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CSF biomarkers in patients with COVID-19 and neurological symptoms: A case series

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Abbreviations used in this paper: CNS, central nervous system; CSF; Cerebrospinal fluid; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; NfL, neurofilament light protein; β_2M, β_2-microglobulin

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Abstract

Objective

To explore whether hospitalized patients with SARS-CoV-2 and neurological symptoms have evidence of CNS infection, inflammation and injury using CSF biomarker measurements.

Methods

We assessed CSF SARS-CoV-2 RNA along with CSF biomarkers of intrathecal inflammation (CSF white blood cell count, neopterin, β_2-microglobulin (β_2M) and immunoglobulin G-index), blood-brain-barrier (BBB) integrity (albumin ratio), and axonal injury (CSF neurofilament light chain protein [NfL]) in six patients with moderate to severe COVID-19 and neurological symptoms who had undergone a diagnostic lumbar puncture. Neurological symptoms and signs included features of encephalopathies (4/6), suspected meningitis (1/6) and dysgeusia (1/6). SARS-CoV-2 infection was confirmed by rtPCR analysis of nasopharyngeal swabs.

Results

SARS-CoV-2 RNA was detected in the plasma of two patients (Cycle threshold [Ct]value 35.0-37.0) and in CSF at low levels (Ct 37.2, 38.0, 39.0) in three patients in one but not in a second rtPCR assay. CSF neopterin (median, 43.0 nmol/L) and β_2-microglobulin (median, 3.1 mg/L) were increased in all. Median IgG-index (0.39), albumin ratio (5.35) and CSF white blood cell count (<3 cells/µL) were normal in all, while CSF NfL was elevated in two patients.

Conclusion

Our results on patients with COVID-19 and neurological symptoms suggest an unusual pattern of marked CSF inflammation in which soluble markers were increased but white cell response and other immunological features typical of CNS viral infections were absent. While our initial hypothesis

centered on CNS SARS-CoV-2 invasion, we could not convincingly detect SARS-CoV-2 as the underlying driver of CNS inflammation. These features distinguish COVID-19 CSF from other viral CNS infections, and raise fundamental questions about the CNS pathobiology of SARS-CoV-2 infection.

Introduction

Neurological manifestations are common features of COVID-19, but questions remain regarding the underlying mechanisms of CNS pathology (1). We have recently reported neuronal injury and glial activation in patients with COVID-19 using plasma markers of axonal and astrocytic damage (2). A variety of CNS disorders, including strokes, seizures and other encephalopathies have been reported, particularly in severe COVID-19 (3-7). By contrast, hyposmia and dysgeusia are relatively common in milder infection, possibly indicating viral invasion of the olfactory bulb (8).

CSF biomarkers are useful in characterizing CNS responses to infection, both by direct detection of invading pathogens and by host inflammatory responses. Indeed, CSF white blood cell (WBC) count is often used as the *sine qua non* indicator of meningitis or encephalitis. Likewise, soluble inflammatory markers can serve as useful measures of the character and consequences of CNS infections. Neopterin (a marker of cellular activation, including macrophage/microglia and astrocytes) and β_2-microglobulin (β_2M) (a component of the major histocompatibility complex [MHC] class I molecule) have proven to be robust and frequently altered in neuroinflammatory diseases (9, 10). Similarly, CNS injury can be sensitively detected with CSF neurofilament light chain protein (NfL), a structural component of myelinated axons (9, 11). Additionally, the immunoglobulin G (IgG) index assesses intrathecal antibody responses and the ratio of CSF albumin to blood concentration (albumin ratio) provides a measure of blood-brain barrier (BBB) disruption (12). Together these biomarkers aid in characterizing the magnitude, character and impact of viral CNS infections.

Here, we present an analysis of these well-established CSF biomarkers in six patients with COVID-19 and neurological symptoms.

Methods

Study population

We included patients with confirmed COVID-19 infection and neurological abnormalities who had undergone a diagnostic lumbar puncture (LP). All patients were admitted to Sahlgrenska University Hospital in Gothenburg, Sweden between March 1st, and April 4th, 2020.

