 44 COVID-19 cases. They found SARS-CoV-2 RNA widely distributed in 84 distinct anatomical locations up to 230 days after infection.[3] Surprisingly, viral persistence was detected by high-sensitivity droplet digital PCR (ddPCR) across multiple tissue samples among all deceased with infection cases despite being undetectable in plasma.[3] These findings suggest that the viral load in patients with COVID-19 might be low but still detectable in biospecimens with the appropriate assays following acute SARS-CoV-2 infection. In addition, the detection of subgenomic RNA, a marker of recent virus replication, and the isolation of replication-competent SARS-CoV-2 from respiratory and non-respiratory tissues, suggest that viral replication might occur for several months after the initial infection.[3] A Spanish group's autopsy study of 27 COVID-19 patients with long disease duration showed the presence of persistent SARS-CoV-2 RNA in autopsy tissue samples from patients that had a median duration of 39 days of infection (ranging 9 to 108 days).[4] These results and other case reports also suggest that immunocompromised patients, such as those with haematological malignancies, are more susceptible to persistent viral infection given their inability to mount a robust immune response. Importantly, Italian researchers reported on 27 consecutive patients who ostensibly recovered from COVID-19 but whose clinical condition progressively worsened despite testing negative for nasopharyngeal swabs or bronchoalveolar lavage samples. Subsequent autopsies revealed continued shedding of SARS-CoV-2 RNA in their lung tissue samples for up to 300 days after remission from acute infection, and people who previously had COVID-19 were more likely to report persistent post-COVID symptoms.[5] These results suggest that the SARS-CoV-2 virus had not been completely cleared from these patients. In addition to autopsy studies, researchers in Singapore found residual viral protein and RNA 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.[6] Similarly, researchers in Spain showed that SARS-CoV-2 was still present in surgically resected intestinal specimens 6 months after COVID-19 rehabilitation, despite negative nasopharyngeal PCR results.[7] Moreover, researchers in the USA and Austria found that viral RNA persisted in the stool of COVID-19 patients 7 months after diagnosis, which might imply that the gut of individuals with long COVID is a persistent viral reservoir.[8,9] Furthermore, a USA-based group reported that gastrointestinal symptoms (abdominal pain, nausea, and vomiting) are associated with the persistence of faecal

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SARS-CoV-2 RNA, 7 months after diagnosis.[8] Slovakian researchers reported the presence of SARS-CoV-2 in the cerebrospinal fluid of patients with long COVID.[10] The persistence of SARS-CoV-2 in the human olfactory neuroepithelium and associated inflammation were the cause of long COVID symptoms or relapses in post-COVID symptoms, such as loss of smell, as reported by researchers in France.[11] Time from the first onset of symptoms to inclusion in the study ranged from 110 to 196 days. A USA-based group described the presence of SARS-CoV-2 RNA and protein in the stools of 14 premature newborn babies delivered with negative nasal PCR results for SARS-CoV-2, whose mothers had been infected during pregnancy.[12] In another USA study, circulating SARS-CoV-2 spike antigen was detected in 60% of a cohort of 37 patients with long COVID within 12 months of diagnosis compared with those with non-long COVID.[13] These patients with non-long COVID were PCR confirmed COVID-19 convalescents with no reported prolonged symptoms sampled after diagnosis.

The presented evidence suggests that the duration of SARS-CoV-2 infection in patients can persist considerably longer than suggested by PCR-negative tests on nasopharyngeal swabs or bronchoalveolar lavage fluids. A US study including 40 947 participants with SARS-CoV-2 reinfection (two or more infections), 443 588 participants with a single SARS-CoV-2 infection, and 5 334 729 participants with no SARS-CoV-2 infection indicated that reinfection further increases risks of death, hospitalisation, and sequelae in multiple organ systems in the acute and post-acute phases.[14] Persistent low-grade multisystem injury in both adults and children might result from lingering SARS-CoV-2 infection or reinfection. In another study, the circulating SARS-CoV-2 components, spike protein, and viral RNA fragments persisted for up to 1 year or longer after SARS-CoV-2 acute infection in patients with long COVID, whereas these viral components decreased or were totally absent in convalescent patients with COVID-19, as shown by ddPCR and spike protein ELISA techniques.[15]

