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Patients with COVID-19 and neurological manifestations show undetectable SARS-CoV-2 RNA levels in the cerebrospinal fluid

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ABSTRACT

We report that patients with COVID-19 displaying distinct neurological disorders have undetectable or extremely low levels of SARS-CoV-2 RNA in the cerebrospinal fluid, indicating that viral clearance precede the neurological involvement. This finding points to the need for the development of more sensitive molecular tests and the investigation of other neurotropic pathogens to exclude concurrent neuroinfection.
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In December 2019, cases of severe acute respiratory syndrome (SARS) emerged from Wuhan, China, and were after associated with a novel Coronavirus (SARS-CoV-2). Since then, reports have described neurological manifestations in addition to the typical clinical symptoms of the Coronavirus disease 2019 (COVID-19), represented by fever, cough, diarrhea, and fatigue. Hospitalized patients with COVID-19, especially those with severe disease, can display neurological disorders as shown by Mao et al. (2020), such as acute cerebrovascular disease in up to 5.7%, impaired consciousness in 14.8%, and skeletal muscle injury in 19.3% of cases. Nonetheless, COVID-19 seems to be associated with a wide spectrum of neurological manifestations, including meningoencephalitis (Bernard-Valnet et al., 2020; Dogan et al., 2020; Moriguchi et al., 2020), encephalomyelitis (Zanin et al., 2020), Guillain-Barré Syndrome (GBS) (Toscano et al., 2020; Coen et al., 2020; Ottaviani et al., 2020; Alberti et al., 2020; Juliao Caamaño and Alonso Beato, 2020), and perfusion abnormalities in brain magnetic resonance imaging (MRI) (Helms et al., 2020). However, opposed to prolonged viral RNA detection in nasopharyngeal swabs (≥20 days after symptoms onset) and in feces (To et al., 2020), attempts of our research group and others to detect SARS-CoV-2 RNA in the cerebrospinal fluid (CSF) of patients with neurological manifestations indicative of central nervous system (CNS) infection has been shown frustrated so far, except for one case of meningoencephalitis (Table 1). CSF analysis showed normal to mild elevated protein levels, and pleocytosis was particularly observed in the cases of meningoencephalitis (Table 2). Indeed, CNS infiltrate was predominantly constituted by mononuclear cells, which is compatible with viral infection (Table 2). Panciani et al. (2020) hypothesized a three-step model to explain the CNS invasion by SARS-CoV-2 and the resulting neurological involvement, suggesting that the viral load in CSF progressively increases and it triggers an inflammatory response, but the viral clearance precede the occurrence of indirect SARS-CoV-2 effects on the CNS. Neurological manifestations in COVID-19 generally arise between 1 to 14 days after the beginning of infectious symptoms (Mao et al., 2020), and it has been predicted a mean incubation period of 5 days (time between infection and symptoms onset) (Li et al., 2020). Therefore, considering the time between infection and the lumbar puncture (Table 1), CSF may be devoid of virus particles, even if patients have a positive result for SARS-CoV-2 RNA on nasopharyngeal swabs. Head computed tomography or MRI data showing distinct patterns of neurological damage (Al Saiegh et al., 2020;

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Table 1
SARS-CoV-2 detection in CSF of COVID-19 patients with neurological manifestations.

Neurological outcomes	Number of cases	Days between CSF withdrawn and symptoms onset	RT-qPCR in nasopharyngeal swab	RT-qPCR in CSF	Other viruses tested negative in CSF	References
Meningoencephalitis (1), Encephalitis (1), Facial palsy (2), delirium (2), intracranial hypertension (1), new daily persistent headache (1)[a]	8	2 to 10	positive	negative	HSV-1/2, VZV, CMV, EBV, HHV-6, Influenza A and B viruses	This study[b]
Meningoencephalitis	1	9	negative[c]	positive	n.i.	Moriguchi et al. (2020)
Meningoencephalitis	2	5 and 17	positive	negative	Enterovirus, HSV-1/2, VZV, CMV, HHV-6, Parechovirus	Bernard-Valnet et al. (2020)
Autoimmune meningoencephalitis	6	n.i.	positive	negative	Common seasonal viruses (not specified)	Dogan et al. (2020)
Acute disseminated encephalomyelitis	1	n.i.	positive	negative	Neurotropic viruses (not specified)	Zanin et al. (2020)
GBS	5	9 to 13	4 positive and 1 negative[c]	negative	n.i.	Toscano et al. (2020)
GBS	1	10	positive	negative	Enterovirus, HSV-1/2, VZV, CMV, HHV-6, Parechovirus	Coen et al. (2020)
GBS	1	10	positive	negative	n.i.	Ottaviani et al. (2020)
GBS	1	7	positive	negative	n.i.	Alberti et al. (2020)
Facial diplegia (GBS variant)	1	11	positive	negative	n.i.	Juliao Caamaño and Alonso Beato (2020)
Perfusion abnormalities in brain MRI	7	n.i.	positive	negative	n.i.	Helms et al. (2020)
Acute cerebrovascular disease	2	7 and 10	positive	negative	n.i.	Al Saiegh et al. (2020)
Encephalitis	1	19	positive	negative	n.i.	Ye et al. (2020)
Encephalitis	1	5	positive	negative	Enterovirus, HSV-1/2, VZV, EBV, HHV-6, HHV-8, Adenovirus	Pilotto et al. (2020)

