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BRIEF REPORT

# Cerebrospinal Fluid Features in Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Reverse Transcription Polymerase Chain Reaction (RT-PCR) Positive Patients

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This study analyzed the cerebrospinal fluid features of 31 coronavirus disease 2019 (COVID-19) patients with neurological complications. We observed neither severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA in the cerebrospinal fluid, nor intrathecal immunoglobulin G (IgG) synthesis but did observe signs of blood-brain barrier disruption. These results might serve as a basis for a better understanding of SARS-CoV-2 related neuropathogenesis.

**Keywords.** SARS-CoV-2; neurological manifestations; COVID-19; intrathecal synthesis; blood-brain barrier.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is responsible for the coronavirus disease 2019 (COVID-19) pandemic. Typical clinical manifestations include cough, dyspnea, fever, and fatigue, with a minority of patients evolving toward bilateral pneumonia, sometimes leading to acute respiratory distress syndrome [1]. Among less typical features, neurological complications have been described in SARS-CoV-2 infected patients, including meningo-encephalitis, encephalopathy, stroke, and Guillain-Barré syndrome [2–5]. Currently, the central nervous system tropism and neuropathogenesis of SARS-CoV-2 have not been fully elucidated. ACE-2 identified as the receptor required for virus entry [6] is expressed not only in the lungs but also in the vascular endothelium and the central nervous system [4]. Several articles have described the neurological manifestations of SARS-CoV-2 in large series of patients, but cerebrospinal fluid (CSF) parameters have only been analyzed in a limited number of patients [4, 5, 7]. The aim of this retrospective observational study was to report CSF features of 31 patients with documented SARS-CoV-2 infection associated with neurological disorders.

## MATERIAL AND METHODS

### Patients

We retrospectively included 31 patients who were hospitalized or consulted in the emergency department between 26 February 2020—the beginning of the epidemic in Switzerland—and 7 May 2020. Inclusion criteria were a positive real-time reverse transcription polymerase chain reaction (rRT-PCR) for SARS-CoV-2 in a respiratory specimen and a lumbar puncture on the same day or thereafter, due to acute neurological manifestations. We retrospectively collected epidemiological and biological data, the patient type (outpatient or hospitalized in an intensive or a nonintensive care unit), and the medical history of COVID-19. Neurological manifestations confirmed by neurologists were reviewed and classified into 5 categories: encephalopathy (confusion, impaired consciousness, delayed awakening), isolated headache, meningeal syndrome, seizure, and peripheral nervous system manifestations (polyneuropathy). The work was approved by the local ethics committee.

### CSF Analysis

Cytological examination of CSF was performed for 33 CSF samples from 31 patients. The blood-brain-barrier (BBB) integrity was evaluated using the albumin quotient (CSF albumin/serum albumin) with age-corrected cutoffs and the Reiber formula.

### Intrathecal Synthesis SARS-CoV-2 IgG

The immunoglobulin G (IgG) antibody index (AI) calculation was determined according to Reiber's method. Eighteen patient's paired CSF and serum, sampled less than 2 days apart, were analyzed on an Agility ELISA system (Dynex, Denkendorf, Germany) with the Euroimmun Anti-SARS-CoV-2-ELISA (IgG) kit (Euroimmun, Luebeck, Germany). An AI > 2.0 reflects a specific anti-SARS-CoV-2 IgG intrathecal production.

## RESULTS

### Patients

The study included COVID-19 patients (n = 31), confirmed by a positive SARS-CoV-2 rRT-PCR in a respiratory specimen,

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who displayed new neurological manifestations at the time of or after diagnosis, and who underwent lumbar puncture. The majority of patients (29/31) were hospitalized, 58% in the intensive care unit (ICU), and 35% in the nonintensive care unit (Table 1). The median age of patients was 66 years (range 39–85 years), and the oldest patients were those in the ICU (69 years; range 42–83 years). The majority were male (71%), particularly in the ICU (83%).

### Neurological Manifestations

Neurological manifestations were classified into 5 groups: 19 patients (61%) had encephalopathy, 3 (10%) isolated headache, 4 (13%) meningeal syndrome, 1 (3%) seizures, 3 (10%) Guillain-Barré syndrome, and 1 (3%) a critical illness polyneuropathy (Table 1). Encephalopathy, the most observed neurological manifestation, was more frequent in patients hospitalized in the ICU (83%). The median time from the onset of symptoms associated with COVID-19 to the time of lumbar puncture was 23 days; it was longer in patients hospitalized in the ICU (26 days), compared to patients not requiring ICU admission (8 days).

