Biomarkers for CNS injury in CSF are elevated in COVID-19 and associated with neurological symptoms and disease severity

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Abstract

Background

Neurological symptoms have been frequently reported in hospitalized patients with coronavirus disease 2019 (COVID-19) and biomarkers of CNS injury are reported to be increased in plasma but not extensively studied in CSF. This study examines CSF for biomarkers of CNS injury and other pathology in relation to neurological symptoms and disease severity in patients with neurological manifestations of COVID-19.

Methods

Nineteen patients with neurological symptoms and mild to critical COVID-19 were prospectively included. Extensive analysis of CSF, including measurement of biomarkers of CNS injury (neurofilament light chain protein (NfL) glial fibrillary acidic protein (GFAp) and total tau) was performed and related to neurological features and disease severity.

Results

Neurological symptoms included altered mental status (42%), headache (42%), central (21%) and peripheral weakness (32%). Two patients demonstrated minor pleocytosis and four patients had increased immunoglobulin G levels in CSF. Neuronal autoantibody testing using commercial tests was negative in all patients. Increased CSF levels of NfL, GFAp and total-tau protein were seen in 63%, 37%, and 16% of patients, respectively. Increased NfL correlated with disease severity, time in intensive care and level of consciousness. NfL in CSF was higher in patients with central neurological symptoms.

Conclusion

Although limited by small sample size, our data suggest that levels of NfL, GFAp and total tau in CSF are commonly elevated in patients with COVID-19 with neurological symptoms. This is in contrast to the standard CSF work-up where pathological findings are scarce. NfL in particular, is associated with central neurological symptoms and disease severity.

Introduction

Coronavirus disease 2019 (COVID-19), is a pandemic caused by the novel severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). A significant number of case reports and case series have described different types of neurological complications in COVID-19 [1-3]. The neurological manifestations are broad and may be caused by a direct effect of the virus on the nervous system or by a para-infectious or post-infectious immune-mediated inflammation [4]. However, neurological complications may also be secondary to critical illness and a long stay in an intensive care unit (ICU).

Recently, neurochemical evidence of acute CNS injury in patients with COVID-19 was shown in the form of increased plasma levels of neurofilament light chain protein (NfL), a marker of axonal injury, and of glial fibrillary acidic protein (GFAp), a marker of astrocytic injury [5, 6]. In this regard, few studies have investigated the CSF in patients with COVID-19 [7, 8] , which is less sensitive than plasma to confounding release of neuromarkers, *e.g.*, from peripheral nerves.

Lumbar puncture (LP) is an important tool to evaluate critically ill patients with neurological symptoms, as it can reveal both the underlying pathology and the severity of injury to the nervous system. There are few reports on results from CSF analysis in patients with COVID-19 and prospective studies with comprehensive CSF and neurological investigations are rare. The aim of this study was to describe clinical characteristics in relation to findings in CSF analyses among patients with COVID-19 and neurological symptoms.

Methods

Patients and study design

This was a prospective single-center study. Patients with confirmed COVID-19 and at least one new-onset neurological symptom were included from April until July 2020. Patients had either a positive PCR for SARS-CoV-2 in upper and/or lower airway samples [9, 10] or SARS-CoV-2-specific immunoglobulin G (IgG) in serum. Clinical neurological evaluation was performed by experienced neurologists. The following findings were documented: cranial nerve affection, central and peripheral paralysis, extrapyramidal, cerebellar and sensory symptoms and altered mental status including confusion, encephalopathy and reduced level of consciousness graded with the Glasgow Coma Scale (GCS). The findings were documented at the worst time point during the disease before the LP and at the time of the LP. Patients with GCS $\leq$ 12 or a central paralysis at any time before the LP were categorized as patients with central neurological symptom.

Included patients were investigated with LP if this was required as part of their routine evaluation. In patients without strong indication for LP, the procedure was optional. The NIH criteria for COVID-19 severity grading were used to classify patients as mild, moderate, severe or critical [11]. As a measure of respiratory status, the lowest PaO_2/FiO_2-ratio at any time before the LP were documented for patients treated in the ICU.

