Cerebrospinal Fluid Analysis Post–COVID-19 Is Not Suggestive of Persistent Central Nervous System Infection

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This study was undertaken to assess whether SARS-CoV-2 causes a persistent central nervous system infection. SARS-CoV-2–specific antibody index and SARS-CoV-2 RNA were studied in cerebrospinal fluid following COVID-19. Cerebrospinal fluid was assessed between days 1 and 30 (n = 12), between days 31 and 90 (n = 8), or later than 90 days (post–COVID-19, n = 20) after COVID-19 diagnosis. SARS-CoV-2 RNA was absent in all patients, and in none of the 20 patients with post–COVID-19 syndrome were intrathecally produced anti–SARS-CoV-2 antibodies detected. The absence of evidence of SARS-CoV-2 in cerebrospinal fluid argues against a persistent central nervous system infection as a cause of neurological or neuropsychiatric post–COVID-19 syndrome.

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection primarily targets the upper and lower respiratory tract, causing dry cough and fever. Neurological and neuropsychiatric manifestations have been associated with coronavirus disease 19 (COVID-19), ranging from mild to fatal at all disease stages irrespective of disease severity.[1,2] Interestingly, immunofluorescence and polymerase chain reaction (PCR) analyses of intestinal biopsies obtained from asymptomatic individuals at 4 months after the onset of COVID-19 revealed persistent detection of SARS-CoV-2 RNA and specific immunoreactivity in the small bowel in 50% of individuals.[3] It appears reasonable to assume that SARS-CoV-2 may also reach the central nervous system (CNS) via several routes, including the transcribial, hematogenous, and lymphatic routes, or via axonal transport or transsynaptic transfer.[4] Histopathological data revealing viral RNA transcripts and particles by transmission electron microscopy in brain tissue may suggest CNS infection.[5] Therefore, symptoms such as cognitive impairment or fatigue persisting for >90 days (post–COVID-19) following acute respiratory COVID-19 might be caused by SARS-CoV-2 persistence in the CNS.

Systematic studies of SARS-CoV-2 RNA detection in cerebrospinal fluid (CSF) from patients with neurological symptoms early during COVID-19 and in patients with post–COVID-19 may help address the question of an acute and/or persistent CNS infection with SARS-CoV-2. SARS-CoV-2 RNA was infrequently detected in the CSF in single cases and case series,[6–10] with all these cases reported within the first 90 days of the respiratory infection.

In addition to molecular assays, the SARS-CoV-2–specific CSF antibody index ($AI_{SARS\text{-}CoV\text{-}2}$) allows calculation of an intrathecally produced antibody fraction and might provide indirect evidence of CNS infection.

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The AI is in clinical use for chronic CNS infections such as herpes virus encephalitis, subacute sclerosing panencephalitis, and neuroborreliosis,[11] and is under investigation for progressive multifocal leukoencephalopathy.[12]

This study aimed to clarify whether SARS-CoV-2 persistently infects the CNS, with SARS-CoV-2 RNA from CSF and the $AI_{SARS\text{-}CoV\text{-}2}$ as outcome measures.

Materials and Methods

Participants

The data and biomaterial were derived from a prospective cohort study at baseline, collected at 2 tertiary university hospitals in Germany (Cologne/Berlin) between April 2020 and April 2021 from patients hospitalized or presenting at the specialized post–COVID-19 outpatient clinic. The study was approved by the institutional review board of the University of Cologne (20-1501) and Berlin (EA2/066/20) and registered in the German Clinical Trials Register (DRKS00024434). Patients between 18 and 99 years of age and with neurological or neuropsychiatric symptoms during or after PCR-confirmed COVID-19 were eligible for the study following written informed consent.