### CSF Analysis

All patients test results were negative for SARS-CoV-2 RNA in the CSF by rRT-PCR (Table 1). Among 33 CSF specimens, elevated white blood cell (WBC) counts were noted for 3 patients. The first had concomitant pneumococcal meningitis, and the second had a Waldenstrom macroglobulinemia and suspicion of Bing-Neel syndrome. Interestingly, the third patient was a 45 year-old female presenting with a meningeal syndrome. CSF WBC count was 99 M/L, with 99% lymphocytes. An extensive microbiological workup was negative, including SARS-CoV-2 rRT-PCR in CSF, as well as the AI.

The predominant cells were lymphocytes. Macrophages were observed in 60% of CSF specimens. CSF protein and albumin levels were increased in 39% and 23%, respectively. CSF/plasma albumin ratio (Qalb), was increased in 58% of 19 tested patients, indicating a disturbance of the BBB integrity (S5). CSF protein, albumin levels, and Qalb were well correlated, but as all patients showed low levels of serum albumin (Supplementary Table 1) following intensive care hospitalization, only Qalb could be used to estimate the BBB integrity. This serum albumin decrease was probably induced by feeding difficulties during prolonged disease.

**Table 1. Clinical and Biological Characteristics of the Patients With Coronavirus Disease 2019 (COVID-19) and Neurological Manifestations**

| Variable | All Patients[a] (N = 31) | ICU Patients (N = 18) | Non-ICU Patients (N = 11) |
|---|---|---|---|
| Epidemiological data | | | |
| Age (median ± IQR) | 66.3 ± 18.8 | 69.5 ± 12.3 | 61.2 ± 25.4 |
| Male (%) | 71 | 83 | 55 |
| Neurological manifestations | | | |
| Encephalopathy n (%) | 19 (61%) | 15 (83%) | 4 (36%) |
| PNS involvement n (%) | 4 (13%) | 1 (5%) | 3 (27%) |
| Headache n (%) | 3 (10%) | 0 (0%) | 1 (9%) |
| Meningeal syndrom n (%) | 4 (13%) | 2 (11%) | 2 (18%) |
| Seizures n (%) | 1 (3%) | 0 (0%) | 1 (9%) |
| Neurological symptoms may be related to COVID-19 n (%) | 28 (90%) | 16 (89%) | 10 (91%) |
| Delay between onset general symptoms and LP median (min-max) | 23 (0–50) | 26 (4–50) | 8 (0–40) |
| CSF analysis | | | |
| WBC (M/L) (median ± IQR) | 2.0 ± 2.0 | 2.0 ± 1.8 | 2.0 ± 4.0 |
| Lymphocytes (%) (median ± IQR) | 92.0 ± 9.2 | 92.0 ± 9.5 | 95.0 ± 9 |
| Monocytes (%) (median ± IQR) | 4.5 ± 6.0 | 4.0 ± 5.5 | 5.0 ± 5.0 |
| Neutrophils (%) (median ± IQR) | 1.0 ± 3.5 | 1.0 ± 3.0 | 1.0 ± 5.0 |
| Detection of plasma cells n (%) | 5 (17%) | 3 (18%) | 2 (18%) |
| Detection of macrophages n (%) | 18 (60%) | 9 (53%) | 8 (72%) |
| Positive SARS-CoV-2 RT-PCR n | 0 | 0 | 0 |
| Protein level (g/L) (median ± IQR) (normal value:0.15–0.45 g/L) | 0.42 ± 0.36 | 0.45 ± 0.36 | 0.39 ± 1.07 |
| Increased albumin quotient (>Qlim) n/n total (%) | 11/19 (58%) | 9/16 (56%) | 2/3 (67%) |
| Detection of CSF anti- SARS-CoV-2 IgG n/n total (%) | 14/18 (77%) | 11/12 (92%) | 3/6 (50%) |
| Presence of oligoclonal bands type IV (same pattern in serum and CSF) n/n total (%) | 16/19 (84%) | 13/16 (81%) | 3/3 (100%) |
| Positive intrathecal anti-SARS-CoV-2 IgG antibody index (> 2.0) n | 0 | 0 | 0 |

Positive intrathecal anti-SARS-CoV-2 IgG antibody index, an AI value <2, means that specific SARS-CoV-2 antibodies were detected in the CSF, but they were not locally produced.

Abbreviations: CSF, cerebrospinal fluid; ICU, intensive care unit; IgG, immunoglobulin G; IQR, interquartile range; LP, lumbar puncture; M/L, Mega per liter (equivalent cells per microliter); PNS, peripheral nervous system; Qlim, albumin quotient cutoff corrected by age; RT-PCR, reverse transcription polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; WBC, white blood cells.

[a]Among the patients, 2 were not hospitalized. Both had headache and were not tested for CSF IgG antibodies nor oligoclonal bands.

