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Cerebrospinal fluid biomarkers in SARS-CoV-2 patients with acute neurological syndromes

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1 **Cerebrospinal fluid biomarkers in SARS-CoV-2 patients**
2 **with acute neurological syndromes**

3
4 **Abstract**

5 **Background and purpose:** Mechanisms underlying acute brain injury in SARS-CoV-2
6 patients remain poorly understood. A better characterization of such mechanisms remains
7 essential to preventing long-term neurological sequelae. Our present aim was to study a
8 panel of biomarkers of neuroinflammation and neurodegeneration in the cerebrospinal fluid
9 (CSF) of NeuroCOVID patients.

10 **Methods:** We retrospectively collected clinical and CSF biomarkers data from 24
11 NeuroCOVID adults seen at the University Hospital of Guadeloupe between March and June
12 2021.

13 **Results:** Among 24 NeuroCOVID patients, 71% had encephalopathy and 29%
14 meningoencephalitis. A number of these patients also experienced de novo movement
15 disorder (33%) or stroke (21%). The CSF analysis revealed intrathecal immunoglobulin
16 synthesis in 54% of NeuroCOVID patients (two with a type 2 pattern and 11 with a type 3)
17 and elevated neopterin levels in 75% of them (median 9.1 nM, IQR 5.6-22.1). CSF
18 neurofilament light chain (NfL) was also increased compared to a control group of non-
19 COVID-19 patients with psychiatric illnesses (2905 ng/l, IQR 1428-7124 versus 1222 ng/l,
20 IQR 1049-1566). Total-tau was elevated in the CSF of 24% of patients, whereas protein 14-
21 3-3, generally undetectable, reached intermediate levels in two patients. Finally, CSF A β 1-42
22 was reduced in 52.4% of patients (median 536 ng/l, IQR 432-904) with no change in the
23 A β 1-42/A β 1-40 ratio (0.082, IQR 0.060-0.096).

24 **Conclusions:** We showed an elevation of CSF biomarkers of neuroinflammation in
25 NeuroCOVID patients and a rise of CSF NfL, evocative of neuronal damage. However,
26 longitudinal studies are needed to determine whether NeuroCOVID could evolve into a
27 chronic neurodegenerative condition.

28 **Keywords:** Encephalitis, Encephalopathy, COVID-19, Neuroinflammation, Neuronal injury,
29 CSF biomarker.

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56 **1. Introduction**

57 The pathogenesis of nervous system damage associated with SARS-CoV-2 infection
58 (NeuroCOVID) remains poorly known. Putative non-exclusive mechanisms at the origin of
59 NeuroCOVID comprise *i*) brain invasion by retrograde progression of the virus along cranial
60 nerve pathways [1,2] or through brain-blood barrier (BBB) disruption and *ii*) deleterious
61 systemic immune response or compartmentalized immune response within the central
62 nervous system (CNS) [3–5]. Unraveling these mechanisms is critical to identifying optimal
63 therapeutic targets and guiding our strategy to prevent long-term neurological sequelae in
64 NeuroCOVID patients [6,7].

65 NeuroCOVID might be associated with an increased risk of developing a
66 neurodegenerative disorder or might hasten its progression [8,9]. Triggering a
67 neurodegenerative cascade in NeuroCOVID patients might involve the combination of
68 several factors, including *i*) a specific vulnerability of some brain regions such as the
69 hippocampus or the midbrain to SARS-CoV-2 infection [10], *ii*) gut microbiome dysregulation
70 induced by SARS-CoV-2 infection and its possible consequences on the brain through the
71 gut-brain axis [11–13], *iii*) and SARS-CoV-2-induced dysregulation of genes critical for
72 neuronal survival [14]. Some undefined factors associated with an extended stay in an
73 intensive care unit (ICU) [15], a depressive state, post-traumatic distress [10], or severe
74 sepsis [16,17] could also influence the risk of cognitive decline after severe SARS-CoV-2
75 infection.