Viral diagnostics

Infection with SARS-CoV-2 was confirmed using real-time polymerase chain reaction (rtPCR) analysis of nasopharyngeal swab specimens. Additional swab specimens and cell-free CSF and plasma samples were analysed using the same protocols. Nucleic acid from 200 µL nasal swab medium, plasma or CSF was extracted by a MagNA Pure LC instrument (Roche Diagnostics, Mannheim, Germany) using the Total Nucleic Acid isolation kit. The nucleic acids were eluted in 100 µL volume, and 5 µL of this were used for real-time PCR. Real-time PCR of a target in the RdRP region (modified from (13)) was performed in a QS6 instrument (Applied Biosystems, Foster City, CA, USA) in 20 µL reactions containing oligonucleotides and Taqman Fast Virus 1-step Mastermix (Applied Biosystems). The sequence of the primers was RdRP_Fi, GTCATGTGTGGCGGTTCACT; RdRP_Ri, CAACACTATTAGCATAAGCAGTTGT,

and RdRP_probe, CAGGTGGAACCTCATCAGGAGATGC. After a reverse transcription step at 46°C for 30 min followed by 10 min of denaturation at 95°C, 45 cycles of two-step PCR was performed (15 s at 95°C, 60 s at 56°C)(13). Plasma and CSF samples with detectable SARS-CoV-2 were reanalysed from stored specimens using the Xpert® - Xpress SARS-CoV-2 test (Cepheid, Sunnyvale, USA) according to manufacurers instructions. Cycle threshold (Ct) values were used to estimate sample viral load using the formula $(47\text{-Ct})/3.4=\log_{10}$ copies/sample. Ct values < 37 were regarded as positive, values > 40 as negative, while values between 37 and 40 were interpreted as indeterminant.

Biomarker analyses

CSF WBC count was performed using routine methods with a limit of detection of 3 cells/µL. CSF and serum β_2M concentrations were measured using the N Latex β_2M kit on the Atellica NEPH 630 System (Siemens Healthcare GmbH. Erlangen, Germany). CSF and serum neopterin concentrations were measured using a commercially available immunoassay (BRAHMS, Berlin, Germany) (9). CSF NfL was measured using a previously described in house sandwich ELISA(14). Since NfL increases with normal ageing, NfL concentrations were age-adjusted to the median age (65 years) of the study group (11). Immunoglobulin G (IgG) and albumin concentrations were measured by immunoturbidimetry on a Cobas instrument (Roche Diagnostics, Penzberg, Germany). IgG-index and albumin ratio were calculated as previously described (12).

Standard protocol approvals, registrations, and patient consents

This study has been approved by the Swedish Ethical Review Authority (2020-01771). All participants provided informed written consent.

Data Availability

Researchers can apply for access to anonymized data from the present study for well-defined research questions that are in line with the overall research agenda for the cohort. Please contact the corresponding author.

Results

Study population

During the study period, a total of 112 patients with COVID-19 were admitted to the clinic. Six patients who had undergone a diagnostic LP on clinical indications were identified and included in the

study. All had respiratory symptoms with hypoxemia requiring hospitalization. Patient characteristics at baseline are shown in Table 1, and an overview of disease courses and timing of LP for each patient is shown in Figure 1. Two patients were previously healthy, one had schizophrenia. Of three patients with hypertension, two had diabetes mellitus, one of whom also had ischemic heart disease. Two patients (2 and 4) required intubation and intensive care; patient 5 had an elevated d-dimer three days after LP and was subsequently diagnosed with pulmonary emboli. Neurological signs and symptoms are summarized down in Table 1. The most common neurological symptoms were various features of encephalopathy, found in patients 1-3 and 6. CT neuroimaging was performed in four patients.

Specifically, patient 1 presented with disorientation and lack of spatial awareness. CT scan showed evidence of small-vessel disease and global cortical atrophy. Patient 2 was disoriented to time and place, exhibited poor memory, difficulty with fluent speech, performed poorly on simple tasks such as holding a fork or visiting restroom independently, complained of extreme fatigue and later developed multiple seizures. He underwent brain CT and CT angiography after the seizures and EEG two days later which showed no epileptic activity, but generalized background slowing while under deep sedation. The CT was normal while CT angiography showed atherosclerotic changes consonante with the patient's underlying risk profile and comorbidities. Patient 3 suffered from cognitive slowing, expressive verbal difficulties and altered personality according to relatives. He had a normal CT scan apart from discrete white matter changes. Patient 4 presented with somnolence, moderate neck stiffness and photophobia suggesting meningities and leading to the LP; a CT scan was normal. Patient 5 had dysgeusia, extreme fatigue and described an altered sence of reality. Patient 6 was disoriented to time, person and situation at admission, unable to perform simple tasks, and had serverely limitied verbal communication. None had clear focal motor or sensory neurological signs by bedside exam. Due to restrictions imposed at the time to prevent transmission to hospital workers and other patients, no MRI scans or additional EEG exams were performed.

