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Cytokine Release Syndrome-Associated Encephalopathy in Patients with COVID-19

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Abstract

Severe disease and uremia are risk factors for neurological complications of coronavirus disease-2019 (COVID-19). An in-depth analysis of a case series was conducted to describe the neurological manifestations of patients with COVID-19 and gain pathophysiological insights that may guide clinical decision-making – especially with respect to the cytokine release syndrome (CRS). Extensive clinical, laboratory, and imaging phenotyping was performed in five patients. Neurological presentation included confusion, tremor, cerebellar ataxia, behavioral alterations, aphasia, pyramidal syndrome, coma, cranial nerve palsy, dysautonomia, and central hypothyroidism. Neurological disturbances were remarkably accompanied by laboratory evidence of CRS. SARS-CoV-2 was undetectable in the cerebrospinal fluid. Hyperalbuminorachy and increased levels of the astroglial protein S100B were suggestive of blood-brain barrier (BBB) dysfunction. Brain MRI findings comprised evidence of acute leukoencephalitis (n = 3, of whom one with a hemorrhagic form), cytotoxic edema mimicking ischemic stroke (n = 1), or normal results (n = 2). Treatment with corticosteroids and/or intravenous immunoglobulins was attempted – resulting in rapid recovery from neurological disturbances in two cases. Patients with COVID-19 can develop neurological manifestations that share clinical, laboratory, and imaging similarities with those of chimeric antigen receptor-T cell-related encephalopathy. The pathophysiological underpinnings appear to involve CRS, endothelial activation, BBB dysfunction, and immune-mediated mechanisms.

Key words: COVID-19, neurological manifestations, kidney disease, cytokine, corticosteroids, intravenous immunoglobulins. clinical practice, physiopathology.

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Abbreviations

BBB: blood-brain barrier

CAR: chimeric antigen receptor

COVID-19: coronavirus disease-2019

CRP: C-reactive protein

CRS: cytokine release syndrome

CSF: cerebrospinal fluid

CT: computed tomography

D: days after symptom onset (D0: date of symptom onset)

DWI: diffusion-weighted imaging

EEG: electroencephalogram

ESRD: end-stage renal disease

FLAIR: fluid-attenuated inversion recovery

GCS: Glasgow Coma Scale

ICANS: immune effector cell-associated neurotoxicity syndrome

IL: interleukin

IVIg: intravenous immunoglobulins

MRI: magnetic resonance imaging

RT-PCR: reverse transcription-polymerase chain reaction

SARS: severe acute respiratory syndrome

SARS-CoV-2: severe acute respiratory syndrome coronavirus-2

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Introduction

Coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) can be complicated by neurological manifestations that have an adverse impact on morbidity and mortality [1-4]. A study from China reported preliminary evidence that neurological manifestations may be present in up to 36% of hospitalized cases [1]. Severe neurological manifestations occurred in 10% of patients during their hospital stay, with severe disease and uremia acting as potential risk factors [1]. Unfortunately, the clinical detection of neurological symptoms in patients with COVID-19 remains challenging because of the severity of concomitant respiratory and systemic manifestations. Moreover, the lack of insight into the presentation and pathogenesis of neurological complications is currently hampering effective therapeutic interventions.

Similar to other coronaviruses, SARS-CoV-2 theoretically has the potential to penetrate into the central nervous system (CNS) through hematogenous or retrograde neuronal routes [5-6]. However, the question as to whether SARS-CoV-2 may cause neurological manifestations through a direct neuropathic effects or by promoting a hyperinflammatory reaction mounted by the host's immune system in the form a cytokine release syndrome (CRS) remains to be established [7]. Noteworthy is also the observation that high serum levels of interleukin (IL)-6 are a strong predictor of mortality in patients with COVID-19 [8].

In this study conducted in a renal unit, we describe a series of five patients with COVID-19 and kidney disease who presented severe neurological manifestations. Extensive clinical, laboratory, imaging phenotyping was performed to gain pathophysiological insights that may guide clinical decision-making – especially with respect to the CRS.

PATIENTS AND METHODS

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Patients

Between March 9 and April 9, 2020, a total of 58 patients with COVID-19 were admitted to the renal unit of the Strasbourg University Hospital (Strasbourg, France). Seven patients (12.7%) developed severe neurological manifestations. In the context of clinical care, five of them underwent an extensive laboratory and imaging characterization. The remaining two (presenting with coma and seizures) were excluded because of the lack of extensive laboratory and MRI data. SARS-CoV-2 infection was confirmed in all cases by RT-PCR assays targeting the RNA-dependent RNA polymerase (*RdRp*) viral gene from nasopharyngeal swab specimens. According to current French laws, ethical approval for retrospective studies conducted in the context of clinical care can be waived.

Clinical work-up

All of the five patients underwent an extensive clinical work-up aiming at excluding other causes of neurological impairment – such as alcohol or drug intoxication, metabolic disorders, hypoxemia, thiamine or vitamin B12 deficiencies, epilepsy, hypothermia, sepsis, antibiotic overdosage, hypercapnic encephalopathy, hepatic encephalopathy, and CNS infections. All patients underwent lumbar puncture to screen for the presence of SARS-CoV-2 in the cerebrospinal fluid (CSF). When serum laboratory findings showed signs of CRS, patients received dexamethasone (20 mg/day for five days in all cases, followed by individualized dosing).[9] Antibiotic and antithrombotic prophylaxis was offered to all participants.

Serum laboratory markers

As of the beginning of the COVID-19 pandemic in France, we conducted as an integral part of our clinical practice longitudinal assessments of numerous laboratory markers in an effort to identify the occurrence of CRS – defined by a peak in levels of proinflammatory molecules (IL-6, C-reactive protein, ferritin) and indices of cytolysis (e.g., lactate dehydrogenase). Markers of

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inflammation, CRS, cell lysis, coagulation, thrombotic microangiopathy, were measured. Levels of serum thyroid hormones were also assessed. Serum levels of S100B protein – an established astroglial marker[10] – were quantified on a Roche e411 analyzer (F. Hoffmann-La Roche AG, Basel, Switzerland), whereas IL-6 was measured with a chemiluminescent immunoassay (Lumipulse G600 II; Fujirebio, Tokyo, Japan) [11].

Laboratory analysis of cerebrospinal fluid

To investigate whether SARS-CoV-2 was detectable in the CSF, RT-PCR assays were conducted according to current guidelines (Institut Pasteur, Paris, France; World Health Organization technical guidance). The threshold limit of detection was 10 copies per reaction. Biochemical and immunological analysis of CSF were also performed.

Brain magnetic resonance imaging

All patients underwent brain magnetic resonance imaging (MRI) on a 3-Tesla MRI scanner. The following protocols were applied: three-dimensional T1-weighted spin-echo, three-dimensional fluid-attenuated inversion recovery (FLAIR; both with and without gadolinium), diffusion-weighted imaging (DWI), susceptibility-weighted imaging (SWI), and two-dimensional FLAIR post-contrast imaging.

RESULTS

General characteristics, clinical course, and MRI findings

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Five patients with severe COVID-19 were included in the study: a 71-year-old woman (case 1), 64-year-old man (case 2), a 53-year-old woman (case 3), a 51-year-old man (case 4), and a 67-year-old man (case 5). No patient had a known history of neurological disease or previous neurological manifestations. Case 3 and 4 had severe COVID-19-related acute kidney injury. Table 1 summarizes the general characteristics, clinical features, neurological disturbances, MRI findings, EEG results, treatment approaches, and outcomes of the five study patients.