76 Here, the consequences of CNS SARS-CoV-2 infection were monitored by measuring
77 different cerebrospinal fluid (CSF) parameters. Previous CSF studies performed in adults
78 with NeuroCOVID – Virhammar et al. (n=19) [18]; Edén et al. (n=6) [19]; Espindola et al.
79 (n=58) [20]; Garcia et al. (n=18) [21]; Paterson et al. (n=21) [22]; Alexopoulos et al. (n=8)
80 [23]; Ziff et al. (n=21) [24]); Guasp et al. (n=60) [25]; Edén et al. (n=23) [26] – reported
81 changes in biomarkers of neuroinflammation [18–21,23–26], astrocytic injury although this
82 point remains debated [18,22,24,26], neuronal injury [18–26], as well as alterations in
83 amyloid processing [22,24]. Most of the previous studies focused on either

84 neuroinflammation, acute neuronal injury, or neurodegeneration, but only a small number
85 analyzed together biomarkers characterizing these different mechanisms. Thus, additional
86 CSF studies in large samples of well-characterized NeuroCOVID patients are needed to
87 further delineate the pathogenesis of CNS damage and prevent its occurrence. Here, we
88 studied a variety of biomarkers associated with neuroinflammation, neuronal injury, and
89 neurodegeneration in the CSF of 24 NeuroCOVID adults with a CNS syndrome during the
90 acute phase of the infection.

91

92 **2. Methods**

93 2.1. Patients and study design

94 We enrolled patients with neurological manifestations of a confirmed COVID-19
95 infection between March 2020 and June 2021 at the University Hospital of Guadeloupe
96 (French West Indies). Patients were considered to have confirmed COVID-19 when real-time
97 protein chain reaction (RT-PCR) for SARS-CoV-2 was positive, either in a nasopharyngeal
98 swab or bronchoalveolar lavage. During the hospital stay, we collected data on medical
99 history and performed clinical (including a detailed neurological examination), biological
100 (including detailed CSF analysis) and neuroradiological (brain and spinal magnetic
101 resonance imaging (MRI), brain computed tomography (CT)) investigations, as well as
102 neurophysiological (electroencephalogram (EEG) and electromyogram (EMG)) recordings.
103 Two types of brain injuries were reported: 1) Encephalopathy defined as an altered mental
104 status lasting ≥ 24 hours (impaired awareness, confusion, delirium with or without
105 hallucinations, cognitive and behavioral disorder) that could be associated with seizure and
106 focal neurologic symptoms, or with electroencephalographic criteria, in the absence of
107 criteria for encephalitis (confer below) [27] and that could not be accounted for by another
108 cause, such as toxic or metabolic factors; 2) Encephalitis/meningitis defined as an altered
109 mental status lasting ≥ 24 hours (encephalopathy) with one of the following criteria: white
110 blood cell count in CSF $\geq 5/\text{mm}^3$, or detection of SARS-CoV-2 by RT-PCR in CSF, or
111 presence of a compatible acute lesion on brain MRI. As previously defined by the United

112 States National Institutes of Health [28], the severity of the illness was classified as mild,
113 moderate, severe, or critical. Patients classified as having encephalopathy or
114 meningoencephalitis could additionally have developed a stroke episode or a movement
115 disorder.

116

117 2.2. Standard plasma and CSF investigations to explore the infectious status of patients

118 A large panel of infections was systematically screened in plasma (serological tests
119 for dengue virus, chikungunya virus, zika virus, human immunodeficiency virus, human T-
120 lymphotropic virus, cytomegalovirus, Epstein Barr virus, leptospirosis, hepatitis B and C
121 viruses) and in CSF (RT-PCR for varicella-zoster virus, herpes simplex virus, enterovirus,
122 *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*, *Legionella*) to search for possible co-
123 infections. Any acute co-infection was a criterion of exclusion.

