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SARS-CoV-2 RNA in the Cerebrospinal Fluid of a Patient with Long COVID

Daša Viszlayová, Martin Sojka, Silvia Dobrodenková, Szabolcs Szabó, Ondrej Bilec, Mária Turzová, Juraj Ďurina, Barbara Baloghová, Zoltán Borbély and Martin Kršák

***Abstract*:** Over 10% of COVID-19 convalescents report post-COVID-19 complications, namely, 'long COVID' or 'post-COVID syndrome,' including a number of neuro-psychiatric symptoms. The pathophysiology of COVID-19 in the central nervous system is poorly understood but may represent post-COVID injury, ongoing sterile maladaptive inflammation, or SARS-CoV-2 persistence. We describe a long COVID patient with SARS-CoV-2 RNA in the cerebrospinal fluid, which seems important, specifically due to recent reports of gray matter volume loss in COVID-19 patients. Further studies of SARS-CoV2 RNA, markers of inflammation, and neuronal damage in the CSF of patients with long COVID would be useful and should address whether the CNS can serve as a reservoir of SARS-CoV-2, clarify the pathway by which COVID-19 contributes to CNS dysfunction, and how best to therapeutically address it.

Keywords: cerebrospinal fluid, COVID-19, long COVID, post-acute sequelae of COVID-19 (PASC), SARS-CoV-2

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Correspondence to:
Martin Kršák
Division of Infectious Diseases, Department of Medicine, School of Medicine, University of Colorado, Anschutz Medical Campus, 12700 East 19th Avenue Box 168, Aurora, CO 80045, USA.
martin.krsak@cuanschutz.edu

Daša Viszlayová
Department of Neurology, Faculty Hospital Nové Zámky, Nové Zámky, Slovakia

Department of Neurology, Faculty of Medicine, Charles University, Hradec Králové, Czech Republic

Martin Sojka
Regional Public Health Authority in Komárno, Komárno, Slovakia

Silvia Dobrodenková
Travel Health Clinic Bratislava, Bratislava, Slovakia

Szabolcs Szabó
Ondrej Bilec
Mária Turzová
Department of Neurology, Faculty Hospital Nové Zámky, Nové Zámky, Slovakia

Juraj Ďurina
Barbara Baloghová
Zoltán Borbély
Department of Internal medicine, Faculty Hospital Nové Zámky, Nové Zámky, Slovakia

Background

Neurological symptoms associated with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections are common. SARS-CoV-2 may not always be present in the cerebrospinal fluid (CSF) of patients with acute neurological manifestations,[1] although some reports of SARS-CoV-2 RNA in the CSF during acute COVID-19 exist.[2] Other studies report inflammatory response with cytokine release.[3] Long COVID is a term to describe the effects of acute coronavirus disease of 2019 (COVID-19) that continue for weeks or months beyond the initial illness. We report the case of a woman with long COVID and SARS-CoV-2 RNA real-time reverse transcriptase polymerase chain reaction (RT-PCR) detection in the CSF, which seems important given that at least two separate recent reports describe gray matter volume loss in various regions of the brain immediately, as well as within a few months after COVID-19.[4,5] Together, these two studies provide both a comparison of patients with and without recent COVID-19, (4 – this should be a supersript referring to reference number 4) and a comparison of brain scans before and after COVID-19. (5 – this should be a supersript referring to reference number 5)

Case report

On 1 October 2020, a 42-year-old woman had a positive SARS-CoV-2 RT-PCR assay from her nasopharyngeal swab. At the time, she reported only cough and diarrhea. After 10 days, repeated nasopharyngeal RT-PCR test was negative and the patient was asymptomatic. On 23 November 2020, she had a recurrence of her previous mild viral illness–related symptoms and also developed a loss of smell and taste: her nasopharyngeal antigen test for SARS-CoV-2 was positive. Two weeks later, she became fatigued and developed anxiety, palpitations, diarrhea, vertigo, and perioral tingling. On 27 January 2021, she was hospitalized with a progressive headache, dizziness, anxiety, palpitations, diarrhea, and panic attacks. Neurological examination revealed hyperreflexia in the lower limbs and malaise. Routine blood tests, basic immunological and serological screening, and anti-neuronal antibodies were all negative. Brain magnetic resonance and electroencephalography showed no pathological changes. Psychological and psychiatric assessment revealed increased tension and depression, which was not present before. A 24-h electrocardiography (ECG) monitoring confirmed sinus tachycardia, and