CSF viral detection and biomarkers

SARS-CoV-2 RNA was detectable in plasma in patients 1 and 2 (Ct values 37.0 and 35.0), and in CSF of patients 3, 4 and 5 (Ct values 39.0, 38.0 and 37.2, respectively). Due to these low levels of viral

detection, all plasma and CSF samples with detectable viral RNA were reanalyzed using the Xpert® assay. Both reruns in plasma confirmed SARS-CoV-2 RNA detection, while SARS-CoV-2 RNA was undetectable in all three CSF samples.

CSF biomarker analyses are shown in Figure 2A-D. None of the patients had CSF pleocytosis (WBC ≤3 cells/µL) (A). The albumin ratio reflecting blood-brain-barrier (BBB) integrity, and IgG-index reflecting intrathecal IgG synthesis, were within the normal range in all (B). The median (range) albumin ratio was 5.35 (4.3-9.7) with a reference value of <10.2 and median (range) IgG-index was 0.39 (0.32-0.43) with a reference value of <0.63. CSF and serum neopterin concentrations were elevated in all patients with median (range) neopterin concentrations of 43.0 (26.7-50.0) in CSF and 41.9 (38.6-44.4) nmol/L in serum with upper normal reference values of 5.8 (CSF) and 8.8 (serum) nmol/l. CSF β_2M was elevated in 5/5 measured CSF samples, and in 6/6 serum samples. Median (range) β_2M concentration was 3.1 (1.6-7.2) in CSF, and 3.75 (2.8-6.0) mg/L in serum, with upper normal reference values of 1.8 mg/L (CSF) and 2.1 (serum). CSF neopterin and β_2M concentrations were increased in all tested cases (C). Age-adjusted CSF NfL was increased in patients 3 and 6 (D). The median (range) age-adjusted CSF NfL (65) was 974 (669-1998) ng/L with an upper normal reference value of 1577 ng/L.

Discussion

In this case series study of CSF biomarkers in six patients with COVID-19 and neurological symptoms, we found marked elevations of the two soluble inflammatory biomarkers in all, and abnormal CSF NfL in two patients, while CSF WBC count, albumin ratio and IgG index were normal in all participants. Two patients had low level plasma viremia, while SARS-CoV-2 RNA was detected at indeterminant levels in only one of two PCR assays in the CSF of three patients. These findings outline an unusual pattern in patients with neurological signs during a viral infection with marked elevation of soluble inflammatory biomarkers in the absence of CSF pleocytosis, BBB disruption or intrathecal IgG synthesis.

It remains unclear if SARS-CoV-2 RNA can reach the CNS compartment, although given previous experience in other coronavirus infections, CNS manifestations in COVID-19 are not unexpected and provided rationale for this initial study (16, 17). Animal models of other coronavirus infections also suggest that viral invasion into the CNS can occur (18). The taxonomic similarities between SARS-CoV and SARS-CoV-2 have led many to believe that the route of entry to the brain is enabled by the membrane-bound ACE II (ACE2) (19). ACE2 has been demonstrated to be expressed in neurons, as well as endothelial and arterial smooth muscle cells in the brain, potentially facilitating SARS-CoV-2 entry across BBB to subsequently affect the CNS (20). However, in these six patients with COVID-19 and clinically apparent neurological symptoms, the lack of a cellular CSF response or signs of intrathecal IgG production usually seen in viral meningitis is interesting and implies that the profound CNS immunoactivation reflected by the high CSF neopterin and β2M levels was not driven by direct neuroinvasion of SARS-CoV-2. CSF viral RNA detection has been challenging, and has so far been described in two singular case reports (21, 22). This discrepancy between the lack of CSF viral detection and consistent neurological abnormalities in different stages of COVID-19 infection suggest an alternate pathophysiology, where the intense systemic inflammatory response induced by SARS-CoV-2 infection may be a driving factor. Neuropathogenesis in COVID-19 is likely multifactorial, where hypoxemia, hypercoagulability and systemic inflammation may all contribute to specific stroke syndromes and to the more general or diffuse encephalopathies seen in a majority of our included patients. Underlying comorbidities seen in 3/6 patients may also play a role in the severity of SARS-CoV-2 infection. The metabolic syndrome has been shown to promote a proinflammatory phenotype in macrophages and other immune cells which may contribute to the hyperinflammatory respone seen in individuals with such underlying conditions and COVID-19 infection (23), and may also increase the risk of vascular complications.