Neurological presentation included confusion (n = 5), tremor (n = 5), cerebellar ataxia (n = 5), behavioral alterations (n = 5), aphasia (n = 4), pyramidal syndrome (n = 4), coma (n = 2), cranial nerve palsy (n = 1), and central hypothyroidism (n = 3). Case 1 died of coma and secondary infection.

A timeline of the neurological and respiratory course according to symptom onset is shown in Figure 1 A, B, C, D, and E. Neurological disturbances occurred in the second week after COVID-19 onset (24−48 h after respiratory degradation) in cases 1, 2, and 5. In cases 2 and 3, the exact date at onset of neurological signs was not assessable because of the patients' critical conditions requiring mechanical ventilation; therefore, their assessment was conducted following extubation. This issue highlights the paramount importance of biomarkers for early identification of neurological disturbances. Neurological disorders may also play a role in the onset of agitation and may result in a prolonged ventilation time.

Brain MRI findings were heterogeneous and included evidence of acute leukoencephalitis (cases 1, 2, and 4; the latter with a hemorrhagic form), cytotoxic edema mimicking an ischemic stroke (case 2), and normal results (cases 3 and 5; Figure 2).

Table 1. General characteristics, clinical features, neurological manifestations, MRI findings, EEG results, treatment approaches, and outcomes of the five patients included in the case series

Characteristic	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Age (years)/sex	71/F	64/M	53/F	51/M	67/M
Medical history					

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Hypertension	Yes	Yes	Yes	No	Yes
Mellitus diabetes	No	Yes	Yes	No	No
Smoking	No	Yes	No	No	Yes (stopped)
Dyslipidemia	Yes	Yes	No	No	No
Sleep apnea	Yes	Yes	No	No	No
BMI (kg/m^2)	31	29	30	31	20
Renal status	ESRD Polycystic kidney disease Peritoneal dialysis	ESRD Diabetic nephropathy Peritoneal dialysis	AKI stage 3, hemodialysis with kidney recovery	AKI stage 3 – kidney recovery	KTR (C3 glomerulopathy) GFR = 33 mL/min/1.73 m^2
Days from symptom onset at hospitalization	7	8	7	7	6
COVID-19 symptoms at hospitalization	Fever, dyspnea, cough, myalgia	Fever, dyspnea, cough, diarrhea, myalgia	Fever, dyspnea	Fever, dyspnea, anorexia, hypotension	Fever, dyspnea, cough, myalgia
Neurological signs at hospitalization	Confusion	Headache, confusion, minor aphasia, tremor	Headache	None	Headache, anosmia, dysgueusia
Severity of respiratory involvement	Severe	Severe	Critical	Critical	Severe
Neurological features	Confusion, agitation, tremor, pyramidal syndrome, coma, dysautonomia, decerebration, Death	Confusion, agitation, tremor, cerebellar ataxia, aphasia, apraxia, pyramidal syndrome, coma, dysautonomia	Confusion, agitation, tremor, cerebellar ataxia, mild aphasia, behavioral alterations, cognitive disturbances	Confusion, agitation, tremor, cerebellar ataxia, pyramidal syndrome, behavioral alterations, cognitive disturbances	Drop in visual acuity, VI cranial nerve palsy, cerebellar ataxia, behavioral alterations, pyramidal syndrome
Central hormonal dysfunction	Central hypothyroidism Low levels of FSH, LH, ACTH	Central hypothyroidism	No	No	Central hypothyroidism
MRI features	Acute leukoencephalitis. Symmetric FLAIR and DWI white matter hyperintensities predominantly in subcortical white matter	Acute leukoencephalitis and cytotoxic edema. FLAIR and DWI white matter hyperintensities in middle cerebellar peduncles, an acute mm-scale cytotoxic edema on the posterior left frontal lobe, that persisted 16 days later excluding ischemic stroke	Normal	Acute hemorrhagic leukoencephalitis. FLAIR hyperintensities and micro-hemorrhagic lesions in the splenium of the corpus callosum	Normal
EEG features	EEG1: diffuse slow wave spikes. EEG2: asymmetric slow wave spikes and right occipital focus without seizure	EEG1: global and diffuse signal slowdown EEG2: slow bilateral delta elements organized in bursts or predominant opposite bifrontal diversions with bilateral 5−6 Hz theta band elements.	Normal	N/A	N/A
Antiviral treatment at hospitalization	No	Lopinavir-ritonavir	Hydroxychloroquine	Hydroxychloroquine	Hydroxychloroquine
Antiepileptic treatment	Levetiracetam	Oxazepam	No	No	No
Corticosteroids (CS), IVig	CS	CS, IvIg	No	No	CS
Neurological outcome	Temporary improvement after CS, relapse, coma and death	Improvement after CS, relapse, rapid improvement with IVIg	Spontaneous and gradual improvement	Spontaneous and gradual improvement	Rapid improvement with CS

Abbreviations: F, female; M, male; BMI, body mass index; CS, corticosteroids; EEG: electroencephalogram; ESRD, end-stage renal disease; KTR, kidney transplant recipient; GFR, glomerular filtration rate; AKI, acute kidney injury. AKI was staged according to the Kidney Disease Improving Global Outcome (KIDGO) criteria. IVig, intravenous immunoglobulins; N/A, not available, DWI: diffusion-weighted imaging, FLAIR: fluid-attenuated inversion recovery

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A

Day from onset	D7	D8	D9	D10	D11	D12	D13	D14	D15	D16	D17	D18	D19	D20	D21	D22	D23	D24
T max (°C)	38.5	38.6	39.0	38.5	38.0	37.0	37.8	38.0	36.8	37.3	37.0	37.3	37.3	38.9	38.0	37.0	38.5	37.8
02 need (L/min)	0	1	1	1	1	1	1	3	4	4	4	4	5	2	2	2	2	5
pm infiltration (%)								7000%										
GCS	14	14	14	14	14	14	14	12	14	14	13	13	8	8	7	10	4	4
Neurological symptoms		confusion											coma				decerebration	
							tremor				tremor							
								pyramidal syndrome										
	LBP										fall	fall						death
Corticosteroids (mg)								MP 80	DM 10	DM 20	DM 20	DM 20	DM 20					
Tests								*					EEG	EEG	MRI, LP		EEG	

B

Day from onset	D8	D9	D10	D11	D12	D13	D14	D15	D16	D17	D18	D19	D20	D21	D22	D23	D24	D25	D26	D27	D28	D29	D30	D31	D32
T max (°C)	38.5	38.1	38.1	39.3	38.4	39.5	37.3	37	37.5	37.1	37	37.3	37.3	37.3	36.2	37	36.3	37	36.8	36.5	36.6	37.1	36.9	36.8	36.8
O2 need (L/mn)	4	2	3	5	6	6	5	6	6	6	3	3	3	0	1.5	1.5	1.5	1	0	0	0	0	0	0	0
pm infiltration (%)	25-50							75																	
GSC	14	14	14	14	13	11	11	11	12	12	12	13	12	10	11	12	13	13	14	14	14	15	15	15	15
Neurological symptoms	Confusion, agitation, tremor, aphasia, incontinency																								
						Coma, pyramidal sd, cerebellar ataxia, aphasia, apraxia, incontinency											amnesia, pyramidal syndrom								
														Cheyne-S											
		LBP														oLBP		fall		fall	oLBP				
Dexamethasone (mg)						20	20	20	10	10				20	20	20									
IV Ig 0.4 g/kg/d																									
Tests					*				EEG	IRM		LP		IRM	EEG										IRM