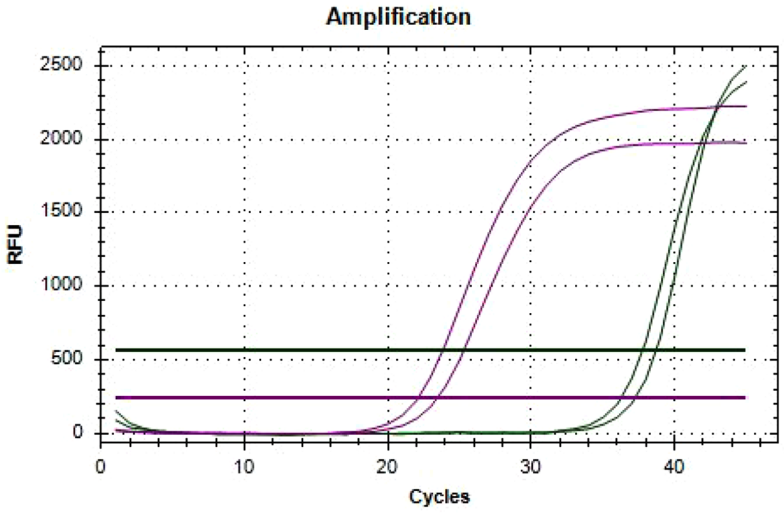

Figure 1. Amplification plot showed RT-PCR detection of SARS-CoV-2 E gene (green curve, Ct 37.73) and RdRp gene (green curve, CT 38.17), together with the human RNA internal standards (purple lines) (Bio-Rad CFX96 instrument, Bio-Rad CFX Maestro 1.1 software).

a transthoracic echocardiogram showed no abnormalities. Due to the new-onset diarrhea, proctoscopy was performed to exclude colitis. Stool studies for common viral (excluding SARS-CoV-2), bacterial, and parasitic pathogens were unrevealing and fecal calprotectin was also negative. Her stool studies showed heavy growth of *Candida glabrata* susceptible to fluconazole.

Antigen and RT-PCR tests for SARS-CoV-2 from nasopharyngeal swabbing were negative at the time of admission, but we confirmed detectable serum nucleocapsid immunoglobulin G (IgG) antibodies against SARS-CoV-2 (the patient had not been previously vaccinated against COVID-19 and anti-spike antibodies were not tested). Her CSF analysis showed mild elevation of protein (0.505 g/l) and lactate dehydrogenase (LDH) (0.57 µkat/l), although other parameters were in normal ranges (neutrophil count, 2 cells/µl; lymphocyte count, 0 cell/µl; erythrocyte count, 10 cells/µl; lactate, 1.55 mmol/l; glucose, 3.75 mmol/l; chloride, 128 mmol/l). CSF studies for bacteria, mycobacteria, fungi, and common neurotropic viruses were negative, as was an evaluation of oligoclonal bands.

RT-PCR for the detection of SARS-CoV-2 RNA in the CSF was performed with Charité/Berlin primers and probes,[6] targeting E gene for the coronavirus screening and RdRp gene as the confirmatory assay. Briefly, viral RNA was stabilized in CSF with equal volume of DNA/RNA Shield solution (ZymoResearch, CA, USA) and aliquoted to prevent RNA degradation and contamination of the original sample. Viral RNA was extracted from the CSF using Quick-RNA Viral Kit (ZymoResearch, CA, USA), and RT-PCR assays were performed using Reliance One-Step Multiplex RT-PCR Supermix (Bio-Rad Laboratories, CA, USA) in CFX96 Touch Real-Time detection system (Bio-Rad). Thermal cycling ran at 55°C for 10 min for reverse transcription, followed by denaturation for 10 min at 95°C and then 45 amplification cycles: 10 s at 95°C and 30 s at 58°C. The CSF was tested in three replicates for each gene and was considered clearly positive at cycle threshold (Ct) of 38.94, 37.22, and 38.17, respectively, for the RdRp gene (Figure 1). The time between the first positive nasopharyngeal SARS-CoV-2 RT-PCR and the positive CSF was 114 days (from the positivity in October) and was 62 days from the separate onset of symptoms on 23 November (since we do not have genomic analysis to distinguish a relapse from a reinfection) – (ZymoResearch: CA, USA), (Bio-Rad Laboratories: CA, USA).