The pathophysiological bases of the markedly elevated CSF concentrations of the immune activation indicators neopterin and β2M, remains uncertain. Although the elevated CSF neopterin and β2M suggest CNS monocytic activation, their dissociation from viral detection, pleocytosis or blood-brain barrier disruption suggest mechanisms other than direct viral invasion and CNS infection. Because this

was a common finding across all six patients, it may be a hallmark of more severe SARS-CoV-2 infection and provide a clue to the neurobiology of CNS disturbance and the 'indirect' effects of systemic infection and immune activation on the CNS. Further studies across a broader spectrum of infection are needed to explore this further.

CSF NfL was elevated in two individuals indicating axonal injury. We suspect this may have been caused by an episode of hypoxia or other event but cannot clearly trace the cause. If more directly caused by viral infection or by vigorous CNS inflammation, this cannot be clearly defined in this small data set.

There are a number of clear limitations in this study. Foremost is the small sample size and the inclusion of only individuals with moderate or severe systemic disease with neurological presentations. These all relate to the demands of patient care including their life-threatening aspects and caregiver protections. Specifically, the study did not include a control group of patients with COVID-19 of comparable severity but without neurological manifestations. Nonetheless, the findings are indeed highly provocative and call for further study. Viral RNA was detectable in CSF at low levels in only one of two assays. Although re-testing of low viral load samples after freezing and thawing often fails, failure to reproduce viral detection on different platforms definitely adds a high degree of uncertainty to this finding. In addition, the timing of LP varied between 6 and 15 days from estimated disease onset, which may have had an impact on the results.

In conclusion, we found an unusual pattern of marked CSF inflammation measured by the biomarkers neopterin and β2M, but without the typical responses of CSF pleocytosis, BBB disruption or intrathecal IgG production seen in many other CNS infections. Although SARS-CoV-2 RNA was found in the plasma of two patients, viral detection in CSF was uncertain and altogether, our data do not indicate direct neuroinvasion by SARS-CoV-2 as the underlying mechanism behind the profound CNS immunoactivation seen in this case series.

Appendix 1 Authors

Name	Location	Contribution
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Henrik Zetterberg, M.D., Ph.D.	Department of Psychiatry and Neurochemistry, Institute of Neuroscience & Physiology, the Sahlgrenska Academy at the University of Gothenburg, Mölndal, Sweden	Study concept and design, data analysis and interpretation, manuscript revision
Magnus Gisslén, M.D., Ph.D.	Department of Infectious Diseases, Institute of Biomedicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden	Study concept and design, patient recruitment, data analysis and interpretation, manuscript revision, study supervision

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Figure legends and tables

Figure 1. Disease course in the six patients.

Details of individual patients' disease courses including viral load in nasopharyngeal swabs as index. Vertical arrow indicates time of lumbar puncture. Geometric symbols on dashed line are individual as also seen in Figure 2. Patient 1 died during the study period. Patient 2 was still admitted at time of manuscript submission. Patients 3-6 were discharged. Patients 1 and 2 were treated with chloroquine phosphate and patient 5 with remdesivir.