Standard protocol approvals, registrations, and patient consent

The study was approved by the Swedish Ethical Review Authority (2020-01883). Informed consent was obtained from each patient, or next-of-kin if a patient was unable give consent. The Declaration of Helsinki and its subsequent revisions were observed.

Biomarker analyses

SARS-CoV-2 PCR was performed on upper and/or lower airway samples using either the Abbott RealTime SARS-CoV-2 assay on the Abbott m2000 platform, or an in-house PCR developed at the Laboratory of Clinical Microbiology, Uppsala University Hospital. SARS-CoV-2 IgG antibodies were analyzed using the CE-labelled SARS-CoV-2 IgG kit with nucleoprotein-based antigen on the Architect i2000SR Analyser (Abbott, Abbott Park, IL, USA) at the Laboratory of Clinical Microbiology, Uppsala University Hospital, as previously described [12].

All routine plasma and CSF analyses including interleukin-6 (IL-6) were performed at the Clinical Chemistry Laboratory at Uppsala University Hospital and analyses of NfL, T-tau, and GFAp at the Clinical Neurochemistry Laboratory at the Sahlgrenska University Hospital. Analyses were performed by board-certified laboratory technicians blinded to clinical data.

CSF NfL and GFAp concentrations were measured using in-house enzyme-linked immunosorbent assays, as previously described in detail [13, 14]. CSF total tau (T-tau) concentration was measured using Lumipulse technology in accordance with the kit insert from the manufacturer (Fujirebio, Ghent, Belgium).

Plasma NfL, GFAp and T-tau measurements were performed using single molecule array (Simoa) assays on an HD-X Analyzer (Human Neurology 4-Plex A assay, N4PA advantage kit, 102153), as previously described [5]. A single batch of reagents was used; intra-assay coefficients of variation were < 8% for all analytes.

Autoantibodies in CSF and serum (NMDAR, LGI1, CASPR2, $GABA_{B1}R$, $GABA_{B2}R$, AMPA1, AMPA2, Ri, Yo, Ma2, CV2, Hu, and amphiphysin) were analyzed using a commercial assay (Euroimmun, Lübeck, Germany).

Statistics

Data are presented as median (IQR) or n (%). The Mann-Whitney U test was used for comparing continuous parameters between groups. Correlations between clinical parameters and CSF findings were tested using Spearman's rank correlation. In the figures, the biomarker data have been log-transformed to achieve near-normal distribution. A p-value of < 0.05 was considered significant. The statistical analysis was performed using SPSS version 27 (IBM Corp, Armonk, NY).

Data availability

Anonymized data from the present study can be made available to researchers with well-designed and defined research questions after contact with the corresponding author.

Results

Covid-19 was confirmed in 32 patients through positive PCR for SARS-CoV-2 in upper and/or lower airway samples and in one patient with IgG for SARS-CoV-2 in serum. Twelve

patients had contraindications for LP (all related to high doses of low molecular weight heparin or oral anticoagulants) and one patient declined the investigation. Therefore, LPs could be performed in 20 out of 33 patients. One patient suffered from a small traumatic subarachnoid hemorrhage and a skull fracture after a head trauma two days before the LP and was excluded. The remaining 19 patients were the main focus of this study. The median time between onset of symptoms and LP was 23 days (IQR: 6–43). Descriptive data are presented in Table 1 and detailed characteristics of each case are given in Table 2.

The most common neurological symptoms at the time of LP were altered mental status (n = 8, 42%) and headache (n = 8, 42%), followed by and peripheral weakness (n = 6, 32%) and anosmia (n = 5, 26%). All neurological symptoms and respiratory support are presented in Table 3.

CSF findings

PCR for SARS-CoV-2 was positive in one patient (5%) and there was a pleocytosis in two patients (11%). CSF albumin level was increased in one patient (5%), denoting disruption of the blood-CSF barrier. Four patients (21%) had elevated intrathecal IgG levels, with normal blood IgG levels and one patient (5%) had CSF-specific oligoclonal IgG bands, denoting intrathecal IgG production. Test for autoimmune encephalitis antibodies was negative in CSF (and serum) of all patients. A majority of patients had increased IL-6 levels (> 0.05 ng/mL), but values were below 20 ng/mL in most cases (14 out of 17 tested, 82%). NfL was above the age-adjusted reference range in 63% of patients, while the corresponding figure for T-tau was 37% and that for GFAp was 16%; for details, see Tables 2 and 4.