These findings provide further evidence that there might be a correlation between the length of time the virus stays in the body and the risk of long COVID (appendix p 1). Therefore, rapid viral elimination in patients with persistent SARS-CoV-2 virus could be crucial. We summarised the approved and ongoing, but unpublished, randomised trials on the nirmatrelvir with ritonavir (a SARS-CoV-2 main protease inhibitor) treatment for long COVID (appendix p 3) or persistence of SARS-CoV-2 in post-COVID-19 patients (appendix p 4) listed on ClinicalTrials.gov. The results of a recent observational study on nirmatrelvir with ritonavir and long COVID are promising and intriguing, and demand further investigation. The use of nirmatrelvir with ritonavir might eliminate viral reservoirs and alleviate long COVID symptoms.

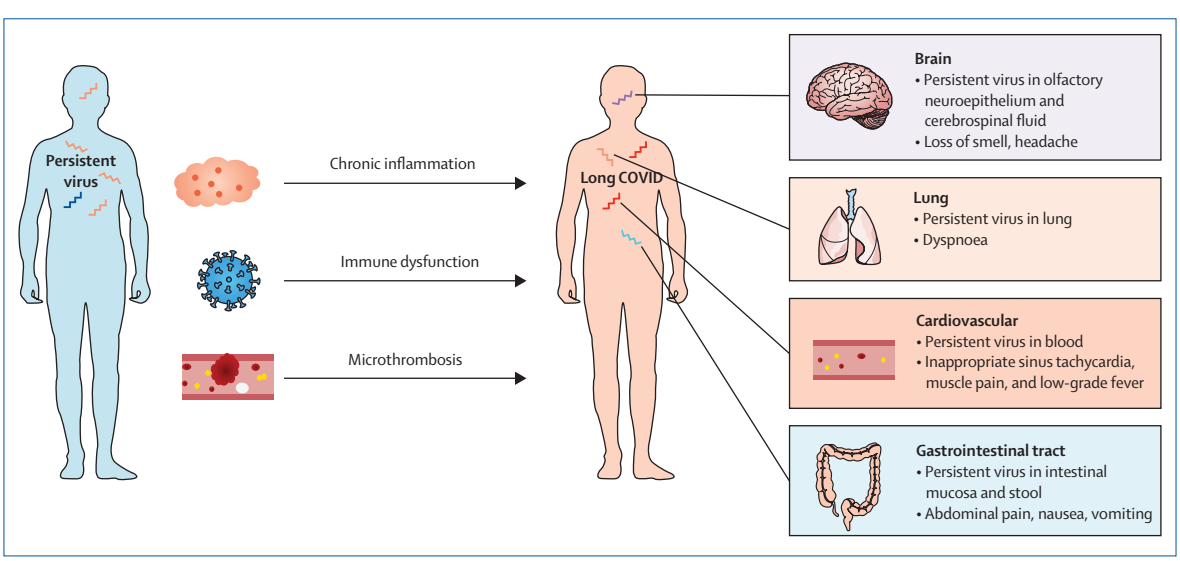

Figure: **Examples of long COVID driven by persistence of SARS-CoV-2**
Possible pathogenic mechanisms of persistent virus that might lead to chronic inflammation, immune dysfunction, microvascular endothelial damage, and microthrombosis seen in long COVID.

Here we present a pathophysiological model of long COVID based on the persistence of SARS-CoV-2 virus that triggers a dysregulation of the immune system, followed by increased release of inflammatory cytokines and abnormal endothelial damage, ultimately leading to the development of chronic inflammation, vascular damage, hypercoagulability, microthrombosis, and multiorgan symptoms (figure). Anti-SARS-CoV-2 and other applicable therapies might contribute to viral clearance or to the reduction of inflammation to improve long COVID symptoms. Here, we present recommendations and future directions for a more effective response to long COVID (appendix p 5). We believe that a comprehensive understanding of the pathogenesis of long COVID will provide an opportunity for better outcomes for all patients experiencing similar conditions.

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