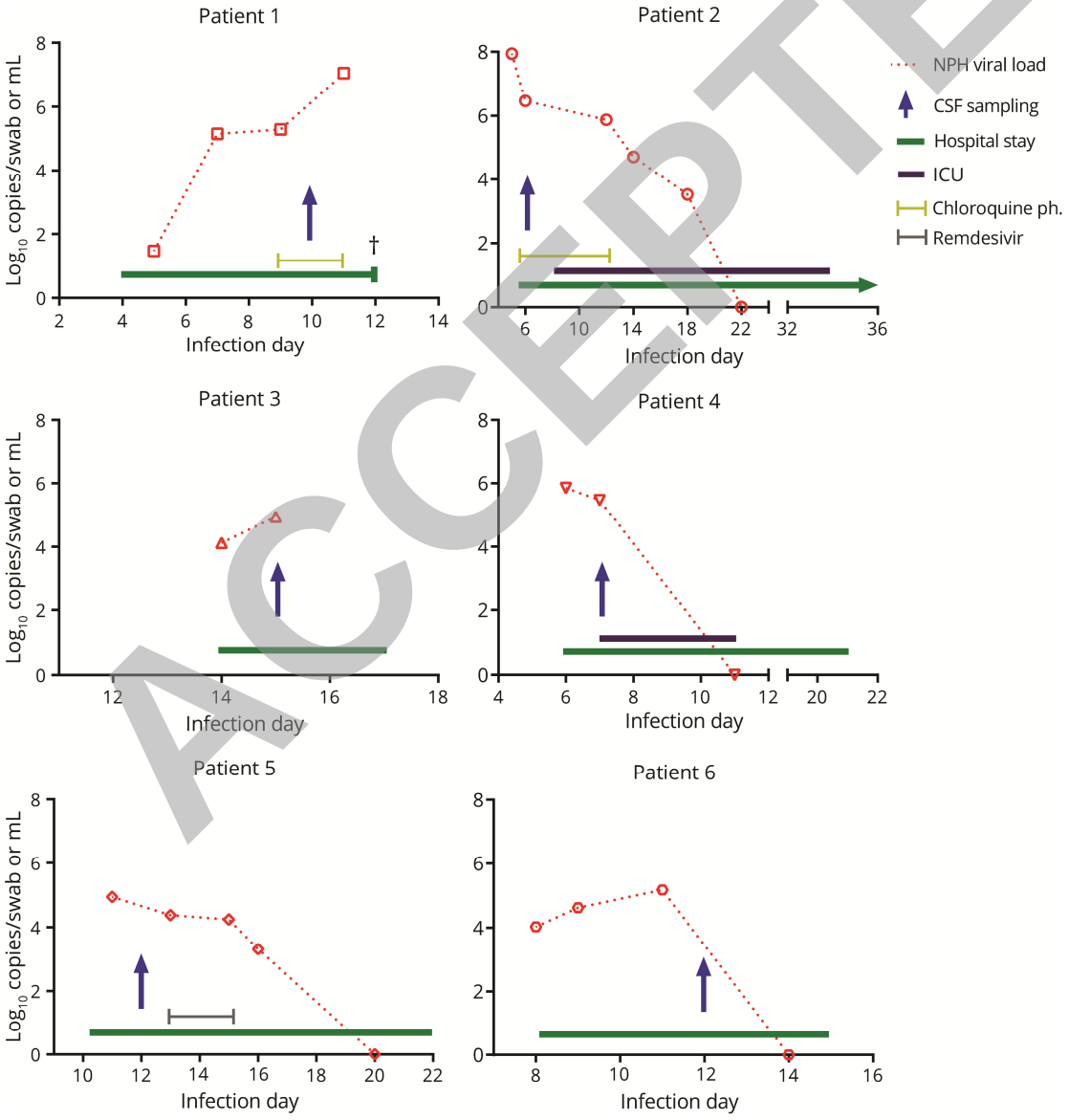

Figure 2. Viral and biomarker measurements

(A) CSF WBC was <3 cells/µL in all patients; symbol shape and colors apply to the patient number and are used in all panels.

(B) Albumin ratios and IgG index values were all in the normal range. The median (range) albumin ratio was 5.35 (4.3-9.7) with a reference value of <10.2 and median (range) IgG-index was 0.39 (0.32-0.43) with a reference value of <0.63.

(C) CSF neopterin concentrations were elevated in all patients. Median (range) CSF neopterin concentrations were 43.0 (26.7-50.0) with an upper normal reference value of 5.8 (CSF) nmol/l. CSF β_2M was elevated in 5/5 measured CSF samples. Median (range) CSF β_2M concentration was 3.1 (1.6-7.2) mg/L, with an upper normal reference value of 1.8 mg/L. Both normal reference values are indicated by dashed lines.