C

Day from onset	D7	D8	D10	D11	D12	...	D22	D23	D24	D25	D26	D27	D28	D29	D30	D31	D33	D37	D39
T max (°C)	39.0	39.7	38.4	38.0	37.8			37.0	37.0	37.0	37.3	37.6	37.0	37.0	37.0	37.2	37.0	36.3	36.3
O2 need (L/mn)	12	Mechanical ventilation						2	2	2	2	1	1	1	1	1	0	0	0
pm infiltration (%)	50-75															50-75			
GSC	15	N/A						13-15	13-15	13-15	13-16	13-17	13-18	15	15	15	15	15	15
Neurologic symptoms		N/A						confusion, agitation, ataxia, tremor							Neurological improvement				
								amnesia, dishinibition, cognitive disturbances											
										fall									
						rash													
Tests					*									MRI	EEG		LP		

D

Day from onset	D7	9	D11	D12	D15	D16	D19	D22	D25	D26	D27	D28	D29	D30	D31	D32	D33
T max (°C)	39	40.6	40.1	39.9	38	37				37.3	38.4	37.4	37.5	37.9	37.3	37.2	37.3
O2 need (L/mn)	2	2	mechanical ventilation							3	3	2.5	2	2	2	2	1
pm infiltration (%)	25																
GSC	15	15	N/A							14	15	15	15	15	15	15	15
Neurologic symptoms			N/A							Confusion							
										Pyramidal sd, ataxia, tremor, nystagmus							
										amnesia, cognitive disturbances, spasmodic tears, depression							
					rash									oLBP		oLBP	
Tests				*									MRI			LP	

E

Day from onset	D6	D7	D8	D9	D10	D11	D12	D13	D14	D15	D16	D17	D18	D19
T max (°C)	37,3°C	38,5°C	38,7°C	39,2°C	38,7°C	36,7°C	36°C	36.3	36.7	36.4	36.4	36.3	36.9	
O2 need (L/mn)	0	0	0	2	2	2	2	2	2	0	0	0	0	0
pm infiltration (%)	10				25									
GSC	15	15	15	15	15	15	15	15	15	15	15	15	15	15
Neurological symptoms						reduced visual acuity								
						left diplopia			partial regression diplopia					
						cerebellar ataxia, tremor, pyramidal sd								
Autre	Atrial fibrillation		oLBP			distal vein thrombosis								
Corticosteroids (mg)					DM 20	MP 500	MP 500	MP 500	DM 20	DM 10	DM 10	DM 10	DM 10	DM 10
Tests					*	MRI				LP				

Figure 1, panels A−E. Temporal course of clinical parameters and laboratory variables in the five study patients.

Diagnostic investigations and administered drugs are also reported. All longitudinal data are shown with respect to the date of symptom onset (D0). Red stars denote the days on which levels of CRS-related inflammatory biomarkers reached a peak.

Abbreviations: CSF, cerebrospinal fluid; Tmax, maximum body temperature; % pm infiltration, percentage of lung infiltration on chest scan; GCS, Glasgow Coma Scale; LBP, low blood pressure; oLBP, orthostatic hypotension; sd, syndrome; MP, methylprednisolone; N/A, not available, DM, dexamethasone; VA, visual acuity.

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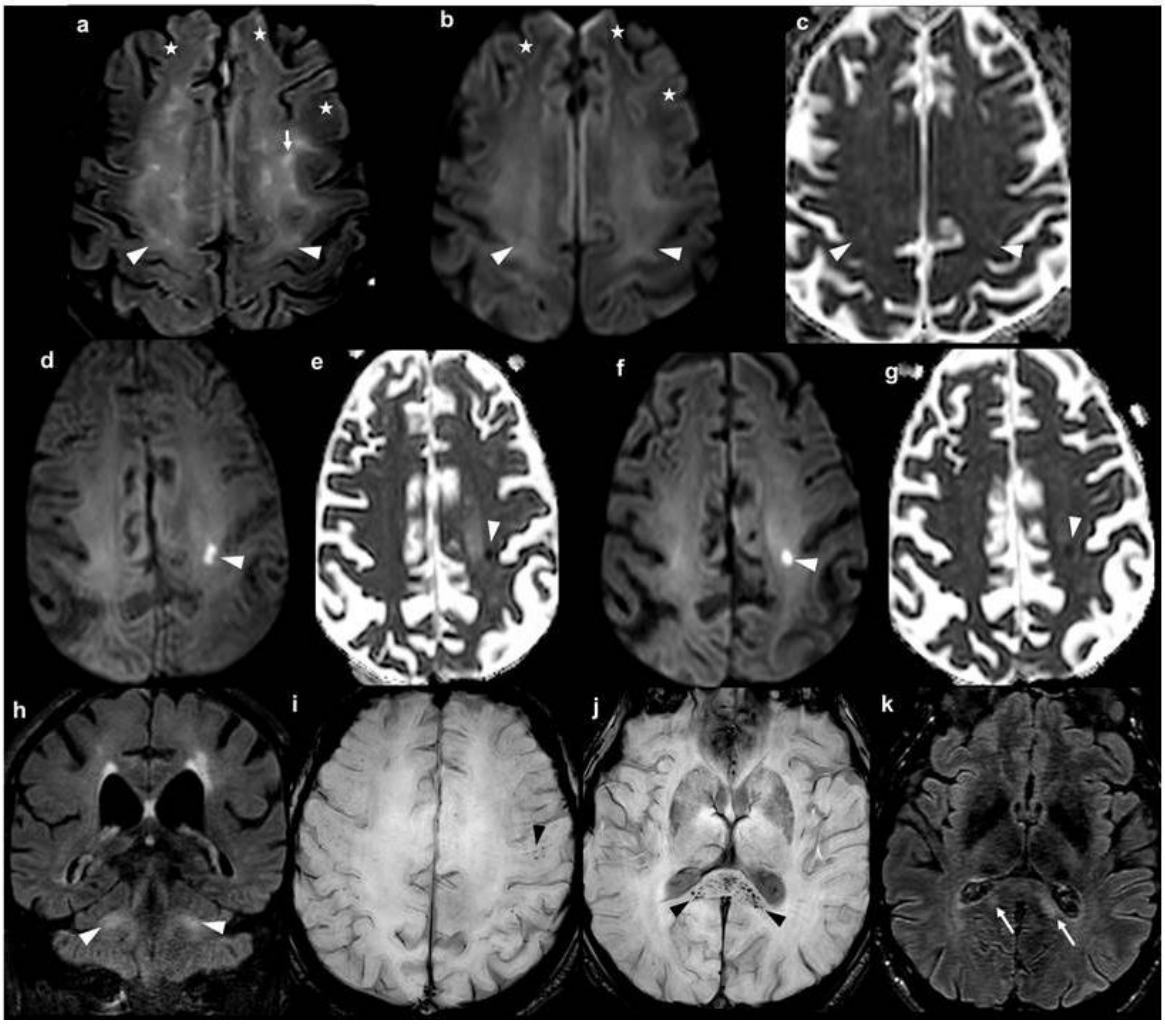