Detection of SARS-CoV-2 RNA in CSF

Viral nucleic acids were extracted from CSF and serum samples (200µl) using the innuPREP Virus DNA/RNA Kit-IPC16 and the automated platform InnoPure C16 touch (20µl eluate volume; Analytik Jena, Jena, Germany). To assess SARS-CoV-2 (N and E gene) RNA reverse transcriptase (RT)-PCR cycle threshold (Ct) levels, samples were analyzed using the LightMix SarbeccoV E gene plus EAV control (TIB Molbiol, Berlin, Germany) and N gene (inhouse primer sets in multiplex PCR) as previously described.[13] Assays were carried out on LightCycler 480 (Roche Diagnostics, Mannheim, Germany). Samples with a weak signal in the RT-PCR assay were reanalyzed using a 1-step RT droplet digital (dd) PCR multiplex assay targeting SARS-CoV-2 E, RdRp, and N with a limit of detection of 5 viral RNA copies per reaction as previously described,[14] and 2 additional commercial tests. The Xpert Xpress SARS-CoV-2 (Cepheid, Sunnyvale, CA) with a limit of detection of 0.005 PFU/ml for N gene and 0.02 PFU/ml for E gene (PFU is defined as plaque-forming unit), and the Cobas SARS-CoV-2 assay on the automated Cobas 6800 (Roche Diagnostics) with a limit of detection of 0.0063 50% tissue culture infectious dose (TCID50)/ml for SARS-CoV-2 ORF1a/b and 0.0082 TCID50/ml for E gene were used.

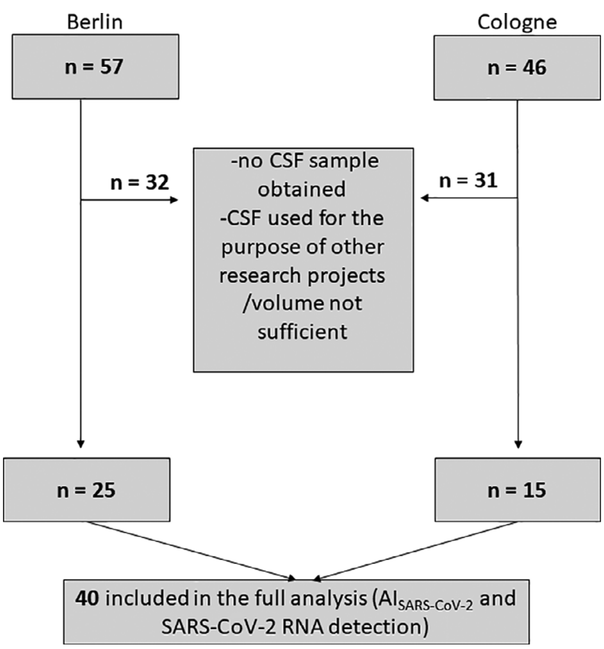

FIGURE 1: Patient enrollment flow chart. The total number of screened patients at 2 tertiary university hospitals in Germany (Cologne/Berlin) between April 2020 and April 2021 are shown as well as the patients excluded, resulting in the total of 40 patients analyzed for the purpose of this study. AI = antibody-specific index; CSF = cerebrospinal fluid.

Assessment of SARS-CoV-2–Specific AI

To determine the $AI_{SARS\text{-}CoV\text{-}2}$, SARS-CoV-2 immunoglobulin class G (IgG) was quantified in diluted CSF and serum samples using the Anti-SARS-CoV-2 QuantiVac ELISA (IgG) targeting the S1 domain of the spike protein (Euroimmun Diagnostik, Lübeck, Germany). Results were expressed semiquantitatively as the ratio of extinction probe and extinction calibrator. CSF samples were generally diluted at 1:2; if antibody concentration exceeded the standards provided, additional 1:20, 1:40, or 1:80 dilutions were required. Serum samples were diluted at 1:101, 1:404, and 1:1010; a few samples required further 1:2020 and 1:4040 dilutions. $AI_{SARS\text{-}CoV\text{-}2}$ was calculated based on SARS-CoV-2 IgG in serum and CSF, and albumin and total IgG to estimate specific intrathecal antibody synthesis as previously described.[11] According to the manufacturer's recommendations, serum SARS-CoV-2–specific IgG values were chosen for calculations for which the optical density (OD) was closest to 1 and closest to the OD detected for the corresponding CSF sample.