There was a correlation between number of days in the ICU and NfL ($r = 0.72$, $p < 0.001$) but not for T-tau and GFAp, Fig. 1A. NfL correlated with level of consciousness on the GCS at time of LP ($r = -0.55$, $p < 0.05$), and worst GCS before LP ($r = -0.64$, $p < 0.01$), Fig. 1B. T-tau and GFAp did not correlate with GCS at time of LP. Worst GCS correlated with T-tau ($r = -0.50$, $p < 0.05$) and GFAp ($r = -0.48$, $p < 0.05$). None of the markers of neuronal injury correlated with time between symptom onset and LP.

NfL were higher in patients with central neurological symptoms (GCS ≤ 12 or central weakness, n = 10), 3250 ng/L (IQR = 1940–5490) compared to patients with other neurological symptoms, 950 ng/L (IQR = 405–1783, $p < 0.05$), Fig. 2. NfL correlated with

COVID-19 severity grade ($r = 0.56$, $p < 0.05$) and were higher in patients with severe or critical COVID-19 (2610 ng/L (IQR = 1280–4705)) compared with mild and moderate disease (420 (IQR = 385–1640, $p < 0.05$)). The PaO_2/FiO_2-ratio did not correlate with any marker of neuronal injury. IL6 did not correlate with disease severity.

If two patients with concomitant diseases with possible effect on the markers of neuronal injury were excluded (one with epilepsy and one with cardiac arrest and resuscitation during ICU-care), all results reported above were still significant except for the correlation between T-tau and GFAp with worst GCS.

Correlation between CSF and plasma biomarkers

In 11 patients, plasma samples were analyzed for NfL, T-tau and GFAp in plasma. There was a strong correlation between CSF and plasma levels for both NfL ($r = 0.98$, $p < 0.001$) and GFAp ($r = 0.97$, $p < 0.001$). For T-tau, no correlation could be demonstrated.

Neuroimaging

A head CT scan or MRI was performed in 17 patients as part of the clinical work-up. No pathological findings could be detected in 8 (47%) patients. A more detailed description of the pathological findings is presented in Table 2.

Discussion

In this prospective study, we present data on biochemical, inflammatory, and neuronal injury biomarkers in the CSF and plasma of patients with COVID-19 and neurological symptoms. The main finding is that a majority of patients had a negative standard CSF work-up, while markers of neuronal injury were increased.

Even though the neurological symptoms were severe in some of the patients, the standard CSF work-up tended to be negative in a majority of patients and no specific pattern for COVID-19 could be identified. Only a few cases had mild pleocytosis and increased IgG in CSF and one patient had oligoclonal bands, which is in line with recent reports [15-18].

Animal models in mice of coronaviruses suggest that viral entry into the CNS can occur [19, 20]. SARS-CoV-2 is known to have a neuro-invasive propensity and there are case reports

with RNA detection in CSF that indicate a direct invasion of the virus into the CNS [21, 22]. In our study, we could detect viral RNA in only one patient, which is in parity with recent reports [8, 22]. The low numbers with detected viral RNA in CSF, in addition to the few findings of inflammatory signs, may suggest that direct CNS invasion is not the main pathogenic mechanism of neurological effects of COVID-19, or at least not for a majority of patients. However, methods such as immunohistochemistry or analysis for detection of spike proteins in CSF might reveal components of autoimmunity and/or CNS invasion which are not possible to detected with the methods we used. The divergent neuroimaging findings does not indicate a common mechanism of neuronal injury related to COVID-19.