Based on the disease course, the presumed diagnosis was long COVID associated with the presence of viral RNA in the central nervous system (CNS). Due to the lack of scientific evidence, we initiated pulse therapy with methylprednisolone (500 mg/day intravenously for three consecutive days), suspecting an autoimmune post-viral reaction to COVID-19 affecting the CNS. We also initiated fluconazole, symptomatic therapy with intravenous multivitamin solutions, alprazolam, beta-blocker, physiotherapy, and psychological support. Eight days later, we repeated the lumbar puncture which again showed mild elevation of proteins (0.451 g/l) and LDH (0.62 µkat/l), and the other parameters remained in normal ranges (neutrophil count, 0 cells/µl; lymphocyte count, 1 cell/µl; erythrocyte count, 4 cells/µl; lactate, 1.51 mmol/l; glucose, 3.21 mmol/l; chloride, 127.6 mmol/l). Repeat RT-PCR for SARS-CoV-2 from the CSF was negative. After the treatment, the patient's symptoms were mostly alleviated, but she continued to have attacks of headache and anxiety.

Discussion

SARS-CoV-2 exhibits neurotropism for CNS and peripheral nervous system.[7,8] The virus could enter the CNS by several possible mechanisms.

Two basic pathways responsible for CNS invasion are hematogenous and neuronal spread.[7] The olfactory neuron dysfunction represents one of the neuronal and non-neuronal pathways for SARS-CoV-2 entry into the brain.[7] In this mode, the unique anatomical organization of olfactory nerves and the olfactory bulb in the nasal cavity and forebrain forms a 'channel' between the nasal epithelium and the brain compartments, especially the brainstem, containing the respiratory and cardiovascular centers. Several analyses indicate that the spike protein of SARS-CoV-2 binds to the angiotensin-converting enzyme 2 (ACE2) protein.[7] The ACE2 receptors have been detected in the glial cells and neurons, particularly in the brainstem and the regions responsible for the regulation of cardiovascular function, including the solitary nucleus, subfornical organ, paraventricular nucleus, and rostral ventrolateral medulla. Other hypotheses of virus entry to the CNS also include peripheral immune cell transmigration (the 'Trojan horse' mechanism).[9] The damage to the CNS and the involvement of neuroimmunological pathways could be particularly relevant for many neurological and neuro-psychiatric symptoms, and these effects do not seem to spare pediatric patients either.[9–11]

Approximately 10% of recovered COVID-19 patients face persistent physical, cognitive, and psychological symptoms well past the acute phase. However, the exact pathophysiology of long COVID and particularly the effects within CNS are not yet understood. Probable mechanisms described in the literature include maladaptive hyperinflammation of various tissues (e.g. vascular endothelium), which may be due to an exaggerated cytokine release. Such response may be triggered by the interaction between SARS-CoV-2 and the immune system, but also other compartments (e.g. endothelium and other cell lines capable of interacting with the virus via ACE2 receptors, prompting complex sequential physiological cascades). Viral reservoirs or lingering fragments of viral RNA/proteins could also contribute to this maladaptive response.[12] Gaebler *et al.*[13] discussed immune evolution and possible influence of immunofluorescence and PCR-confirmed SARS-CoV-2 persistence in intestinal biopsies from asymptomatic individuals 4 months after the onset COVID-19. These reservoirs could repeatedly stimulate the immune system and be responsible for the fluctuating course of symptoms in long COVID patients. Due to numerous neurological symptoms in the long COVID patients, the question remains whether the virus persists in the CNS, how such persistence contributes to the symptomatology, and subsequently how best to address it.