Results

Characteristics of Study Participants

We analyzed 40 patients after PCR-confirmed SARS-CoV-2 infection treated for neuropsychiatric manifestations of COVID-19, and an available matching CSF–serum pair (Fig 1). CSF was assessed between days 1 and 30 (acute COVID-19, n = 12), between days 31 and 90 (ongoing COVID-19, n = 8), or later than 90 days (post–COVID-19, n = 20) after the COVID-19 diagnosis. Patients in the acute COVID-19 group were older ($p < 0.001$), and the frequency with a severe or critical COVID-19 disease course was higher as compared to during ongoing and post–COVID-19 (10 of 12, 83.3% vs 7 of 28, 25.0%). A majority of the patients in the post–COVID-19 group complained of cognitive deficits (17 of 20, 85.0%), verified using a screening test in 4 of 15 tested patients (26.7%),

TABLE 1A. Demographics of Acute and Ongoing Patients

Case	Sex	Age, Decade	Symptom Onset, Days	COVID-19 Severity[a]	Neuropsychiatric Symptoms	MMST[b]	MOCA[b]	NPT[b]
Acute COVID-19								
Case 1	M	81–90	0	Mild	Headache, gait disturbance			
Case 2	M	61–70	6	Severe	Flaccid paraparesis, delirium, inflammatory neuropathy			
Case 3	M	61–70	13	Critical	Delirium			
Case 4	F	81–90	9	Critical	Delirium, myoclonus, transient hemiparesis			
Case 5	F	31–40	21	Mild	Cognitive deficits, headache, dizziness, fatigue		27	
Case 6	F	71–80	21	Critical	Delirium, aphasia, impaired consciousness			
Case 7	F	71–80	20	Severe	Cognitive deficits, delirium, change in personality			
Case 8	M	51–60	28	Critical	Delirium, generalized seizure, critical illness weakness			
Case 9	M	61–70	13	Critical	Gaze saccades, ataxia, delirium			
Case 10	F	61–70	1	Critical	Delirium			
Case 11	M	51–60	29	Critical	PRES, intracranial hemorrhage			
Case 12	F	81–90	30	Critical	Paresis left arm			
Median (range)		63 (56–86)	16.5 (0–30)					
Ongoing COVID-19								
Case 13	M	21–30	43	Critical	Cognitive deficits, delirium, delayed polyneuropathy	29		
Case 14	F	51–60	55	Mild	Myelitis with paraparesis			
Case 15	F		63	Mild	Myelitis with paraparesis			
Case 16	F	41–50	43	Mild	Dizziness, limb weakness			
Case 17	M	71–80	39	Severe	Cognitive deficits, delirium, ocular motility dysfunction			
Case 18	M	31–40	53	Mild	Cognitive deficits, fatigue, depression	30		
Case 19	F	71–80	66	Mild	Transient ischemic attack, dizziness			
Case 20	M	71–80	37	Severe	Guillain–Barré syndrome			
Median (range)		48 (24–77)	48.0 (37–66)					

[a]COVID-19 severity: mild: any of the various signs and symptoms of COVID-19 but no shortness of breath, dyspnea, or abnormal chest imaging; moderate: evidence of lower respiratory disease during clinical assessment or imaging and an oxygen saturation (SpO2) ≥ 94% on room air at sea level; severe: SpO2 < 94% on room air at sea level, a ratio of arterial partial pressure of oxygen to fraction of inspired oxygen < 300mmHg, respiratory frequency > 30 breaths/min, or lung infiltrates >50%; critical: respiratory failure, septic shock, and/or multiple organ dysfunction (https://www.covid19treatmentguidelines.nih.gov/, accessed October 3, 2021).

[b]Blank: testing not performed.

F = female; M = male; MMST = Mini-Mental Status Test; MOCA = Montreal Cognitive Assessment, considered pathological for values < 26; NPT = neuropsychological testing battery covering several cognitive domains including learning and memory, attention, executive functioning, language, and visuoconstruction; PRES: posterior reversible encephalopathy syndrome.