124 CSF protein concentrations were analyzed with a Cobas®-Roche automated analyzer.
125 Abnormal protein levels in CSF were considered if >0.4 g/l. CSF/serum albumin ratios were
126 analyzed and evaluated as abnormal when ≥ 0.0075 . CSF white and red cell countings were
127 performed using Kova Slides®. CSF immunoglobulin G (IgG) index was determined and
128 considered increased when >0.7 . Isoelectric focusing was performed on CSF and serum
129 samples using the Sebia Capillarys® system. Five patterns have been previously described
130 [29]: Type 1: no specific band in CSF and serum (normal); Type 2: specific oligoclonal IgG
131 bands in the CSF and no corresponding band in serum (intrathecal IgG synthesis); Type 3:
132 IgG oligoclonal bands in CSF and additional identical bands in the CSF and serum
133 (intrathecal IgG synthesis); Type 4: similar oligoclonal bands in the CSF and serum (systemic,
134 no intrathecal IgG synthesis); Type 5: monoclonal bands in CSF and serum (no IgG
135 synthesis in CNS). CSF COVID-19 serology (IgG and IgM) was assessed using a Standard
136 Q COVID-19 IgM/IgG Combo Test (SD Biosensor via Orgentec) and RT-PCR with a
137 EurobioPlex SARS-CoV-2 Multiplex kit (Eurobio Scientific). The presence of onconeural
138 antibodies was also analyzed in blood and CSF by immunohistochemistry and a cell-based
139 assay (French reference center).

140

141 2.3. CSF neopterin as a marker of neuroinflammation

142 CSF neopterin was quantified by ultra performance liquid chromatography (UPLC)
143 with fluorometric detection and Empower software for calculation and quantification
144 (Waters®). The upper average reference value for neopterin was previously determined to
145 be 5 nmol/l by Perret Liaudet et al. [30].

146

147 2.4. CSF biomarkers of neuronal injury and neurodegeneration

148 CSF collection, sampling, and storage were performed in a single laboratory using
149 standard procedures prescribed in a consensus paper [31]. According to preanalytical
150 recommendations, CSF samples were collected and aliquoted in polypropylene test tubes
151 (Sarstedt, reference 62.610.201 and 62.558.201).

152 CSF neurofilament light chain (NfL) measurements were performed using an Nf-
153 light® ELISA kit from Uman Diagnostics. Non-COVID patients with psychiatric illnesses
154 (n=20) – patients suffering from depressive syndrome associated with a cognitive complaint
155 without progression during a two-year follow-up, and a normal CSF biomarker profile) [32] –
156 were taken as a control group for NeuroCOVID patients in this assay. For detecting 14-3-3
157 protein, a Peggy Sue® automated Western blot system (Protein Simple, San Jose, CA, USA)
158 was used. According to Fourier et al. [33], qualitative results interpreted were expressed as
159 negative, positive, or intermediate.

160 Core Alzheimer's disease (AD) CSF biomarker assays (T-tau, P-tau 181, A β 1-42, and
161 A β 1-40) were performed using a Lumipulse G600II automated analyzer (Fujirebio®). Typical
162 cut-off values for parameters associated with AD risk were based on international criteria [34].
163 These values were determined locally and are as follows: T-tau >400 ng/l, P-tau >60 ng/l,
164 A β 1-42 <550 ng/l and/or A β 1-42/A β 1-40 ratio <0.055.

165

166 2.5. Statistical analyses

167 All results are expressed in median and interquartile range (IQR). Non-parametric
168 statistical analyses (Mann-Whitney, Spearman's rho correlation) were performed due to the
169 small sample size. The significance level was defined as $p < 0.05$. Statistical analysis was
170 performed using version 19.1 of the MedCalc Statistical Software (MedCalc Software bv,
171 Ostend, Belgium) and the R Statistical Software (v4.1.1; R Core Team 2021).

172

173 2.6. Standard protocol approvals, registrations, and patient consent

174 The study was classified as an observational study according to French health
175 regulations. The study was approved by the local ethics committee (number A17200704),
176 and oral informed consent was obtained from all participants after providing them with written
177 explanations. The study was performed according to the approved protocol.

178 The control group of non-COVID patients with psychiatric illnesses came from a study
179 (NCT-04001270) published and approved by the institutional review board of the Université
180 Claude Bernard Lyon 1 and Hospices Civils de Lyon [32].

181

182 2.7. Data availability

183 Data will be made available by the corresponding author upon reasonable request.
184 The data are not publicly available because they contain information that could compromise
185 our patients' privacy.

186

187 2.8. Literature summary

188 To facilitate the discussion through a global overview of CSF findings in patients with
189 NeuroCOVID, we presented our results in a table together with a summary of data from
190 previous studies (Table 1).