Little is known about the role of CSF analysis in COVID-19 patients with neurological symptoms. Several CSF studies have not found a consensus on how COVID-19 can be associated with these neurological symptoms.[1,14] Some investigators have found anti-SARS-CoV-2 spike IgG antibodies in several patients with encephalopathy.[15] SARS-CoV-2 antibodies may or may not represent an actual presence of SARS-CoV-2 in the CNS at any point during or after COVID-19. Even if such antibodies cross the blood–brain barrier hematogenously or are brought in via the Trojan horse mechanism, their presence may signify certain capacity for spill-over of the systemic SARS-CoV-2 infection or inflammation into the CNS, where such process may be able to contribute to potentially deleterious processes. Other possible CNS damages could be due to the direct effect of SARS-CoV-2 binding to the ACE2 expressed in capillary endothelium of blood–brain barrier to gain access to the CNS or by indirect effect of the cytokine storm on mitochondria or on the nerve fibers.[16]

Other investigators reported inflammatory markers or signs of neuronal damage.[3]

Currently, the paucity of available data mainly includes reports on the CSF analysis in patients with neurological manifestations during acute COVID-19, and no study to our knowledge has evaluated various pertinent parameters in the CSF of patients with long COVID. Further input on possible pathways leading to dysfunction and how to counteract these effects could also lead through studies of neuroglia (e.g. astrocytes), vascular pericytes, and autoantibodies against cerebral structures and specific inflammatory patterns (also found in children with long COVID).[17–20]

The persistence of a replicable virus in the CNS is only one of the possible explanations for our patient's presentation, particularly since the typical COVID-19 symptoms (loss of smell and taste) were accompanied by other, less specific symptoms of fatigue, anxiety, headaches, and tingling.

Furthermore, the relevance of SARS-CoV-2 presence in the CSF is unclear also because the RNA was detected at relatively high Ct values. False-positive results are possible with RT-PCR technology, although it is quite infrequent.[6,21] CSF sample was collected under standard aseptic conditions and was without blood contamination. All the routine preventive measures were taken to avoid possible laboratory cross-contamination of CSF. Moreover, the sample was then aliquoted into three separate analyses (different RNA isolation and PCR runs) to further minimize the possibility of a false-positive result. Although we cannot strictly exclude the possibility of a false positive result, it is plausible to consider CNS viral persistence as a possible mechanism of long-term symptoms at least in some patients.

Conclusion

To our knowledge, this is the first report to confirm the occurrence of SARS-COV-2 RNA in the CSF of a patient with long COVID specifically. This case raises the possibility that SARS-CoV-2 may persist in the central nervous system weeks after respiratory infection.

Further studies of SARS-CoV2 RNA, markers of inflammation, and neuronal damage in the CSF of patients with long COVID would be useful and should address the following questions:

Is the CNS a possible reservoir of SARS-CoV-2 persistence, and if so, what are the consequences to the overall and neuropsychiatric health?
With or without the contribution of viral persistence, what are the characteristics of the inflammatory response in the CNS and how can it be therapeutically addressed?
Is there a clear clinical and physiological distinction between the post-COVID syndrome (the damage caused by the CNS inflammation during acute COVID-19) and long COVID, or is persistent inflammation with or without viral persistence necessary for long COVID to develop?

Author contributions

All authors reviewed and approved the final manuscript prior to submission. DV, MS, SS, OB, MTJD, BB and ZB collected patient-level data. MS performed the PCR analysis and evaluated the results. DV, SD and MK performed literature search and review. All authors' writing contributed equally to the development of the manuscript.

Conflict of interest statement

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Informed consent

The patient provided verbal informed consent for the publication of this case report.

ORCID iDs

Ondrej Bilec https://orcid.org/0000-0002-3735-6356

Martin Kršák https://orcid.org/0000-0002-1746-7462

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