TABLE 1B. Demographics of Post–COVID-19 Patients

Case	Sex	Age, Decade	Symptom Onset, Days	COVID-19 Severity[a]	Neuropsychiatric Symptoms	MMST[b]	MOCA[b]	NPT[b]
Post–COVID-19								
Case 21	M	31–40	175	Mild	Cognitive deficits, fatigue	29		●
Case 22	F	21–30	119	Mild	Cognitive deficits, fatigue, depression, anxiety, myalgia		26	
Case 23	M	51–60	284	Severe	Cognitive deficits, fatigue, anxiety		26	
Case 24	F	21–30	244	Mild	Cognitive deficits, fatigue, headache		26	
Case 25	F	51–60	255	Mild	Cognitive deficits, fatigue, depression		29	
Case 26	F	31–40	286	Mild	Cognitive deficits, hypoesthesia of left arm, left face, right leg		25	
Case 27	F	61–70	113	Mild	Rapid progression of preexisting polyneuropathy		27	
Case 28	F	41–50	329	Mild	Fatigue		26	
Case 29	F	51–60	349	Mild	Cognitive deficits		26	
Case 30	F	21–30	138	Mild	Cognitive deficits		28	
Case 31	M	41–50	100	Mild	Cognitive deficits, myalgia		27	
Case 32	M	61–70	143	Mild	Cognitive deficits, headache, parkinsonian syndrome		24	
Case 33	F	41–50	138	Mild	Cognitive deficits, fatigue, dizziness	30		●
Case 34	M	51–60	226	Mild	Cognitive deficits, fatigue	29		●
Case 35	F	41–50	133	Mild	Cognitive deficits, fatigue, myalgia, sensory deficit, insomnia	29		●
Case 36	M	51–60	120	Severe	Cognitive deficits, fatigue		20	
Case 37	F	41–50	303	Mild	Fatigue		28	
Case 38	M	51–60	387	Mild	Cognitive deficits		24	
Case 39	F	51–60	340	Severe	Cognitive deficits		28	
Case 40	F	51–60	324	Severe	Cognitive deficits, depression	30		●
Median (range)		50.5 (23–70)	225.3 (100–387)					

● indicates patients with pathological findings in at least 1 NPT domain.

[a]COVID-19 severity: mild: any of the various signs and symptoms of COVID-19 but no shortness of breath, dyspnea, or abnormal chest imaging; moderate: evidence of lower respiratory disease during clinical assessment or imaging and an oxygen saturation (SpO2) ≥ 94% on room air at sea level; severe: SpO2 < 94% on room air at sea level, a ratio of arterial partial pressure of oxygen to fraction of inspired oxygen < 300mmHg, respiratory frequency > 30 breaths/min, or lung infiltrates >50%; critical: respiratory failure, septic shock, and/or multiple organ dysfunction (https://www.covid19treatmentguidelines.nih.gov/, accessed October 3, 2021).

[b]Blank: testing not performed.

F = female; M = male; MMST = Mini-Mental Status Test; MOCA = Montreal Cognitive Assessment, considered pathological for values < 26; NPT = neuropsychological testing battery covering several cognitive domains including learning and memory, attention, executive functioning, language, and visuoconstruction.