Figure 2, panels a-l. Brain MRI findings in the five study patients.
Panels a, b, c: Case 1. Axial FLAIR weighted MRI (a), axial diffusion-weighted MRI (b) and axial Apparent Diffusion Coefficient (ADC) map (c). MRI images obtained on D21; cross-sections through motor areas, frontal and parietal lobes. Diffuse bilateral, symmetric white matter FLAIR hyperintensities with mild hyperintense "ground glass" areas (arrow heads) and frank hyperintense areas (conventional radiological FLAIR hyperintensities) (arrows). All abnormal FLAIR areas appeared hyperintense in the diffusion sequence and were characterized by a gradient: frank hyperintense FLAIR lesions had a higher intensity than "ground glass" areas. On the ADC map, "ground glass" areas were iso- or hypointense, whereas frank hyperintense FLAIR areas were hyperintense. Abnormal FLAIR hyperintensities were preferentially localized to subcortical white matter of motor areas and showed a bilateral symmetric distribution. The anterior frontal white matter appeared normal on FLAIR and DWI sequences (a, b: stars).
Panels d, e, f, g, h: Case 2. Axial DWI (d) and axial ADC map (e): cross-sections through frontal and parietal lobes on D17. Axial DWI (f) and axial ADC map (g): cross-sections through frontal and parietal lobes on D33. Coronal FLAIR weighted MRI on D33 (h): cross-section through middle cerebellar peduncles. The first MRI (on D17) revealed an acute hyperintense DWI lesion, with hypointensity on ADC map, suggestive of a cytotoxic edema in the left frontal lobe (d, e) and deemed initially compatible with an acute stroke. However, this lesion maintained a similar aspect following 16 days (f, g), casting doubts on its ischemic origin. Persistence of middle cerebellar peduncles hyperintensities was also evident (h).
Panels i, j, k: Case 4. Axial SWI weighted MRI at D31: cross-section through motor areas, frontal and parietal lobes (i) and the splenium (j). Axial FLAIR weighted MRI (k): cross-section through the splenium. Multifocal microbleeds were evident in the splenium (j: black arrow heads)

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and in the white matter/gray matter junction (i: black arrow head), with an apparent perivascular distribution in the Virchow-Robin spaces. These lesions were associated with hyperintensities on FLAIR weighted sequence (k: arrows).

Serum laboratory markers

The temporal course of CRS biomarkers is presented in Figure 3 A, B, C, D, and E. Neurological manifestations occurred simultaneously with the peak in CRS serum markers (i.e., CRP, IL-6 and LDH) in cases 1, 2, and 5. The peak in CRS markers for cases 2 and 3 occurred while the patients were under mechanical ventilation and serve as potential biomarkers. Serum levels of the astroglial marker, S100B protein, were increased at the time of CRS – reflecting an increased permeability of the blood-brain barrier (BBB) – and returned to their reference range when neurological symptoms and signs of hyperinflammation regressed (Figure 4). Detailed laboratory data are reported in Table 1S. Circulating anticoagulant was detected in cases 1, 2, and 4 but not in case 5. Antiphospholipid antibodies were present in case 1 but undetectable in the remaining four cases. Antinuclear antibodies were assessed in cases 2 and 5 and found to be positive. Serum total complement as well as C3 and C4 were within the reference ranges.

CSF analysis

SARS-CoV-2 was undetectable in the CSF for all patients. According to data from our Department of Virology, SARS-Cov-2 was undetectable in 88 of the 90 patients with COVID-19 who underwent RT-PCR testing of CSF (personal communication).

CSF/serum albumin index was measured in four patients and found to be increased in three – suggesting the presence of BBB dysfunction. Neither CSF cells nor IgG intrathecal synthesis were evident. A CSF increase in IL-6 levels was observed in two patients at the time of lumbar puncture. With the only exception of case 5, oligoclonal bands were undetectable. Antineuronal antibodies were absent in all patients (Table 2S).

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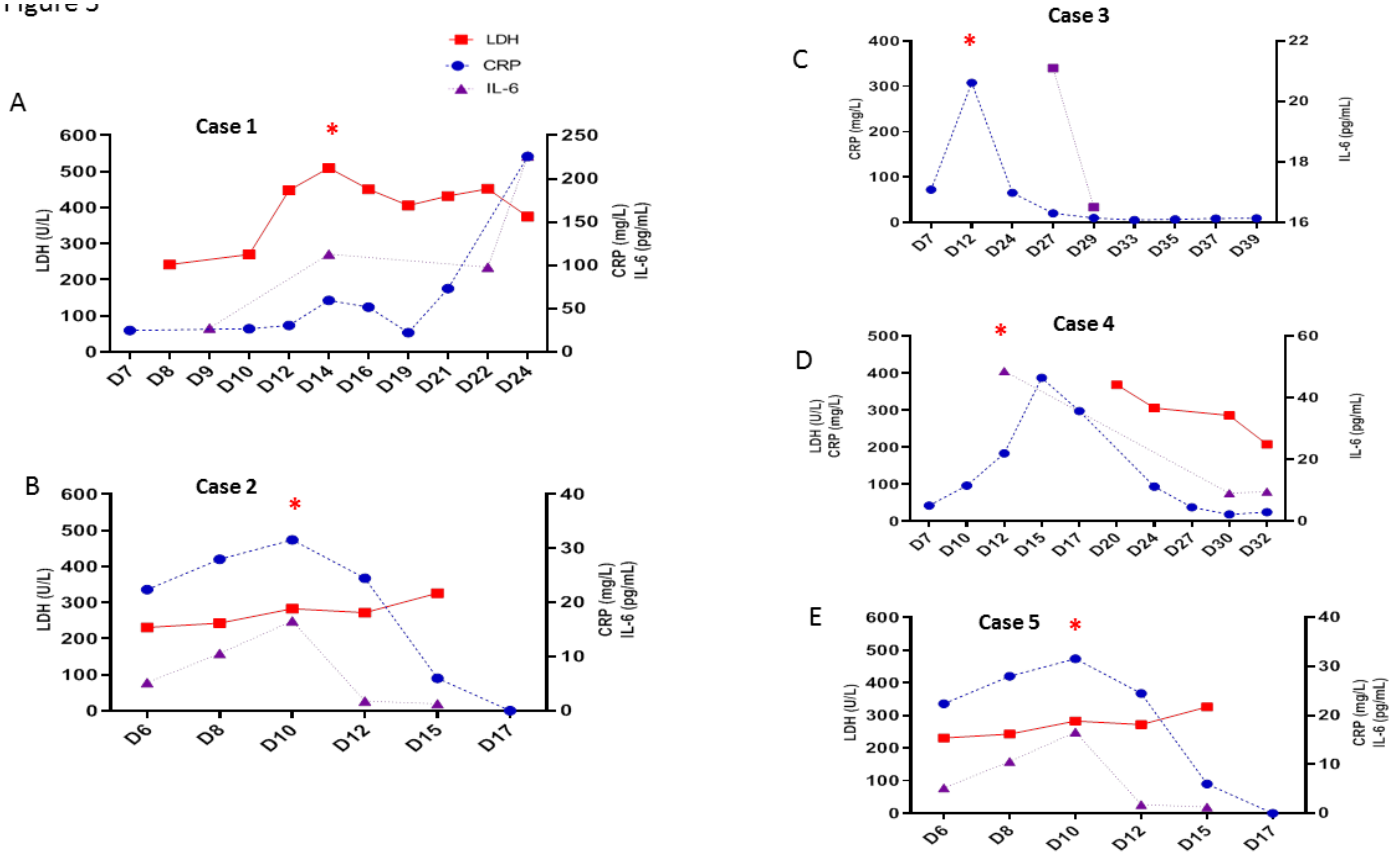

Figure 3. Temporal course of biomarkers of cytokine release syndrome in the five study patients. Red stars denote the days on which levels of CRS-related inflammatory biomarkers reached a peak. Reference values: IL-6, <4 ng/L; lactate dehydrogenase (LDH), 120−246 UI/L; CRP <4 mg/L.