A substantial proportion of the patients had increased CSF levels of the neuronal injury markers NfL (63%) and T-tau (37%) and, to a lesser extent, the glial activation marker GFAp (16%). Increased levels of NfL and GFAp in plasma have recently been reported in hospitalized patients with COVID-19 and in a group of patients with mild to moderate disease. [5, 6]. NfL were higher in patients with central neurological symptom and correlated with disease severity, level of consciousness and time at ICU. However, NfL did not correlate with lowest PaO_2/FiO_2-ratio at time in ICU before the LP, indicating that the increased levels of NFL was not only directly attributable to severity of respiratory deficit

In the absence of direct findings of viral meningitis or encephalitis in the vast majority of patients with COVID-19, the mechanism of the brain injury implied by increased NfL and T-tau remains to be elucidated. Increased plasma and CSF levels of NfL have previously been shown in patients with sepsis-associated encephalopathy [23]. Surgery and anesthesia may cause increased plasma levels of both NfL and T-tau [24]. No patient in the study underwent surgery the weeks before inclusion and only three patients were anesthetized and treated with invasive ventilation at the time of LP but 10 patients at some point before the LP. However, respiratory dysfunction as measured by the lowest PaO_2/FiO_2-ratio during invasive ventilation before the LP did not correlate with any of the markers of neuronal injury. Other confounders such as co-morbidity may also be an issue. One patient had epilepsy and autism but continuous EEG did not reveal seizure activity at the time of the study. Another patient suffered from a short cardiac arrest in the ICU (33 days before LP) but were resuscitated within 60 seconds. Exclusion of these two patients from statistical analysis did not alter any of the main results or conclusions.

Previous studies on herpes encephalitis (HSE) have shown that NfL levels often far more elevated than what is seen in this study [25]. Further, the time-series data recently published by Westman et. al [26] illustrate that the kinetics of NfL after acute infectious encephalitis is relatively slow with a peak approximately one month after onset of disease. This means that timing of the CSF sampling in relation to onset of disease (as well as in relation to ICU care and other confounders) is an important covariate when assessing NfL levels.

We found a strong correlation between plasma and CSF levels of NfL and GFAp, suggesting that plasma levels of these biomarkers parallel CSF levels in patients with COVID-19. The strong correlation indicates a steady-state passage across the blood-CSF barrier irrespective of disease severity, and is consistent with a negligible barrier injury. This is further supported by the findings that only one patient had increased albumin in CSF. This is in contrast to in other viral meningitis or encephalitis, where differences in disease severity cause the level of barrier injury to vary between patients [27].

The raised levels of T-tau in CSF and plasma have only rarely been explored in relation to COVID-19 [8]. Since T-tau indicate cortical neuronal injury [28] the increase suggests ongoing neuronal damage in some of our patients. We found no correlation between T-tau in CSF and plasma, and in other studies the correlation have varied depending on the underlying neurological disorder [28].

IL-6 levels are commonly analyzed in CSF from patients with COVID-19. In line with earlier findings, a minority (18%) of our patients displayed values above 20 ng/ml in CSF. In addition, IL-6 could neither discriminate between mild/moderate and severe/critical COVID-19, nor was there any correlation between plasma and CSF IL-6 levels. Measurement of IL-6 in CSF therefore appears to be of limited value when assessing neurological damage in patients with COVID-19.

Our study has several limitations. It included a small number of patients and since the inclusion was not consecutive, some inclusion bias is possible. All care units in the study hospital treating COVID-19 patients were screened at least once every week for patients who fulfilled inclusion criteria. Even so, some patients had been discharged before they could be included causing a selection bias. Approximately 470 patients with COVID-19 were treated

at the hospital of the study during the time of inclusion. Thirteen of the included patients did not undergo LP thereby making the cohort less representative for the COVID-19 disease. Importantly, the study was not designed to assess incidence or prevalence of neurological symptoms related to COVID-19 – but rather to select patients with whom we had optimal access to information on the clinical course and were able to perform LP. Still, not all patients had the exact same set of investigations performed due to the clinical situation. Furthermore, the effective half-life is reported to be 12–24 hours for GFAp, about 10 hours for T-tau but several weeks for NfL and patients were included at different timepoints along the disease trajectory, which may have affected the results [29, 30]. The study is cross-sectional without longitudinal follow-up data from CSF, final neurological diagnosis and outcome.