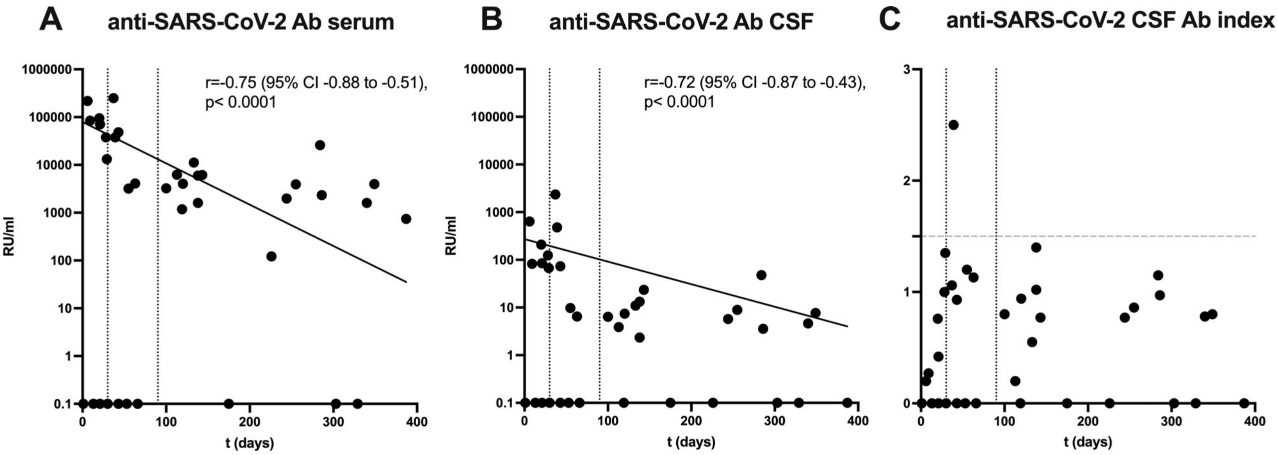

FIGURE 2: Anti–SARS-CoV-2 antibodies (Ab) in serum and cerebrospinal fluid (CSF) over time. (A) Anti–SARS-CoV-2 serum and (B) CSF specific antibodies significantly decrease when assessing patients within the first 30 days (acute COVID-19, n = 12), between days 31 and 90 (ongoing COVID-19, n = 8) and later than 90 days (post–COVID-19, n = 20) after their SARS-CoV-2 infection. (C) Intrathecally produced antibodies could not be identified for any of the post–COVID-19 patients. CI = confidence interval; RU = relative units.

and confirmed in 5 of 5 patients (100%) when applying multidomain cognitive testing (Tables 1A and 1B).

Detection of SARS-CoV-2 RNA in CSF

SARS-CoV-2 RNA (E and/or N gene) was detected in the CSF of 5 patients at low levels with a median Ct value of 39.21 (37.97–40.00), of whom 3 patients were in the acute phase of COVID-19, 1 patient had ongoing COVID-19, and 1 patient had post–COVID-19. None of these results was confirmed by the RT-ddPCR assay or the 2 additional commercial diagnostic tests.

Assessment of SARS-CoV-2–Specific AI

Comparing SARS-CoV-2–specific serum antibodies, 11 patients in the acute or ongoing phase of COVID-19 with detectable antibodies had higher levels than the 16 patients with post–COVID-19 (median relative units [RU] = 48,274 vs 3,581, $p < 0.001$). Anti–SARS-CoV-2 antibody levels in serum inversely correlated with time since the detection of SARS-CoV-2 RNA in the respiratory tract (Fig 2). Regarding SARS-CoV-2–specific antibodies in CSF, 11 patients within the first 90 days of infection and with detectable antibodies had higher levels than 13 patients with post–COVID-19 (median RU = 84.7 vs 7.4, $p < 0.001$). Anti–SARS-CoV-2 antibody levels in CSF inversely correlated with time since the detection of SARS-CoV-2 RNA in the respiratory tract (see Fig 2).

In 1 patient, an intrathecally produced anti–SARS-CoV-2 antibody fraction was determined as assessed by $AI_{SARS\text{-}CoV\text{-}2}$. This was noted 39 days after the detection of SARS-CoV-2 RNA in the respiratory tract (Tables 1A and 2A). In this patient, CSF was taken to further evaluate delirium and ocular motility dysfunction. At the time of sampling, the patient suffered from acute respiratory distress syndrome due to ongoing COVID-19, complicated by multiple organ dysfunction and septicemia. The same patient showed borderline CSF AIs to measles and rubella (1.42 and 1.37, respectively, negative for varicella-zoster virus).