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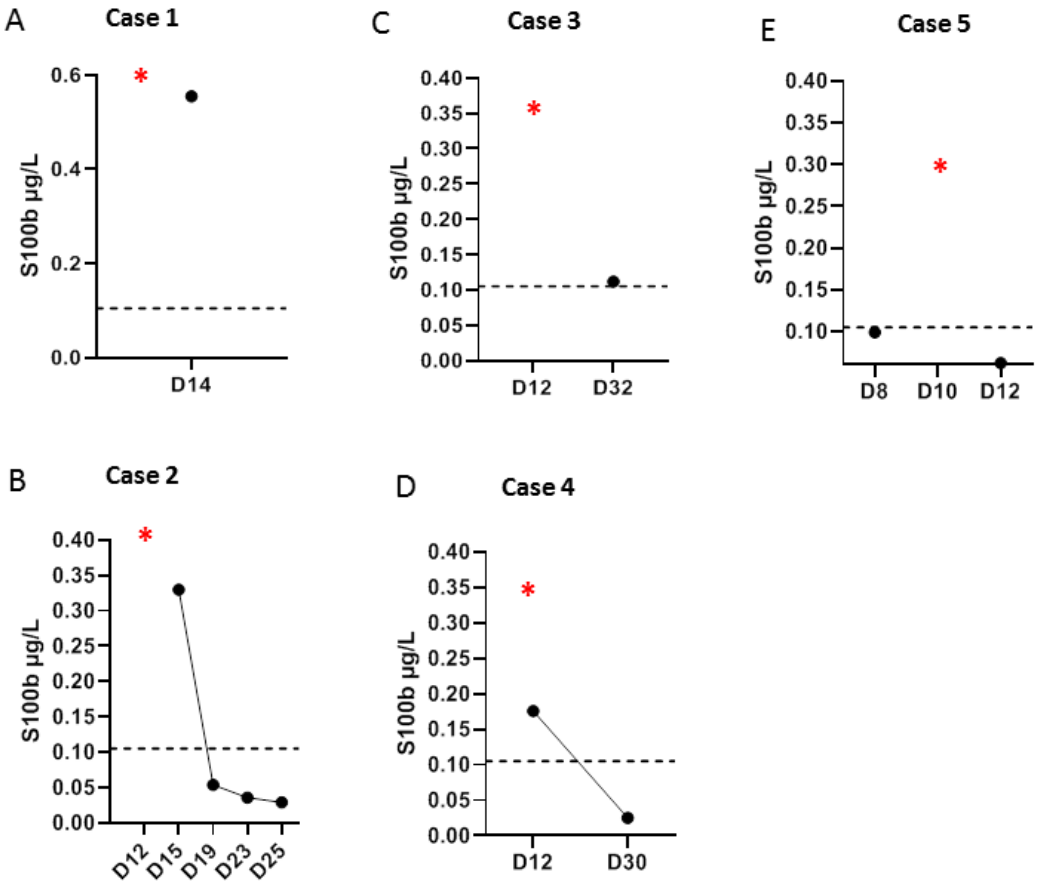

Figure 4. Temporal course of serum S100B (µg/L) levels in the five study patients.

Values above the dotted line indicate high values (>0.105 µg/L). Red stars denote the days on which levels of CRS-related inflammatory biomarkers reached a peak.

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Treatment approaches and outcomes

The treatment approaches and outcomes of the five study patients are shown in Figure 1. Therapeutic attempts with corticosteroids and intravenous immunoglobulins are described below.

Case 1

Corticosteroids were given four days after the symptom onset (D14) to tackle CRS, followed by a temporary improvement with regression of neurologic disturbances (Figure 1A). Unfortunately, the patient experienced a second worsening of her neurological state on D19 (Glasgow Coma Scale [GCS] = 8) and shed died of coma and secondary infection on D24.

Case 2

Two days after admission (on D10), the patient was switched on hemodialysis in an effort to decrease blood urea nitrogen but no improvement in neurological conditions was observed (ultimately excluding uremic encephalopathy). Further deterioration occurred on D13, when he presented with a diffuse pyramidal syndrome, mixed fluent and non-fluent aphasia, anterograde amnesia, cerebellar ataxia, a dysexecutive syndrome, and dysautonomia. Following a 5-day course of dexamethasone, an improvement of both consciousness and respiratory conditions was observed (Figure 1B). Four days after withdrawal of dexamethasone, there was a further neurological relapse with appearance of Cheyne-Stokes breathing (GCS = 10). Because of the life-threatening neurological conditions, dexamethasone was reintroduced along with intravenous immunoglobulins (IVIg) for 5 days. A complete and rapid clinical response was observed. A second cycle of IVIg has been planned.

Case 3

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The neurological state spontaneously and gradually improved until discharge on D40.

Case 4

Neurological disturbances gradually and spontaneously regressed with recovery of orientation, memory function and motor function. Although ataxia and nystagmus improved partially, pyramidal syndrome and affective symptoms persisted on discharge on day 35.

Case 5

A 67-year-old man with a history of KT was admitted on D6 with moderate dyspnea. Mycophenolate mofetil was withdrawn followed by administration of hydroxychloroquine. On D9, worsening of respiratory conditions with signs of CRS was evident – prompting dexamethasone administration. On D11, he developed acute neurologic alterations consisting of bilateral reduced visual acuity, horizontal diplopia on the left side, predominant right cerebellar ataxia, tetrapyramidal syndrome, and personality change. Visual assessment revealed 7/10 right visual acuity drop, 1/10 left visual acuity drop in a previously amblyopic eye, a left abduction deficiency, and a left cervical stiff neck caused by sixth nerve palsy. Pupillary light reflexes and fundus examination were normal. Signs of neurological deterioration were paralleled by laboratory evidence of CRS. Upon treatment with methylprednisolone (500 mg/day for three days), both neurological disturbances and respiratory symptoms improved. Dexamethasone was subsequently given for ten days to tackle CRS (Figure 1E). On D60, all neurological signs have regressed except slight palsy of left nerve VI.

DISCUSSION

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Severe disease and uremia are potential risk factors for neurological complications in patients with COVID-19 [1]. Hypertension, obesity, and diabetes – which are known risk factors for severe forms of COVID-19[12] – occur frequently in patients with renal disorders.