In conclusion, our results show that the standard CSF work-up is normal in a majority of patients with COVID-19 and new onset neurological symptoms. CSF biomarkers related to CNS injury are increased indicating COVID-19-related brain damage. NfL in particular is indicative of disease severity and may be a valuable tool for monitoring neuro-protective effects of new therapies. Future studies in larger samples are needed to explore and understand the genesis of neurological injury in COVID-19 patients.

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References

[1]. Filatov A SP, Hindi F, Espinosa PS. Neurological Complications of Coronavirus Disease (COVID-19): Encephalopathy. *Cureus* 2020 **12:** e7352.

[2]. Ellul MA, Benjamin L, Singh B*, et al.* Neurological associations of COVID-19. *Lancet Neurol*. 2020 **19:** 767-783.

[3]. Virhammar J, Kumlien E, Fällmar D*, et al.* Acute necrotizing encephalopathy with SARS-CoV-2 RNA confirmed in cerebrospinal fluid. *Neurology*. 2020**:** 10.1212/WNL.0000000000010250.

[4]. Nordvig AS, Rimmer KT, Willey JZ*, et al.* Potential neurological manifestations of COVID-19. *Neurology: Clinical Practice*. 2020**:** 10.1212/CPJ.0000000000000897.

[5]. Kanberg N, Ashton NJ, Andersson L-M*, et al.* Neurochemical evidence of astrocytic and neuronal injury commonly found in COVID-19. *Neurology*. 2020**:** 10.1212/WNL.0000000000010111.

[6]. Ameres M, Brandstetter S, Toncheva AA*, et al.* Association of neuronal injury blood marker neurofilament light chain with mild-to-moderate COVID-19. *J Neurol*. 2020.

[7]. Edén A, Kanberg N, Gostner J*, et al.* CSF biomarkers in patients with COVID-19 and neurological symptoms: A case series. *Neurology*. 2020.

[8]. Espíndola OM, Brandão CO, Gomes YCP*, et al.* Cerebrospinal fluid findings in neurological diseases associated with COVID-19 and insights into mechanisms of disease development. *Int J Infect Dis*. 2020.

[9]. Mao L, Jin H, Wang M*, et al.* Neurologic Manifestations of Hospitalized Patients With Coronavirus Disease 2019 in Wuhan, China. *JAMA Neurol*. 2020.

[10]. Rogers JP, Chesney E, Oliver D*, et al.* Psychiatric and neuropsychiatric presentations associated with severe coronavirus infections: a systematic review and meta-analysis with comparison to the COVID-19 pandemic. *Lancet Psychiatry*. 2020 **7:** 611-627.

[11]. COVID-19 Treatment Guidelines Panel. Coronavirus Disease 2019 (COVID-19) Treatment Guidelines. National Institutes of Health. Available at https://www.covid19treatmentguidelines.nih.gov/. Accessed Oct 22, 2020.

[12]. Lidström A-K, Sund F, Albinsson B, Lindbäck J, Westman G. Work at inpatient care units is associated with an increased risk of SARS-CoV-2 infection; a cross-sectional study of 8679 healthcare workers in Sweden. *Upsala Journal of Medical Sciences*. 2020**:** 1-6.

[13]. Gaetani L, Hoglund K, Parnetti L*, et al.* A new enzyme-linked immunosorbent assay for neurofilament light in cerebrospinal fluid: analytical validation and clinical evaluation. *Alzheimers Res Ther*. 2018 **10:** 8.

[14]. Rosengren LE, Ahlsen G, Belfrage M, Gillberg C, Haglid KG, Hamberger A. A sensitive ELISA for glial fibrillary acidic protein: application in CSF of children. *J Neurosci Methods*. 1992 **44:** 113-119.

[15]. Chougar L, Shor N, Weiss N*, et al.* Retrospective Observational Study of Brain Magnetic Resonance Imaging Findings in Patients with Acute SARS-CoV-2 Infection and Neurological Manifestations. *Radiology*. 2020**:** 202422.

[16]. Helms J, Kremer S, Merdji H*, et al.* Neurologic Features in Severe SARS-CoV-2 Infection. *N Engl J Med*. 2020.