All analyzed patients tested positive for anti-SARS-CoV-2 IgG antibodies in sera, and 78% of them also showed these specific antibodies in CSF (Supplementary Table 1). Sixteen of 19 tested patients (84%) showed IgG oligoclonal bands (OBs) with an identical electrophoretic pattern in serum and CSF (OB IgG type IV pattern). None of the tested patients presented CSF-specific IgG OBs (type II or III) nor an elevated IgG index indicating intrathecal IgG synthesis (data not shown). In addition, no intrathecal IgG-anti-SARS-CoV-2 synthesis was detected.

## DISCUSSION

Neurological complications associated with SARS-CoV-2 are frequent and diverse, and they involve both the central and the peripheral nervous systems [2]. To date, no specific CSF pattern in SARS-CoV-2 infected patients has been described. This retrospective study, despite the limited number of patients included, is the largest to date to describe CSF features of SARS-CoV-2 infected patients with neurological complications in detail, to the best of our knowledge.

Our findings suggest that SARS-CoV-2 infection is an indirect cause of the neurological complications, independently of the neurological diagnosis. Indeed, CSF analysis did not reveal any evidence of direct viral infection of the central nervous system: (1) SARS-CoV-2 RNA was not detected in CSF using rRT-PCR; (2) no sign of CSF inflammation except in rare case of coinfection; (3) absence of intrathecal synthesis using IgG Index and electrophoretic IgG oligoclonal bands; and finally, (4) absence of specific intrathecal anti-SARS-CoV-2 IgG synthesis. Currently, there are few case reports of patients with encephalitis associated with SARS-CoV-2 RNA positive in CSF [4, 8, 9]. One could argue that SARS-CoV-2 rRT-PCR assays are not validated in CSF, and that their sensitivity may be affected by delays in storage time. However, CSF is generally a type of sample for which RNA viruses rRT-PCR assays are easy to validate. Furthermore, because the virology laboratory is located inside the hospital in which the patients included were hospitalized, there has not been any particular delay in sample acquisition, and the storage modalities were not altered, in respect with the pre-COVID-19 era. Thus, there is no theoretical reason why the rRT-PCR assay should yield false negative results in this material. The concomitant absence of total IgG and specific SARS-CoV-2 intrathecal synthesis strengthen the hypothesis that the neurological manifestations observed during COVID-19 are probably induced by indirect mechanisms in the majority of cases [4, 10], as already demonstrated for other viruses [4].

Interestingly, in our study, most of the CSF specimens analyzed showed a type IV oligoclonal pattern. This pattern, already reported in some patients with neurologic disorders in severe SARS-CoV-2 infection [5, 7], is consistent with passive diffusion of oligoclonal IgG from a systemic inflammatory state such as a systemic viral infection. The vast majority (92%) of patients had both type IV oligoclonal pattern and anti-SARS-CoV-2 antibodies in CSF, leading to the hypothesis that IgG oligoclonal bands are at least in part anti-SARS-CoV-2 IgG.

Most of our patients (58%) with neurological manifestations during their COVID-19 illness showed BBB leakage. BBB disruption could be induced by a direct effect of SARS-CoV-2 but is more likely caused by indirect effects via generalized endotheliitis [11]. The abnormal presence of macrophages in 60% of the CSF specimens suggests a microglial activation known to induce the expression of pro-inflammatory cytokines, chemokines and matrix metalloproteases leading to the BBB disruption [12, 13]. This mechanism resembles that described in septic encephalopathy [14].

Limitations of this study include selection biases during inclusion of patients with neurological manifestations. Indeed, the short study period in the initial phase of the pandemic that allowed only patients with rapid onset of neurological disorders to be targeted. Furthermore, patients with neurological manifestations who did not undergo lumbar puncture were not included. Finally, CSF analyses must be performed rapidly and requires CSF and serum samples collected <2 days apart, which prevented the retrospective inclusion of a large cohort.

In summary, our results mainly showed the absence of SARS-CoV-2 RNA detection in the CSF and specific intrathecal IgG synthesis in the tested samples, and they highlighted a reproducible CSF pattern in SARS-CoV-2 infection: normal WBC count, presence of macrophages, signs of BBB permeability, and type IV oligoclonal pattern.

Neurological manifestations associated with COVID-19, especially encephalopathy, are more likely due to a systemic process, such as exacerbated inflammation resulting in endothelitiis, cytokine storm, and microglia activation, rather than direct invasion of the CNS by SARS-CoV-2. If confirmed by other groups, these findings could orientate therapeutic strategies toward anti-inflammatory, rather than antiviral drugs, with regards to COVID-19 related neurological manifestations.

### Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

### Notes

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