The present case series provides an in-depth clinical, laboratory, and imaging characterization of five patients with COVID-19 who developed severe neurological disturbances. The main findings can be summarized as follows. First, the clinical presentation of CNS involvement included confusion, agitation, tremor, impaired consciousness, dysexecutive syndrome, pyramidal syndrome, cerebellar ataxia, cranial nerve palsy, dysautonomia, and central hormonal dysfunction (mainly in the form of hypothyroidism). Noteworthy is also the observation that the onset of neurological manifestations occurred in the second week after COVID-19 symptoms onset, i.e., simultaneously with the worsening of respiratory conditions. The systemic presentation was reflective of clinical symptoms related to the CRS (i.e., fever, headache, myalgia, occasional rash, respiratory failure, and – in some cases – multiorgan failure) [13]. Second, the laboratory work-out demonstrated that SARS-CoV-2 was undetectable in the CSF in our patients (as it was in 98% of patients who performed this test in our hospital). Signs and symptoms of CNS impairment were accompanied by evidence of CRS in serum samples with marked elevation of serum IL-6. Third, we found evidence of an increased BBB permeability as attested by the presence of hyperalbuminorachy. Moreover, elevation of serum levels of the S100B protein – an astroglial marker – was found to occur simultaneously with CRS. Finally, MRI findings were heterogeneous and included evidence of acute leukoencephalitis (n = 3, of whom one with a hemorrhagic form), cytotoxic edema mimicking an ischemic stroke (n = 1), or normal results (n = 2). Notably, MRI findings are frequently normal both in influenza-related acute encephalitis and autoimmune encephalitis [14].

Our findings of CRS-associated neurological disturbances in COVID-19 expand previous observations showing that hyperinflammation is associated with deterioration of respiratory function and death [7, 8]. Our data may have relevant implications with respect to clinical management. Because a reliable detection of neurological symptoms may be unfeasible in

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patients on ventilation support, laboratory evidence of CRS should alert clinicians about a potential risk of CNS involvement. Notably, the onset of neurological manifestations was also paralleled by an increase in serum levels of S100B protein (reflecting an increased BBB permeability) [10]. Elevated serum S100B levels have been previously reported in other acute encephalitis and immune effector cell neurotoxicity syndrome (ICANS) [15-17].

CRS is a potentially fatal complication of different infectious (e.g., influenza, SARS, Epstein-Barr virus-associated hemophagocytic lymphohistiocytosis) and noninfectious (e.g., multiple organ dysfunction syndrome, multiple sclerosis) diseases. Moreover, it can have a iatrogenic origin during the course of T and B lymphocyte engaging therapies like chimeric antigen receptor (CAR)-T-cell immunotherapy, rituximab, and immune check-point inhibitors [18,13]. CRS is triggered by an initial release of proinflammatory cytokines from activated T and/or B cells; this event in turn activates bystander immune cells and endothelial cells to produce proinflammatory molecules. Engagement of other immune cells follows in the context of a self-perpetuating positive feedback loop. In this scenario, excess production of IL-6 from activated macrophages is a key event. CRS-driven neurological disturbances have been described for the first time following CAR-T cell therapy and termed ICANS. Remarkably, the clinical, laboratory, and imaging features of severe ICANS closely resemble those shown by the five patients with COVID-19 described in our series [15, 19, 20]. Brain MRI in ICANS revealed the presence of acute T2/FLAIR hyperintensities suggestive of interstitial edema of varying severity and small (mm-scale) ischemic strokes [15]. One of our cases had a lesion in the splenium of the corpus callosum – a finding in accordance with previous data reported in ICANS[21] and encephalitis complicating acute viral infections (e.g., influenza and mumps) [14]. In ICANS, hemorragic forms (similar to the findings of patient 4 in our series) have been described.[20] More importantly, proinflammatory cytokines like IL-6 have been shown to lead to endothelial damage and BBB dysfunction in this clinical entity [20,15].

All of these findings, coupled with the extant evidence, allow offering a pathogenetic model (Figure 5) according to which COVID-19-associated neurological manifestations are driven by

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CRS and consequent immune response. Some neurological complications of COVID-19 may be caused by endothelial activation with subsequent increased BBB permeability, edema,[22] and penetration of proinflammatory cytokines that can activate microglial cells in the CNS. The resulting neuroinflammatory response may yield to reactive gliosis accompanied by infiltration of CD68+ monocytes/macrophage [23] and release of S100B protein. This pathophysiological model is in accordance with an autopsy report in COVID-19 showing a range of white matter pathology with an anatomical distribution compatible with MRI lesions observed in our patients (i.e., subcortical areas, corpus callosum, middle cerebellar peduncle) and the lack of typical features of viral encephalitis. Rare neocortical microscopic organizing infarcts were also identified, suggesting a vascular origin followed by secondary myelin loss [24].

In keeping with our management strategies to tackle CRS, three of our patients with neurological disturbances received corticosteroids and one IVIg with improvement. This in accordance with two case of encephalopathy in COVID-19 with improvement after steroid [25], and IVIg [26].

Blockade of IL-6 with tocilizumab – an anti-IL-6 receptor (IL-6R) monoclonal antibody – has been also approved to treat CRS complicating CAR-T cell therapy in the absence of neurological manifestations. Although tocilizumab may be effective against CRS, this molecule is not clinically useful to treat neurotoxicity in the context of CAR-T cell therapy and can even exacerbate this condition[13,15]. This paradoxical effect may be explained by the transient rise in serum IL-6 observed following tocilizumab administration and by the poor penetration of this antibody into the cerebrospinal fluid[27].

Administration of IVIg during the early course of severe COVID-19 may improve clinical outcomes through their immunomodulatory effects against the self-perpetuating positive inflammatory loop typical of CRS [18,19]. Interestingly, high-dose IVIg may reduce *in vitro* and *in vivo* production of IL-6 in other infectious or autoimmune diseases [20,21]. In the current series, a rapid recovery was observed following administration of IVIg (0.4 g per kg of weight per day for five consecutive days) in case 2.

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Our findings need to be interpreted in the context of some limitations. Notably, this is a case series and some neurological manifestations occurring in patients with COVID-19 – especially related to neurovascular dysfunction – might have been absent in our patients.

In conclusion, we provide a pilot description of the peculiar clinical, laboratory, and imaging findings of COVID-19-associated neurological disturbances. The clinical manifestations were chiefly driven by peripheral CRS, absent direct CNS invasion by SARS-CoV-2. Pending future confirmation, our data indicate that corticosteroids aimed at tackling CRS [28] and IVIg may be effective to control severe neurological disturbances in patients with COVID-19.