[17]. Senel M, Abu-Rumeileh S, Michel D*, et al.* Miller-Fisher syndrome after COVID-19: neurochemical markers as an early sign of nervous system involvement. *Eur J Neurol*. 2020.

[18]. Bellon M, Schweblin C, Lambeng N*, et al.* Cerebrospinal fluid features in SARS-CoV-2 RT-PCR positive patients. *Clin Infect Dis*. 2020.

[19]. Netland J, Meyerholz DK, Moore S, Cassell M, Perlman S. Severe acute respiratory syndrome coronavirus infection causes neuronal death in the absence of encephalitis in mice transgenic for human ACE2. *J Virol*. 2008 **82:** 7264-7275.

[20]. Li K, Wohlford-Lenane C, Perlman S*, et al.* Middle East Respiratory Syndrome Coronavirus Causes Multiple Organ Damage and Lethal Disease in Mice Transgenic for Human Dipeptidyl Peptidase 4. *J Infect Dis*. 2016 **213:** 712-722.

[21]. Moriguchi T, Harii N, Goto J*, et al.* A first case of meningitis/encephalitis associated with SARS-Coronavirus-2. *Int J Infect Dis*. 2020 **94:** 55-58.

[22]. Helms J, Kremer S, Merdji H*, et al.* Delirium and encephalopathy in severe COVID-19: a cohort analysis of ICU patients. *Critical Care*. 2020 **24:** 491.

[23]. Ehler J, Petzold A, Wittstock M*, et al.* The prognostic value of neurofilament levels in patients with sepsis-associated encephalopathy - A prospective, pilot observational study. *PLoS One*. 2019 **14:** e0211184.

[24]. Evered L, Silbert B, Scott DA, Zetterberg H, Blennow K. Association of Changes in Plasma Neurofilament Light and Tau Levels With Anesthesia and Surgery: Results From the CAPACITY and ARCADIAN Studies. *JAMA Neurol*. 2018 **75:** 542-547.

[25]. Studahl M, Rosengren L, Günther G, Hagberg L. Difference in pathogenesis between herpes simplex virus type 1 encephalitis and tick-borne encephalitis demonstrated

by means of cerebrospinal fluid markers of glial and neuronal destruction. *J Neurol*. 2000 **247:** 636-642.

[26]. Westman G, Aurelius E, Ahlm C*, et al.* Cerebrospinal fluid biomarkers of brain injury, inflammation and synaptic autoimmunity predict long-term neurocognitive outcome in herpes simplex encephalitis. *Clin Microbiol Infect*. 2020.

[27]. Salimi H, Klein RS. Disruption of the Blood-Brain Barrier During Neuroinflammatory and Neuroinfectious Diseases. *Neuroimmune Diseases: From Cells to the Living Brain*. 2019**:** 195-234.

[28]. Zetterberg H. Review: Tau in biofluids - relation to pathology, imaging and clinical features. *Neuropathol Appl Neurobiol*. 2017 **43:** 194-199.

[29]. Hepner A, Porter J, Hare F*, et al.* Serum Neurofilament Light, Glial Fibrillary Acidic Protein and Tau Are Possible Serum Biomarkers for Activity of Brain Metastases and Gliomas. *World J Oncol*. 2019 **10:** 169-175.

[30]. Thelin EP, Zeiler FA, Ercole A*, et al.* Serial Sampling of Serum Protein Biomarkers for Monitoring Human Traumatic Brain Injury Dynamics: A Systematic Review. *Front Neurol*. 2017 **8:** 300.

Table 1. Patient characteristics.

	Patients with LP, n = 19
Age, median (range), years	64 (34–76)
ICU days, median (IQR)	8 (0–25)
NIH severity: n (%)	
Mild Covid-19	2 (11)
Moderate Covid-19	4 (21)
Severe Covid-19	4 (21)
Critical Covid-19	9 (47)
Comorbidity: n (%)	
Diabetes mellitus	2 (11)
Obesity	9 (47)
Hypertension	8 (42)
Smoking	4 (21)
Cardiac disease	2 (11)
Chronic lung disease	4 (21)
Cerebrovascular disease	1 (5)
Chronic kidney disease	1 (5)
Immunosuppression	2 (11)

LP, lumbar puncture; ICU, intensive care unit; IQR, interquartile range.

