



# First case of SARS-COV-2 sequencing in cerebrospinal fluid of a patient with suspected demyelinating disease

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## Abstract

The association between coronaviruses and central nervous system (CNS) demyelinating lesions has been previously shown. However, no case has been described of an association between the novel coronavirus (SARS-COV-2) and CNS demyelinating disease so far. SARS-COV-2 was previously detected in cerebrospinal fluid (CSF) sample of a patient with encephalitis. However, the virus identity was not confirmed by deep sequencing of SARS-COV-2 detected in the CSF. Here, we report a case of a patient with mild respiratory symptoms and neurological manifestations compatible with clinically isolated syndrome. The viral genome of SARS-COV-2 was detected and sequenced in CSF with 99.74–100% similarity between the patient virus and worldwide sequences. This report suggests a possible association of SARS-COV-2 infection with neurological symptoms of demyelinating disease, even in the absence of relevant upper respiratory tract infection signs.

**Keywords** COVID-19 · SARS-COV-2 · Clinically isolated syndrome · Cerebrospinal fluid

## Background

The novel coronavirus (SARS-COV-2) is associated with respiratory symptoms. There have been reports of COVID-19 associated neurological manifestations. The viral genome was demonstrated by RT-PCR technique in cerebrospinal fluid sample (CSF), suggesting that the virus has the ability to infect central nervous system (CNS) [1]. The association between other coronaviruses and CNS demyelinating lesions has been studied [2, 3]. However, no case has been described of an association between SARS-COV-2 and CNS demyelinating disease so far.

## Case report

A 42 year-old patient, resident in São Paulo, sought neurological consultation due to paresthesias of the left upper limb, later progressing to left hemithorax, and hemiface. Upon neurological examination, she had hypoesthesia in the above-mentioned regions. The patient also had mild respiratory symptoms that included coryza and nasal obstruction without fever for 3 weeks. RT-PCR for SARS-COV-2 of nasal and pharyngeal swab and cerebrospinal fluid (CSF) was carried out. She had a similar neurological clinical picture 3 years ago with spontaneous full recovery of symptoms. As the symptoms were exclusively sensitive and due to the association with SARS-COV-2 infection, the patient was not treated with corticosteroids. The patient had full recovery after 3 weeks. Specific SARS-COV-2 RNA primers and probe directed to RDRP-2 gene described WHO (Charité, Berlin) were used. A control CSF examination was carried out 16 days later.

Blood cell counts, transaminases, bilirubin, CPK, coagulogram, electrolytes, renal function, and C-reactive protein were all normal. CSF analysis showed 1 WBC/mm<sup>3</sup>, protein of 32 mg/dl, and glucose of 68 mg/dl. No CSF oligoclonal bands were demonstrated. Brain magnetic resonance

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**Fig. 1** Cervical spinal cord with a small left lateral ventral lesion with T2/STIR hypersignal, without mass effect, without gadolinium enhancement, measuring about 0.4 cm in its sagittal plane

imaging (MRI) was normal. Cervical MRI is shown in Fig. 1. Chest tomography was normal. Serology for HIV, viral hepatitis, syphilis, as well as antinuclear antibodies, anti-SSA, and anti-SSB antibodies were all negative. Vitamin B12 and folic acid levels were within normal ranges. The clinical diagnostic hypothesis was a clinically isolated syndrome (CIS). RT-PCR for SARS-COV-2 was positive in the first CSF sample, negative in nasal and pharyngeal samples, and negative in control CSF.

To confirm the identity of the virus in CSF identified in the CSF sample, we deep sequenced the material using the MinION platform from Oxford Nanopore technology as described in (<https://www.protocols.io/view/ncov-2019-sequencingprotocol-bbmuik6w>). Reads were mapped against MN908947.3 reference genome using CLC genomic workbench v.16 (Qiagen). Due to the low viral load present on the LCR, a full-genome consensus was not obtained. Regions having the better coverage of the genome (> 200) were used to the analysis. Therefore, two fragments from ORF1a were obtained and concatenated resulting in a 1580-nucleotide long sequence that was multiple-aligned together to 200 worldwide representative SARS-COV-2 reference genomes (available at GISAID). An identity matrix was generated, and revealed 99.74–100% similarity between the patient virus and worldwide sequences. No additional regions from the patients SARS-COV-2 genome other than the used for

similarity analysis were obtained with enough quality to allow a more detailed investigation on putative nucleotide or aminoacid particular substitutions. Institutional Ethical Board approval and written consent were obtained.

## Discussion

Here, we report a case of SARS-COV-2 infection with a clinical presentation compatible with CIS [4]. The diagnosis of CIS was established, since the patient had a clinical attack involving a single anatomical region and did not have dissemination in space either clinically or by MRI, no oligoclonal bands were found, and no better explanation was found by clinical investigation [4]. The lesion site seems to justify the symptoms, including facial symptoms, due to possible involvement of spinal trigeminal nucleus. To the best of our knowledge CNS demyelinating disease has not been associated with COVID-19 so far; however, other coronaviruses were previously associated with CNS demyelinating autoimmune diseases, including MS exacerbations [5] and autoreactive T cells able to recognize myelin antigens [6, 7]. A possible explanation is that SARS-COV-2 entry into the CNS may have led to this exacerbation.

One single report describes CSF positivity for SARS-COV-2 by the RT-PCR technique [1]. To the best of our knowledge, this is the first report to confirm the identity of SARS-COV-2 in CSF with deep sequencing. There are multiple proposed mechanisms for SARS-COV-2 entry into the CNS. As already studied for other coronaviruses, SARS-COV-2 could move via olfactory nerve [8] or by hematogenous spread [9]. The fact that the PCR was negative in the oropharyngeal swab may be due to the duration of the symptoms, since the patient had respiratory symptoms for 3 weeks when the swab was collected. However, the present case does not suggest chronic CNS infection, since the CSF control RT-PCR was negative. One possibility is that SARS CoV-2 infection is more persistent in the CNS, since it is a more immunoprivileged site. Another possibility is that after the initial stage of replication in cells of the respiratory system, the SARS-COV-2 infects blood cells that can cross blood–brain barrier allowing virus to pass into the CNS [10].

This case report suggests a possible association between CNS focal symptoms compatible with demyelinating disease and SARS-COV-2 infection. This report should alert clinicians to this possible association, even in the absence of relevant upper respiratory tract infection signs.

## Compliance with ethical standards

**Conflicts of interest** On behalf of all authors, the corresponding author states that there is no conflict of interest.

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