191

192 3. Results

193 3.1. Clinical findings and management

194 We analyzed data from 24 NeuroCOVID patients. The median age was 62 years
195 (IQR: 56-70), and males were more represented (62.5%) (Table 2). No patient had a
196 previous medical history of neurological disease. Among these patients, encephalopathy was
197 the main neurological syndrome (70.8%, n=17) compared to meningoencephalitis (29.2%,
198 n=7). Eight of these patients (33%, six with encephalopathy and two with
199 meningoencephalitis) also developed movement disorders, and five of them (21%, four with
200 encephalopathy and one with meningoencephalitis) experienced a stroke. Disease severity
201 in these patients was estimated to be either moderate (16.7%), severe (25%), or critical
202 (58.3%). All patients hospitalized in ICU received mechanical ventilation (Table 2).

203

204 3.2. CSF immune reaction and neuroinflammation

205 All patients had a lumbar puncture (LP) a median five days (IQR 3-12) after the onset
206 of neurological symptoms. CSF findings are shown in Table 3. Except for CSF pleocytosis,
207 protein levels, and albumin ratios, which were higher in patients with meningoencephalitis, no
208 significant difference was observed in biomarkers of the immune response and
209 neuroinflammation between the two clinical subgroups.

210 Isoelectric focusing patterns 2 and 3, which indicate intrathecal IgG synthesis, were
211 identified in two (8.7%) and 11 (47.8%) of the patients, respectively. Type 2 or 3 patterns
212 were observed in 25% of patients with moderate forms, 50% of patients with severe forms,
213 and 70% of those with critical forms, without a statistical relationship between disease
214 severity and band pattern ($p=0.275$). CSF neopterin was increased in 75% (n=18) of patients
215 (median 9.1 nmol/l, IQR 5.6-22.1). No correlation was found between CSF neopterin level
216 and the delay of LP ($\rho=0.25$, $p=0.25$), nor between CSF neopterin levels and duration of
217 ICU stay ($\rho=0.152$, $p=0.62$).

218

219 3.3. CSF biomarkers of neuronal injury and neurodegeneration

220 CSF NfL levels were significantly higher than in the control group of non-COVID-19
221 patients with psychiatric illnesses (2905 ng/l, IQR 1428-7124 versus 1222 ng/l, IQR 1049-

222 1566) (Fig. 1, Table 3). There was no correlation between CSF NfL level and age ($\rho=0.337$,
223 $p=0.135$), CSF NfL level and the delay of LP ($\rho=0.09$, $p=0.71$), and CSF NfL level and
224 duration of ICU stay ($\rho=-0.442$, $p=0.17$).

225 Total-tau protein levels were increased in 5/21 patients (24%, four with
226 encephalopathy, one with meningoencephalitis). One patient with a concomitant elevation of
227 T-tau (2577 ng/l) and P-tau (64 ng/l) also demonstrated high NfL levels (66,560 ng/l) and
228 intermediate levels of 14-3-3 protein, while amyloid markers (A β 1-42 level and A β 1-42/A β 1-
229 40 ratio) were not significantly modified. There was a positive correlation between T-tau and
230 NfL CSF levels ($\rho=0.510$ and $p=0.036$) in the whole cohort.

231 CSF 14-3-3 protein was negative in 21/23 patients and intermediate in 2/23 (one
232 encephalopathy with additional stroke and one meningoencephalitis).

233 A β 1-42 peptide was lowered in 11/21 (52.4%) patients (median 536 ng/l, IQR 432-
234 904). The A β 1-42/A β 1-40 amyloid ratio was, however, normal in the 21 patients analyzed
235 (0.082, IQR 0.060-0.096). Among the 11 patients with low levels of A β 1-42, nine had
236 encephalopathy, and two had meningoencephalitis. Three of the patients with reduced CSF
237 A β 1-42 levels had a concomitant increase in CSF/serum albumin ratio. The median CSF NfL
238 concentration was increased in these patients (2852 ng/l, IQR 1948-5626), but T-tau or P-tau
239 levels remained normal. Overall, none of the patients had a typical CSF pattern evocative of
240 Alzheimer's disease pathophysiology.