Discussion

As the key finding of our study, neither fundamental CSF findings, nor various PCR protocols, nor IgG-based SARS-CoV-2–directed antibody measures were suggestive of replicative CNS infection as the cause of neuropsychiatric symptoms in post–COVID-19. These post–COVID-19 patients had suffered from a mild course of the acute infection, and cognitive deficits were among the leading complaints. The median age of 50 years was within the range of published post–COVID-19 cohorts.[15–17] We noted an elevated $AI_{SARS\text{-}CoV\text{-}2}$ in 1 patient with severe ongoing COVID-19 infection, possibly explained by polyspecific immune activation, matching the absence of SARS-CoV-2 RNA from CSF, and borderline AI indexes toward other viruses.

The current evidence for direct viral brain invasion in COVID-19 is conflicting; the frequent detection of SARS-CoV-2 in brain reported by one group[5] was not confirmed by others.[18,19] These autopsy studies included older individuals that deceased from COVID-19, demographics that substantially differed from our post–COVID-19 patients. The same is true for published CSF studies assessing only the acute or ongoing phases of COVID-19,[20] and lacking systematic antibody analyses.

TABLE 2A. CSF Findings in Acute and Ongoing Patients

Case	CSF Lymphocytes, µl	Total CSF Protein, g/l	Anti–SARS-CoV-2 IgG CSF	Serum	$AI_{SARS\text{-}CoV\text{-}2}$	SARS-CoV-2 RNA (E or N gene), Ct Value
Acute COVID-19						
Case 1	0	0.57	Not det.	Not det.	—	37.97[a]
Case 2	1	0.34	638.44	217,210.60	0.20	40.00[a]
Case 3	1	0.43	Not det.	Not det.	-	Not det.
Case 4	2	0.29	82.50	84,114.82	0.27	Not det.
Case 5	1	0.22	Not det.	Not det.	—	39.21[a]
Case 6	1	0.21	84.71	70,504.06	0.42	Not det.
Case 7	1	0.35	208.40	95,046.05	0.76	Not det.
Case 8	2	0.14	124.02	37,796.22	1.00	Not det.
Case 9	1	0.52	Not det.	Not det.	—	Not det.
Case 10	3	0.33	Not det.	Not det.	—	Not det.
Case 11	0	0.46	67.23	13,183.33	1.35	Not det.
Case 12	2	0.41	Not det.	Not det.	—	Not det.
Median (range)	1.00 (0–3)	0.34 (0.14–0.57)	104.37 (67.23–638.44)	77,309.44 (13,183.33 – 217,210.60)	0.59 (0.20–1.35)	
Ongoing COVID-19						
Case 13	1	0.24	73.41	48,273.96	0.93	Not det.
Case 14	4	0.37	9.76	3,201.80	1.20	Not det.
Case 15	2	0.23	6.49	4,086.26	1.13	Not det.
Case 16	1	0.21	Not det.	Not det.	—	40.00[a]
Case 17	1	0.56	479.14	37,622.50	2.50	Not det.
Case 18	6	0.39	Not det.	Not det.	—	Not det.
Case 19	1	0.46	Not det.	Not det.	—	Not det.
Case 20	0	0.88	2,350.40	249,768.96	1.06	Not det.
Median (range)	1.00 (0–6)	0.38 (0.21–0.88)	73.41 (6.49–2,350.40)	37,622.50 (3,201.80 – 249,768.96)	1.13 (0.93–2.50)	

Oligoclonal band status was available in 33 of the 40 patients and 17 of the 20 patients with post–COVID-19 syndrome, with none of the patients showing type 2 or 3 oligoclonal bands suggestive of intrathecally produced antibodies.

[a]Not confirmed using alternative polymerase chain reaction protocols; for details, see Materials and Methods section.

AI = antibody index; CSF = cerebrospinal fluid; Ct = cycle threshold; IgG = immunoglobulin class G; Not det. = not detected.

Owing the limitations to our study, we cannot definitely preclude CNS infection; the sample size is small, a CSF PCR may fail to detect virus latently infecting brain tissue, and an IgG-based $AI_{SARS\text{-}CoV\text{-}2}$ directed against the spike protein may miss other immune responses.