(D) CSF NfL was increased in two patients. The median (range) age-adjusted CSF NfL (65) was 974 (669-1998) ng/L with an upper normal reference value of 1577 ng/L.

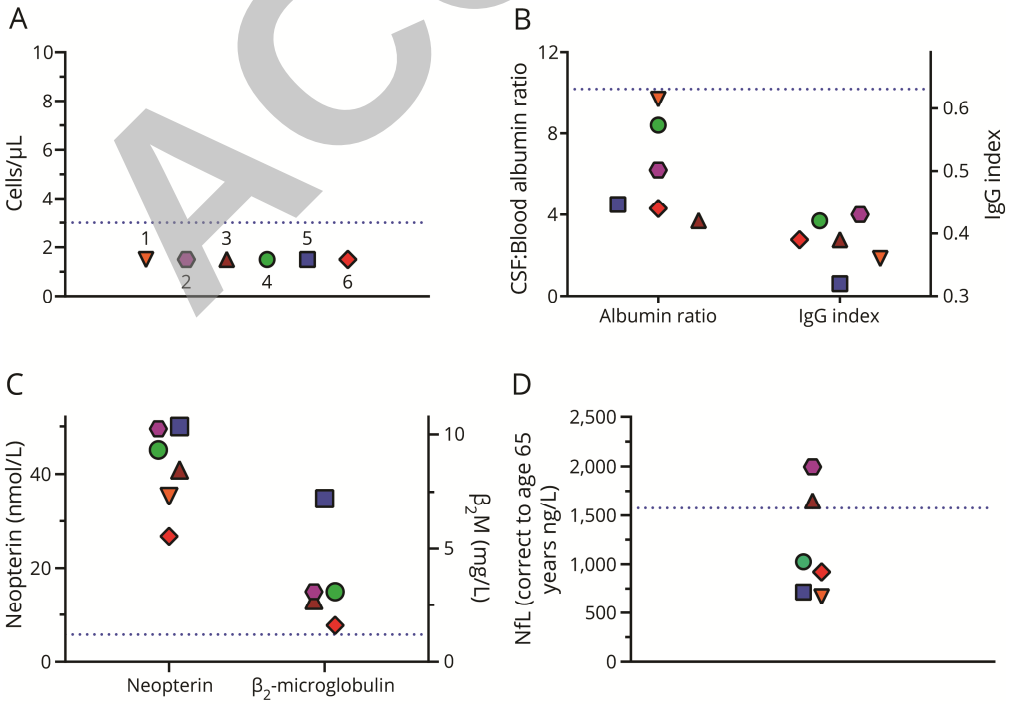

Table 1. Patient characteristics.

					At time of LP				
Patient number	**Sex**	**Age**	**Comorbidities**	**Neurological signs and symptoms**	**POX (O_2 l/min)**	**CRP ; mg/L**	**B-lymph; $x10^9$ cells/L**	**Creatinine; µmol/L (GFR, mL/min/1.73m^2)**	**ALT; µkat/L**
1	F	80s	DM, HT	Encephalopathy	91% (4.5)	160	0.8	76 (55)	0.67
2	M	60s	CHD, DM, HT, obesity	Encephalopathy, Extreme fatigue, memory loss	90% (7)	109	0.6	71 (80)	0.5
3	M	60s	None	Encephalopathy, personality changes	95% (2)	190	1.1	96 (68)	0.5
4	M	60s	Schizophrenia	Moderate neck stiffness, photophobia, somnolence	95% (15)	120	1.3	242 (22)	2.3
5	M	40s	None	Extreme fatigue, dysgeusia, disorientation	96% (2.5)	120	0.7	78 (92)	1.1
6	M	70s	HT	Encephalopathy	94% (1)	110	0.7	67 (79)	2.4

LP, lumbar puncture; POX, pulse oximeter oxygen saturation; O_2, oxygen; CRP, C-reactive protein; B-lymph, Total blood lymphocyte count; GFR, glomerular filtration rate; ALT, alanine aminotransferase; DM, diabetes mellitus; HT, hypertension; CHD, coronary heart disease.

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