241

242 **4. Discussion**

243 We analyzed CSF biomarkers in a group of 24 well-characterized SARS-CoV-2
244 infected patients developing acute CNS injury. We found features consistent with active
245 neuronal damage and immune reaction restricted to the CNS in most patients. Consistent
246 with previous studies, our findings provide evidence that CNS immune activation occurs in
247 NeuroCOVID patients together with neuronal injury and impaired amyloid processing. This,
248 further sheds light on disease pathogenesis and mechanisms of neurological sequelae

249 secondary to SARS-CoV-2 infection while raising concerns about the long-term impact of
250 NeuroCOVID on brain function.

251 A large proportion of the SARS-CoV-2 patients developing CNS abnormalities
252 demonstrated CNS immune activation. In particular, agarose gel isoelectric focusing allowed
253 us to show that CSF oligoclonal bands of IgG were present in 57% of our patients. By
254 contrast, in previous studies, intrathecal IgG synthesis was inconstantly reported and
255 observed in only 2 to 8% of patients [18,20,35]. In the present study, the median delay
256 between the onset of neurological symptoms and LP was much shorter (median of five days)
257 compared to 14 days [21] and 11 days [19] in the two studies in which intrathecal IgG
258 synthesis was not detected (Table 1), suggesting that the delay between neurological
259 manifestations and LP might account for such differences.

260 CSF neopterin is a well-established immune activation marker with elevated
261 concentrations seen in many inflammatory states, including infections, autoimmune
262 disorders, and primary CNS lymphoma [36–38]. Our study found that CSF neopterin was
263 increased in 75% of all NeuroCOVID cases. Our finding agrees with the data reported by
264 Eden et al. [19], in which 6/6 NeuroCOVID patients (four with encephalopathy and two with
265 altered mental status) exhibited high levels of CSF neopterin. Under inflammatory conditions,
266 neopterin in the brain is produced by microglia and astrocytes in response to stimulation by
267 interferon gamma [39]. An elevation of CSF neopterin was reported when brain
268 neuroinflammation results from viral infections, especially in herpes virus encephalitis,
269 enterovirus meningoencephalitis [40], and in HIV-1-associated neurocognitive disorders [36].
270 Overall, biomarkers of focal immune reaction and neuroinflammation could provide valuable
271 tools for diagnosing NeuroCOVID, for example, to distinguish between stroke due to SARS-
272 CoV-2 infection versus non-inflammatory/infectious etiologies.

273 A substantial proportion of our patients had abnormal CSF levels of biomarkers that
274 reflect neuronal injury, including NfL, T-tau, and to a lesser extent, protein 14-3-3. NfL is a
275 cytoskeletal protein mainly expressed in large myelinated axons [41]. The positive correlation
276 that normally exists between elevated CSF NfL and age in healthy individuals [42] was

277 absent in NeuroCOVID patients suggesting that ongoing neuronal insults may cover up the
278 age effect. Accordingly, this correlation is also absent in inflammatory, neurodegenerative,
279 traumatic, and cerebrovascular diseases [43–45] where NfL is thought to be passively
280 released into the CSF. CSF T-tau is also a well-studied biomarker that can be taken as a tool
281 not only for prediction but also for diagnosing AD [46]. Of twenty-one patients, five had
282 elevated levels of CSF T-tau. Among them, one patient with encephalopathy associated with
283 stroke demonstrated high T-tau levels (2577 ng/l, i.e. five times higher than the cut-off value)
284 and an intermediate level of protein 14-3-3, suggesting more extensive neuronal damage in
285 this individual. Changes in CSF biomarkers of neuronal injury have been reported during
286 various CNS infections. In a large retrospective study of 281 patients with CNS infections
287 [40], T-tau and protein 14-3-3 were reported abnormally high in the CSF of patients
288 developing herpes simplex virus (HSV) encephalitis. High levels of T-tau were also observed
289 in patients with HSV encephalitis undergoing LP within seven days after the onset of
290 symptoms suggesting that a sharp increase in T-tau occurs in the first days following HSV
291 infection [40]. In NeuroCOVID patients with CNS injury, previous studies reported increased
292 levels of NfL [18,19,21,22], whereas T-Tau was found inconstantly increased [18,22] (Table
293 1). Elevated CSF 14-3-3 protein was reported in four of eight critical COVID-19 patients with
294 encephalopathy in the study from [23] (Table 1). Overall, our results confirm that neuronal
295 damage is significant during the acute phase of NeuroCOVID and suggest that CSF NfL is
296 the most reliable biomarker of neuronal injury in this context.