Table 2. Characteristics, neuroimaging findings, and biomarkers in CSF in each case.

Age (years)	**LP at COVID day**	**ICU No. days**	**NIH-score**	**Respiration***	**Primary neurological symptom***	**Neuroimaging**	**WBC (10^6/L)**	**NfL (ng/L)**	**Tau (ng/L)**	**GFAp (ng/L)**	**IL-6 (ng/L)**
49	30	39	Critical	Invasive vent	Central & peripheral weakn	See A) below	0	**219000**	**11900**	**4200**	**35**
55§	18	25	Severe	Invasive vent	Central weakness	ANE WMC	3	**32800**	**2900**	**3800**	19
64	43	42	Critical	Invasive vent	Central & peripheral weakn	See B) below	**8**	**5490**	333	760	8
67	73	20	Critical	Invasive vent	Altered mental status	Negative CT	0	**3920**	249	**2900**	6
75	29	10	Critical	HFNO	Altered mental status	Negative CT	1	**3710**	**794**	333	11
73	22	27	Critical	Invasive vent	Peripheral weakness	Microbleed WMC	1	**3250**	397	500	11
64	23	4	Severe	HFNO	Altered mental status	See C) below	1	**2610**	**546**	840	**54**
74	44	29	Critical	Invasive vent	Central & peripheral weakn	Infarcts & hemorrhages	0	**2210**	229	700	7
76	33	16	Critical	Invasive vent	Central & peripheral weakn	Unspecific WMC	3	**1940**	449	300	10
75	5	0	Moderate	Air	Confusion	N/A	3	**1860**	**486**	620	8
71	4	0	Severe	HFNO	Altered mental status	Negative CT	**13**	1550	**602**	810	11
71	4	0	Moderate	Air	Altered mental status	Unspecific WMC	0	1420	**771**	710	6
39	37	21	Critical	Invasive vent	Peripheral weakness	Negative CE-MRI	4	**1010**	.	220	6
34	78	8	Critical	Invasive vent	Cranial nerve affection	Negative CE-MRI	2	**890**	307	530	5
39	12	0	Mild	Air	Headache	N/A	0	420	150	190	15
47	75	0	Moderate	Air	Headache	Unspecific WMC†	0	400	168	360	4
41	6	0	Severe	HFNO	Headache	Negative CE-CT	1	370	244	250	.
40	15	5	Moderate	Invasive vent	Central weakn	Negative CE-MRI	31‡	370	150	250	**102**
54	4	0	Mild	Air	Sensory symptoms	Negative CT	1	.	.	.	.

LP, lumbar puncture; ICU, intensive care unit; WBC, white blood cell count; NfL, neurofilament light protein; GFAp, glial fibrillary acidic protein; IL, interleukin; O_2, oxygen with nasal cannula or mask; HFNO, high-flow oxygen; ANE, acute necrotizing encephalopathy; WMC, white matter change; CE, contrast-enhanced; N/A, not available; vent., ventilator; weakn., weakness.

Reference ranges: NfL: age 30–40 years, < 560 ng/L; age 40–60 years, < 890 ng/L; age > 60 years, < 1,850 ng/L; Tau: age < 50 years, < 360 ng/L; age > 50 years, < 479 ng/L; GFAp: age 20–60 years, < 750 ng/L, age > 60 years, < 1,250 ng/L; WBC: < 5 10^6/L.

Bold numbers are values above reference range.

Specific imaging findings: A) Cortical and subcortical infarcts with contrast enhancement and hemorrhagic components; B) Numerous punctate findings in deep white matter on susceptibility weighted imaging, representing microbleeds or microthrombosis; C) Focal cortical diffusion restriction attributable to epileptic seizures, and faint meningeal enhancement after LP.

* = Neurological findings and highest respiratory support during the patient's disease course before the lumbar punctures

† = Imaging was performed several weeks after hospitalization.