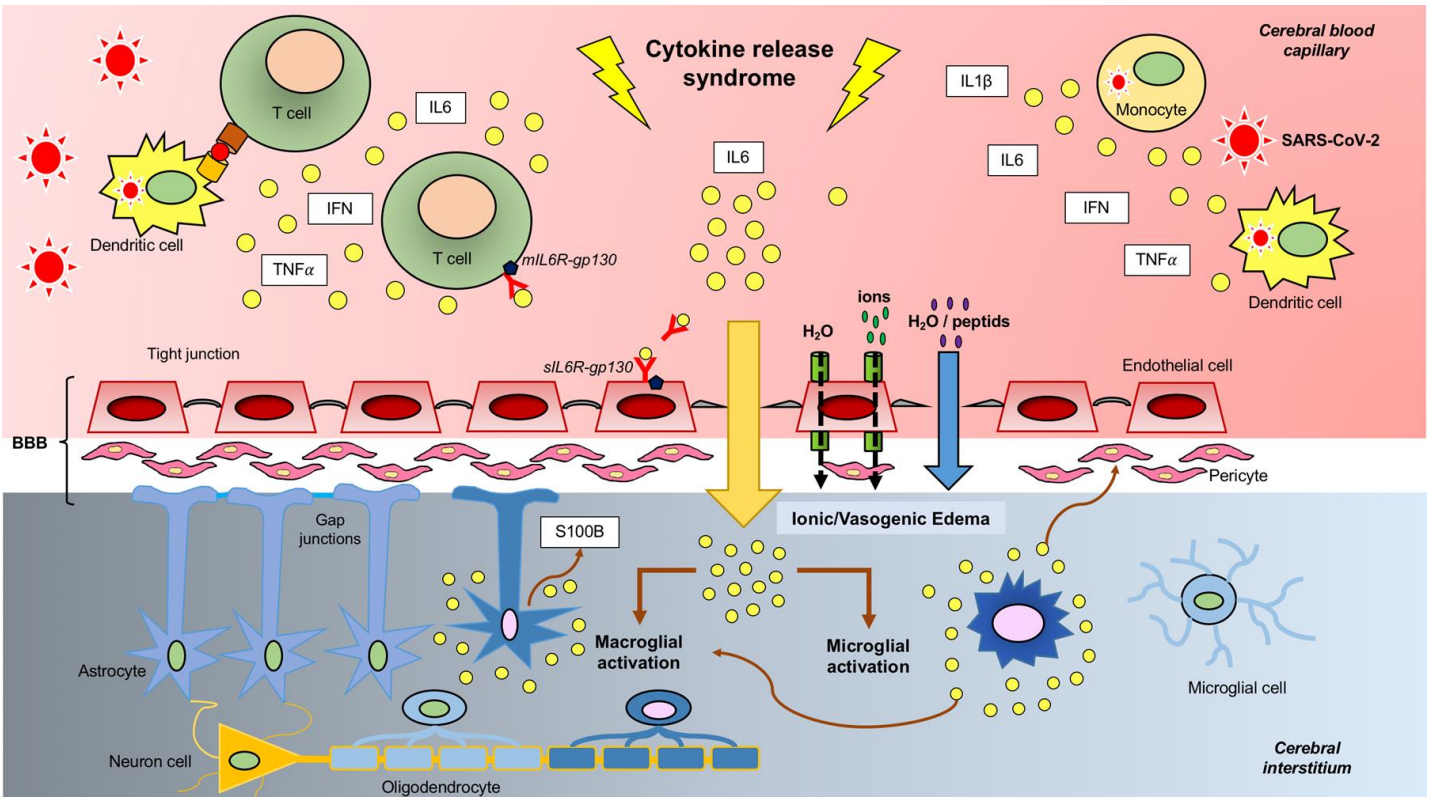

Figure 5. Cytokine release syndrome-associated encephalitis in COVID-19: pathophysiological model

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Abbreviations: BBB, blood-brain barrier; sIL6R, soluble interleukin-6 receptor; gp130, glycoprotein 130; ACE-2, angiotensin-converting enzyme-related carboxypeptidase; TNF, tumor necrosis factor; IL, interleukin; IFN, interferon; H_2O: water.

In severe cases of COVID-19, SARS-CoV-2 induces a cytokine release syndrome during the second week of infection. Following viral infection, macrophages, dendritic cells, other immune cells, and endothelial cells become activated and produce large amounts of proinflammatory molecules (including IL-6, IL-1beta, TNF-alpha, IFN-alpha/beta, and IFN-gamma). IL-6 acts as a master mediator of a self-perpetuating proinflammatory loop that results in lymphocyte activation (via the cis-pathway) followed by a massive release of cytokines (cytokine storm). The trans-pathway of activation may alter endothelial permeability – resulting in blood-brain barrier (BBB) dysfunction [28, 22]. In turn, an altered BBB permeability may lead to edema and even red blood cells extravasation (potentially accounting for the hemorrhagic form of acute leukoencephalitis observed in case #4). This sequence of events closely resembles those occurring in immune-effector cell neurotoxicity syndrome [20]. Proinflammatory cytokines can leak through a dysfunctional BBB, ultimately activating microglial cells (brain tissue-resident macrophages). This may in turn lead to a secondary inflammatory response in the macroglia accompanied by the release of S100B, an astroglial protein reflecting both glial activation and BBB dysfunction. The resulting neuroinflammatory response can yield to reactive gliosis accompanied by infiltration of CD68+ monocytes/macrophage [23, 24].

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Authors' contributions

Conceptualization, Peggy Perrin, Nicolas Collongues, Seyyid Baloglu; data acquisition, Peggy Perrin, Seyyid Baloglu, Dimitri Bedo, Xavier Bassand, Gabriela Gautier, Nicolas Keller, Stephane Kremer, Samira Fafi-Kremer, Ilies Benotmane; Data analysis, Peggy Perrin, Nicolas Collongues, Seyyid Baloglu, Dimitri Bedo, Thomas Lavaux, Stephane Kremer, Bruno Moulin, Ilies Benotmane, Sophie Caillard; Writing – Original draft preparation; Writing – review and editing, Nicolas Collongues, Seyyid Baloglu, Bruno Moulin, Sophie Caillard, Ilies Benotmane; Software (graphics), Ilies Benotmane; Figure 5 design, Dimitri Bedo; Supervision, Sophie Caillard.

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Supplementary Materials

Table 1S. Laboratory findings of the five study patients at hospitalization and during follow-up.

All longitudinal data are shown with respect to the date of symptom onset (D0). For each case, three columns are presented. The first column shows the value measured on the day of hospitalization. The second column reports the nadir or peak levels observed during follow-up (as appropriate). The third column shows the day (measured from D0) on which the nadir or peak level was registered. *Nadir level during follow-up **Peak level during follow-up.