Nevertheless, despite these limitations, CSF studies such as ours are needed to further explore the still elusive pathogenesis of post–COVID-19. Whereas neuropsychiatric symptoms during acute COVID-19 could be explained by hyperinflammation, hypoxemia, hypoperfusion, dehydration, glucose dysregulation, and sedation,[1] they remain unexplained in post–COVID-19.[15–17] Latent infection, viral persistence, virus-induced autoimmunity, persistent structural, functional, or metabolic changes following

TABLE 2B. CSF Findings in POST–COVID-19 Patients

Case	CSF Lymphocytes, µl	Total CSF Protein, g/l	Anti–SARS-CoV-2 IgG CSF	Serum	$AI_{SARS\text{-}CoV\text{-}2}$	SARS-CoV-2 RNA (E or N gene), Ct Value
Post–COVID-19						
Case 21	0	0.42	Not det.	Not det.	—	Not det.
Case 22	3	0.19	Not det.	1,182.71	—	Not det.
Case 23	1	0.25	47.76	25,994.37	1.15	38.20[a]
Case 24	5	0.36	5.74	1,983.64	0.77	Not det.
Case 25	7	0.36	8.95	3,916.38	0.86	Not det.
Case 26	1	0.24	3.59	2,340.37	0.97	Not det.
Case 27	2	0.26	3.88	6,233.72	0.20	Not det.
Case 28	2	0.28	Not det.	Not det.	—	Not det.
Case 29	1	0.33	7.69	3,975.46	0.80	Not det.
Case 30	1	0.16	2.35	1,600.14	1.40	Not det.
Case 31	4	0.33	6.40	3,244.73	0.80	Not det.
Case 32	4	0.55	23.50	6,144.03	0.77	Not det.
Case 33	0	0.31	13.23	5,984.96	1.02	Not det.
Case 34	0	0.26	Not det.	121.71	—	Not det.
Case 35	1	0.22	10.99	11,228.37	0.55	Not det.
Case 36	0	0.39	7.41	4,023.44	0.94	Not det.
Case 37	2	0.29	Not det.	Not det.	—	Not det.
Case 38	1	0.25	Not det.	739.52	—	Not det.
Case 39	8	0.41	4.63	1,598.22	0.78	Not det.
Case 40	1	0.21	17.32	13,146.16	0.95	Not det.
Median (range)	1.00 (0–8)	0.29 (0.16–0.55)	7.55 (2,35–47.76)	3,916.38 (121.71–25,994.37)	0.83 (0.20–1.40)	

Oligoclonal band status was available in 33 of the 40 patients and 17 of the 20 patients with post–COVID19 syndrome, with none of the patients showing type 2 or 3 oligoclonal bands suggestive of intrathecally produced antibodies.

[a]Not confirmed using alternative polymerase chain reaction protocols; for details, see Materials and Methods section.

AI = antibody index; CSF = cerebrospinal fluid; Ct = cycle threshold; IgG = immunoglobulin class G; Not det. = not detected.

infection, and psychosocial stress are among the alternative nonexclusive explanations.[1]

Currently, post–COVID-19 is defined as “signs and symptoms that develop during or after an infection consistent with COVID-19, continue for more than 12 weeks and are not explained by an alternative diagnosis” (www.nice.org.uk/guidance). Such a definition based on a temporal association with preceding COVID-19 illustrates the need for biomarker studies to more precisely differentiate post–COVID-19 from pre- or coexisting other conditions, given the relatively young patient population with complaints of cognitive deficits several months after SARS-CoV-2 infection.

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Author Contributions

F.S., Y.G., C.F., S.S., V.D.C., and C.W. contributed to the conception and design of the study; all authors contributed to the acquisition and analysis of data. F.S., Y.G., and C.W. contributed to drafting the text or preparing the figures; all authors critically revised the manuscript for important intellectual content.

Potential Conflicts of Interest

CW received personal compensation from BioNTech for participating in an educational discussion. The other authors have nothing to report.

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