‡ = Traumatic lumbar puncture with 19 000 10^6/L erythrocytes. Not considered as a pleocytosis.

§ = Patient published as a case report (ref 3)

Table 3. Respiratory support and neurological symptoms among the 19 patients investigated with LPs.

Respiratory support, n (%)	*At time of LP*	*Most severe before LP**
None	8 (42)	4 (21)
O_2	5 (26)	1 (5)
HFNO	3 (16)	4 (21)
Invasive ventilation	3 (16)	10 (53)
Neurological symptoms, n (%)		
Cranial nerve affection	2 (11)	4 (21)
Central paralysis	4 (21)	6 (32)
Peripheral paralysis	6 (32)	6 (32)
Extrapyramidal symptoms	0 (0)	0 (0)
Cerebellar symptoms/ataxia	1 (5)	0 (0)
Sensory symptoms	2 (11)	4 (21)
Altered mental status	8 (42)	13 (68)
Headache	8 (42)	
Vertigo	4 (21)	
Anosmia	5 (26)	
GCS, median (IQR)	14 (8–15)	13 (7–15)

LP, lumbar puncture; O_2, oxygen with nasal cannula or mask; HFNO, high-flow oxygen; GCS, Glasgow Coma Scale; IQR, interquartile range.

* = Highest respiratory support or most severe neurological symptom during the patient's disease course before the lumbar puncture.

Table 4. Laboratory findings.

		Above reference range n (%)
CSF (n = 19)		
WBC (n = 19), 10^6/L	1 (0–3)	3 (15)
Albumin (n = 19), mg/L	206 (175–285)	1 (5)
IgG (n = 17), mg/L	34 (27–57.5)	4 (21)
T-tau (n = 17), ng/L	397 (237–687)	7 (37)
GFAp (n = 18), ng/L	660 (288–930)	3 (16)
NfL (n = 18), ng/L	1,900 (773–3,763)	12 (63)
IL-6 (n = 17), ng/L	9.6 (6.3–17.2)	†

Autoantibodies (n = 18), n	0	0 (0)
OCB (n =18), n	1	1 (5)
Blood		
GFAp (n = 10), pg/L	152 (97–384)	†
NfL (n = 11), pg/L	42 (11–103)	6 (55)
T-tau (n = 10), pg/L	2.2 (0.9–3.3)	†
IL-6 (n = 16), ng/L	17.8 (4.7–31.8)	†
CRP (n = 16), mg/L	64 (8–1114)	13 (81)
WBC (n = 16), 10^9/L	7.7 (4.7–11.4)	6 (38)
Autoantibodies (n = 14), n	0	0 (0)

CSF, cerebrospinal fluid; WBC, white blood cell count; NfL, neurofilament light protein; GFAp, glial fibrillary acidic protein; IL, interleukin; OCB, oligoclonal bands; CRP, C-reactive protein.
Continuous variables are presented as median (interquartile range); categorical variables as number (%).
Reference ranges – CSF-WBC: < 5 10^6/L; Albumin: age < 45 years, < 320 mg/L, age > 45 years, < 420 mg/L; IgG: < 56 mg/L; CSF-NfL: age 30–40 years, < 560 ng/L, age 40–60 years, < 890 ng/L, age > 60 years, < 1,850 ng/L; CSF-Tau: age < 50 years, < 360 ng/L, age > 50 years, < 479 ng/L; CSF-GFAp:, age 20–60 years, < 750 ng/L, age > 60 years, < 1,250 ng/L; plasma-NfL: age < 61 years, < 20 ng/L, age 61–76 years, < 35 ng/L, age > 76 years, < 55 ng/L; CRP: < 5 mg/L; WBC: < 5 10^9/L.
† Reference range not established.

Figure legends

Figure 1.
Correlation between neurofilament light protein (NfL) in cerebrospinal fluid and **(A)** number of days in intensive care unit (ICU), and **(B)** worst Glasgow Coma Scale (GCS) before the lumbar puncture.

Figure 2.
The levels of NfL in CSF given as logarithmic data, in patients with central neurological symptoms and other neurological symptoms.