	Case 1			**Case 2**			**Case 3**			**Case 4**			**Case 5**		
	D7	**Follow-up**	***Delay***	**D8**	**Follow-up**	***Delay***	**D7**	**Follow-up**	***Delay***	**D7**	**Follow-up**	***Delay***	**D6**	**Follow-up**	***Delay***
IL-6 (ng/L)**, reference <4 ng/L	N/A	97.8	*D22*	N/A	61	*D13*	N/A	21.1	*D21*	N/A	N/A		N/A	14.8	*D10*
C-reactive protein (mg/L)**	24.6	225.8	*D24*	64	116	*D11*	72.2	308	*D6*	42.4	388	*D15*	22.4	31.6	*D10*
Procalcitonin (µg/L)**	0.45	1.73	*D14*	0.78	1.66	*D11*	0.21	3.02	*D6*	0.07	1.56	*D32*	0.08	0.23	*D10*
Ferritin (µg/L)**	N/A	9250	*D12*	1263	2593	*D15*	N/A	176	*D21*	N/A	1030	*D30*	442	695	*D13*
Albuminemia (g/L)*	35	32	*D21*	40	33	*D18*	42	30	*D6*	N/A	30	*D20*	48	41	*D12*
Lymphocytes ($\times 10^9$/L)*	0.78	0.16	*D21*	0.17	0.11	*D14*	1.19	1.08	*D6*	0.99	0.57	*D12*	0.94	0.61	*D14*
Hemoglobin (g/dL)*	10.7	10.2	*D22*	10.9	10.4	*D18*	14	7.6	*D20*	15.1	9.6	*D21*	14.2	12.4	*D13*
Platelet count ($\times 10^9$/L)*	98	74	*D10*	65	65	*D8*	337	244	*D23*	188	188	*D14*	214	189	*D12*
Haptoglobin**	N/A	3.53	*D16*	2.82	3.61	*D18*	N/A	N/A		N/A	N/A		N/A	2.43	*D8*
High-sensitivity troponin (ng/L)**	89	289	*D19*	115	186	*D13*	N/A	3.8	*D23*	N/A	22.2	*D12*	18.6	29.8	*D10*
Creatine kinase (U/L)**	N/A	289	*D12*	902	2624	*D15*	77	29	*D3*	N/A	191	*D12*	56	163	*D10*
Lactate dehydrogenase (U/L)**	242	522	*D14*	386	612	*D12*	N/A	250	*D23*	N/A	369	*D20*	231	283	*D10*
Aspartate aminotransferase (U/L)**	20	130	*D14*	34	100	*D15*	21	34	*D13*	37	63	*D20*	20	23	*D10*
Alaline aminotransferase (U/L)**	31	120	*D14*	37	63	*D17*	28	108	*D15*	29	54	*D20*	23	24	*D10*
Lactic acid (mmol/L)**	N/A	2.75	*D19*	0.73	1.34	*D13*	1.3	2.05	*D8*	0.6	1.37	*D15*	1.85	0.78	*D8*
Fibrinogen (g/L)**	4.55	7.89	*D24*	6.96	10.63	*D13*	6.32	8.45	*D5*	N/A	10.5	*D16*	5.66	6.23	*D10*
D-dimer (µg/L)**	740	790	*D12*	2310	6340	*D18*	N/A	3080	*D23*	N/A	2610	*D21*	870	870	*D6*
Blood urea nitrogen (mmol/L)**	33.4	39.2	*D22*	28	59	*D18*	17.8	43.4	*D16*	6.1	26.7	*D24*	12.5	28	*D12*
Creatinine (µmol/L)	Dialysis			Dialysis			170	569	*D15*	101	296	*D22*	145	234	*D9*
Proteinuria/creatininuria (g/g)**	0.9	0.9	*D7*	5.3	5.3	*D8*	1.3	1.3	*D7*	N/A	1.3	*D19*	0.2	0.2	*D6*
Natremia (mmol/L)*	133	127	*D19*	130	126	*D16*	138	134	*D14*	139	135	*D32*	139	136	*D10*
Kaliemia (mmoL/L)*	3.7	3.02	*D20*	3.56	3.56	*D8*	3.9	3.19	*D8*	3.91	3.07	*D17*	3.67	3.67	*D6*
Magnesemia (mmol/L)*	N/A	0.6	*D10*	0.63	0.63	*D8*	0.68	0.56	*D7*	N/A	0.82	*D32*	0.75	0.75	*D6*
Calcemia (mmol/L)*	1.78	1.78	*D7*	1.66	1.66	*D8*	2.18	2.18	*D3*	N/A	2.02	*D12*	2.35	2.24	*D12*
TSH (mUI/L)*	N/A	0.03	*D21*	N/A	0.12	*D18*	N/A	0.06	*D7*	N/A	1.56	*D32*	N/A	0.18	*D10*
T4L (ng/L)*	N/A	5.68	*D21*	N/A	8.2	*D18*	N/A	7.53	*D7*	N/A	N/A		N/A	11.4	*D10*
Total complement (U/mL)*	N/A	66	*D22*	N/A	65	*D25*	N/A	47	*D31*	N/A	N/A		N/A	>96	*D10*
C3 (g/L)*	N/A	0.84	*D22*	N/A	1	*D25*	N/A	1.39	*D31*	N/A	N/A		N/A	1.19	*D10*
C4 (g/L)*	N/A	0.25	*D22*	N/A	0.29	*D25*	N/A	0.41	*D31*	N/A	N/A		N/A	0.38	*D10*
Circulating anticoagulant		Positive			Positive			N/A			Positive			Negative	
Antiphospholipid antibodies		Positive [a]			Negative			Negative			Negative			Negative	
Anti-nuclear antibodies		N/A			Positive 1/320e			N/A			N/A			Positive 1/160e	

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Abbreviations: D, days; N/A, not available. [a] Anticardiolipin IgM and anti-β2-glycoprotein IgM were detected. Electrolyte analysis in cases 1 and 2 revealed hypocalcemia (in the context of cinacalcet use for secondary hyperparathyroidism) and hyponatremia (in the context of hyperosmolality induced by icodextrin used for peritoneal dialysis).

Table 2S. Laboratory analysis of cerebrospinal fluid in the five study patients

	Case 1	Case 2	Case 3	Case 4	Case 5
Biochemistry					
Aspect	Clear	Hemorrhagic*	Clear	Clear	Clear
Glucose (g/L)	1.08	1.05	0.7	0.51	0.53
Proteins (g/L)	0.57	1.1	0.29	0.58	0.67
Albumin (mg/L), reference <250 mg/L	N/A	386	180	374	485
IgG (mg/L), reference <42 mg/L	N/A	55.7	20.7	61.5	53
CSF/serum albumin index (CSF × 10^3/serum albumin), normal<8	N/A	14.6	5.9	11.8	15.2
CSF IgG index, normal <0.7	N/A	0.49	0.42	0.4	0.51
Oligoclonal bands	N/A	Negative	Negative	Negative	IgG oligoclonal bands (mirror pattern)
Red blood cells (number/mm^3)	<1	31800	N/A	6	2.4
White blood cells (number /mm^3)	<1	2	N/A	<1	1
IL-6 (ng/L), reference < 4 ng/L	30	6.1	2	5	4
Anti-neuronal antibodies	N/A	Negative	N/A	Negative	Negative
Microbiology					
SARS-CoV-2 RT-PCR	Negative	Negative	Negative	Negative	Negative
HSV-1 PCR	Negative	Negative	Negative	Negative	Negative
HSV-2 PCR	Negative	Negative	Negative	Negative	Negative
VZV PCR	Negative	Negative	Negative	Negative	Negative
JCV PCR	N/A	Negative	N/A	Negative	N/A
Enterovirus PCR	N/A	N/A	Negative	N/A	N/A
Direct exam and bacterial culture	N/A	N/A	N/A		N/A
Direct exam and fungal culture	Negative	Negative	N/A	Negative	N/A
Toxoplasma gondii PCR	Negative	N/A	N/A	Negative	N/A
Aspergillus antigen	Negative	N/A	N/A	Negative	N/A
Candida antigen	Negative	N/A	N/A	N/A	N/A
Cryptococcus antigen	Negative	Negative	N/A	Negative	N/A

Abbreviations: CSF, cerebrospinal fluid; N/A, not available; IL, interleukin; SARS-CoV-2, severe acute respiratory syndrome coronavirus-2; PCR, polymerase chain reaction; HSV, herpes simplex virus; VZV, varicella-zoster virus; JCV, JC polyomavirus. *History of traumatic lumbar puncture.

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