Notes. CSF, cerebrospinal fluid; RT-qPCR, reverse transcriptase quantitative polymerase chain reaction; HSV-1/2, herpes simplex virus 1 and 2; VZV, varicella zoster virus; EBV, Epstein-Barr virus; CMV, cytomegalovirus; HHV-6, human herpes virus 6; GBS, Guillain-Barré Syndrome; n.i., not informed. [a] Number of cases of each neurological manifestation observed in our cohort of COVID-19 patients is shown inside parenthesis; [b] Detection of SARS-CoV-2 RNA was carried out with the Biomanguinhos (E + P1) RT-qPCR kit (FIOCRUZ, Brazil); [c] Confirmed by serologic test in serum sample.

Table 2
Characteristics of the CSF of COVID-19 patients with distinct neurological manifestations.

Neurological manifestations	Number of cases	CSF cell count (cells/μL)[a]	Mononuclear cells (%)	Polymorphonuclear cells (%)	Protein (mg/dL)[b]	Glucose (mg/dL)[c]	References
Meningoencephalitis	1	18	100	0	60.0	43.0	This study
Encephalitis	1	2	100	0	23.0	96.0	This study
Facial palsy	2	3	100	0	40.5	56.5	This study
Delirium	2	3	100	0	51.5	115.0	This study
Intracranial hypertension	1	1	100	0	19.0	56.0	This study
New daily persistent headache	1	1	100	0	27.0	63.0	This study
Meningoencephalitis	1	12	83	17	n.i.	n.i.	Moriguchi et al. (2020)
Meningoencephalitis	2	19	93	7	46.4	normal	Bernard-Valnet et al. (2020)
Autoimmune meningoencephalitis	6	0	-	-	69.4	116.3	Dogan et al. (2020)
Acute disseminated encephalomyelitis	1	normal	n.i.	n.i.	normal	normal	Zanin et al. (2020)
GBS	5	0[d]	n.i.	n.i.	83.2[e]	normal	Toscano et al. (2020)
GBS	1	n.i.	n.i.	n.i.	n.i.	n.i.	Coen et al. (2020)
GBS	1	0	-	-	108	n.i.	Ottaviani et al. (2020)
GBS	1	9	n.i.	n.i.	54	n.i.	Alberti et al. (2020)
Facial diplegia (GBS variant)	1	0	-	-	44	n.i.	Juliao Caamaño and Alonso Beato (2020)
Perfusion abnormalities in brain MRI	7	0	-	-	normal (except for one patient)	n.i.	Helms et al. (2020)
Acute cerebrovascular disease	2	n.i.	n.i.	n.i.	n.i.	n.i.	Al Saiegh et al. (2020)
Encephalitis	1	1	n.i.	n.i.	27	56.5	Ye et al. (2020)
Encephalitis	1	18	n.i.	n.i.	69.6	n.i.	Pilotto et al. (2020)

Notes. Mean values are shown for cerebrospinal fluid (CSF) cell counts, protein, and glucose levels in groups of neurological manifestations with more than 1 case; [a] Normal CSF cell counts: ≤5 cells/μL; [b] Normal range for CSF protein: 15 – 40 mg/dL; [c] Normal range for CSF glucose: 40 – 80 mg/dL; [d] Five patients presented no cells and one patient had 3 cells/mm^3. [e] Two patients had normal CSF protein levels without specified values; therefore, a concentration of 30 mg/dL was assumed to calculate a mean value. GBS, Guillain-Barré Syndrome; n.i., not informed.

Dogan et al., 2020; Helms et al., 2020; Zanin et al., 2020) and the undetectable levels of SARS-CoV-2 RNA in CSF promptly collected at the onset of neurological symptoms (Table 1) corroborate with this picture. Moreover, the suggestive para-infectious process of GBS cases (Alberti et al., 2020; Coen et al., 2020; Ottaviani et al., 2020; Toscano et al., 2020) and the spontaneous recovery of encephalitis in patients with COVID-19 also supports the idea of transient SARS-CoV-2 dissemination and extremely low CSF viral load (Ye et al., 2020). Thus, considering the sole report of a severe case of meningoencephalitis with detectable SARS-CoV-2 RNA in CSF (Moriguchi et al., 2020), likely presenting a low viral load since just one of two RT-qPCR assays turned positive at a cycle threshold of 37, we can assume that whether SARS-CoV-2 is detectable in CSF, it may depend on disease severity, the time of sample collection or the sensitivity of the molecular test used. On the other hand, herpes simplex viruses 1 and 2 (HSV-1/2), varicella zoster virus (VZV), and enterovirus are responsible for the greatest number of CNS infection in immunocompetent hosts (Bookstaver et al., 2017). Therefore, CSF investigation of COVID-19 patients presenting with GBS or CNS manifestations to exclude concurrent neuroinfection with other neurotropic viruses should be encouraged.

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Ethical statement

This study was approved by the Brazilian National Committee of Ethics in Research (CAAE: 30611720.6.0000.5262), and written informed consent was obtained from all patients.

Conflict of interest statement

The authors declare no conflict of interest.

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