297 In our group of patients, 52.4% (nine with encephalopathy, two with encephalitis)
298 developed changes in CSF amyloid biomarkers, but none presented abnormalities
299 suggestive of Alzheimer's disease amyloidosis. A β 1-42 levels were significantly reduced in
300 these patients but with no concomitant reduction of A β 1-42/A β 1-40 ratios as expected in
301 amyloidosis [47]. Besides, no concomitant increase of T-tau and P-tau was observed in
302 these particular patients. Changes in CSF amyloid biomarkers were also previously reported
303 in two other studies describing NeuroCOVID patients with CNS and peripheral nervous
304 system (PNS) damages. Paterson et al. [22] reported a decrease in the A β 1-42/A β 1-40 ratio

305 but normal T-tau or P-tau levels in three patients with Guillain-Barre syndrome and two with
306 encephalopathy (Table 1). CSF amyloid A β 1-40, A β 1-42, and A β 1-42/A β 1-40 ratio, as well
307 as soluble amyloid precursor protein metabolites (sAPP α and sAPP β), were significantly
308 reduced in another study describing 21 COVID-19 patients with PNS and CNS injuries [24].
309 Overall, these results suggest that SARS-CoV-2 infection may possibly induce a down-
310 regulation of amyloid precursor protein processing, possibly resulting in a global reduction in
311 β -amyloid peptide production [24] that is not found in the pathophysiology of AD. This is
312 consistent with previous reports on neuroinflammatory conditions and CNS infections where
313 decreases in both A β 1-40 and A β 1-42 were reported [40,48,49]. One may also assume that
314 the clearance and elimination of amyloid metabolites might be enhanced in patients with
315 higher CSF/serum albumin ratios traducing increased BBB permeability. Note, however, that
316 CSF/serum albumin ratios were elevated in only one-third of patients with reduced CSF A β 1-
317 42. From a more general point of view, the amyloid precursor protein being considered as
318 an innate antiviral defense factor [50–52], alterations in its metabolism are not totally
319 unexpected in NeuroCOVID patients.

320 Beyond acute infection, there is still a challenge to distinguish neurological sequelae
321 of SARS-CoV-2 infection from early neurodegenerative processes [53]. Post-infectious
322 immune response generated by anti-neuronal autoantibodies [54] or persistent viral
323 replication in tissue reservoirs such as the olfactory mucosa [2] could mediate residual and
324 chronic neuroinflammation. Therefore, longitudinal clinical, biological, and neuropathological
325 studies are needed to better understand long-term consequences of these processes.

326 Our study has some limitations, such as the small sample size and the lack of a
327 prospective control group of COVID-19 patients without neurological symptoms. Also, the
328 variable delay between the onset of neurological symptoms and LP could be a possible
329 confounding factor. Nevertheless, one of the strengths of our study was that participants
330 were well-characterized and formed a relatively homogeneous group. Another strength is the
331 harmonization of sample collection and handling, assuring robust and comparable results
332 between patients.

333 Overall, our study showed that CSF biomarkers of neuroinflammation and neuronal
334 injury are elevated in acute NeuroCOVID patients. We speculate that neuroinflammation,
335 demonstrated by elevation of CSF neopterin and intrathecal synthesis of IgG as well as BBB
336 disruption, could trigger neuronal damage and compromise amyloid precursor peptide
337 processing. Our results confirm the idea that anti-inflammatory drugs are essential at an
338 early phase of the disease [55]. While their effects appear to improve outcomes in the acute
339 phase of the disease [56], the impact on residual neurological disability is still poorly
340 understood and requires better understanding.

341

342

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Table 1 - Literature summary of NeuroCOVID studies with CSF analysis.

NeuroCOVID Studies	Delay (in days) between neuro. symptoms and LP med. [IQR]	Patients with neurological manifestations (n)	Neuroinflammation (CSF)					Neuronal injury (CSF)				Amyloid markers (CSF)		Astrocyte reactivity (CSF GFAP)
			OCB (n)	Neopterin (n)	IgG index (n)	Albumin ratio (n)	Inflammatory cytokines (n)	NfL (ng/l) med. [IQR]	T-tau (ng/l) med. [IQR]	P-tau	14-3-3 protein	Aβ1-42	Ratio Aβ1-42/Aβ1-40	
Espindola et al.	NA	58	↑ (3/38)	NA	NA	NA	NA	1694 [1091–3358]**	318 [173–457]**	NA	NA	NA	NA	NA
Paterson et al.	NA	34 21 CNS 10 PNS 3 others	NA	NA	NA	NA	NA	↑ CNS 1510 [857-14800] vs controls 872 [654-1200]	→ CNS 585 [220-1788] vs controls 289 [243-356]	→*	NA	NA	↓ (5/32)	↓* CNS and PNS vs controls
Garcia et al.	«early CSF collection group» n=8 4 [1-6] «late CSF collection group» n=10 20 [13-27]	18	→ (5/18)	NA	→ (7/18)	→ (7/18)	↑ IL-6, TNFα, IL-10, IL-12p70 Stroke group (n=7) vs controls ↑ IL-10, IL-12p70 critical illness group (n=8) vs controls	↑* 8657 [1400–18333]	NA	NA	NA	NA	NA	NA
Edén et al. (2020)	11 [7-12]	6	→ (6/6)	↑ (6/6)	→ (6/6)	→ (6/6)	NA	↑ (2/6) 974 [669–1998]	NA	NA	NA	NA	NA	NA
Virhammar et al.	NA	19	↑ (1/18)	NA	↑ (4/17)	↑ (1/19)	↑*	↑ (12/18) 1900 [773–3763]	↑ (7/17)	NA	NA	NA	NA	↑ (3/18)
Alexopoulos et al.	NA	8	NA	NA	↑ (1/8)	↑ (3/8)	NA	NA	NA	NA	Positive (4/8)	NA	NA	NA
Ziff et al.	NA	21	NA	NA	NA	NA	↑* TNFα, IL6, IL1β, IL8 vs controls	↑* vs controls	NA	NA	NA	↓* vs controls	↓* vs controls	↓* vs controls
Guasp et al.	NA	60	→ (27/27)	NA	NA	NA	↑ MCP-1, G-CSF, IL18, IL6, IL8, MIG (n=27) vs controls	↑ Encephalopathy (n=16) 1543 [740-2083] vs controls (n=24) 764.5 [472.5-896.5]	NA	NA	NA	NA	NA	NA
Edén et al. (2022)	NA	23	NA	↑ vs controls	→	→	↑ TNFα, IL6, IL2 vs controls	↑* vs controls	NA	NA	NA	NA	NA	→ vs controls
Chaumont et al.	5 [3-12]	24	↑ (13/24)	↑ (18/24)	↑ (6/23)	↑ (7/23)	NA	↑* 2905 [1428-7124]	↑ (5/21)	↑ (1/21)	Intermediate (2/23)	↓ (11/21)	→ (20/20)	NA

Abbreviations: CNS: patients with “central nervous system” injury; CSF: cerebrospinal fluid; GFAP: glial fibrillary acid protein; IgG: immunoglobulin G; IL: interleukin; IQR: interquartile range; LP: lumbar puncture; NA: not available; NfL: neurofilament light chain; OCB: oligoclonal bands; PNS: patients with “peripheral nervous system” injury; P-tau: phosphorylated-tau; TNF: tumor necrosis factor; T-tau: total-tau; Vs: versus; →: normal; ↑: increased; ↓: decreased; *: the total number of patients is not available; **: NeuroCOVID patients not compared to healthy controls.

Table 2 - General characteristics of 24 COVID-19 patients with neurological manifestations.

	n=24 ¹
Age (in years)	62 [56-70]
Sex	
Female	9 (37.5%)
Male	15 (62.5%)
Comorbidities	
Hypertension	13 (54.2%)
Diabetes mellitus	10 (41.7%)
Obesity	7 (29.2%)
Cancer	4 (16.7%)
Chronic alcoholism	2 (8.3%)
Chronic kidney disease	0 (0.0%)
Chronic cardiac disease	1 (4.2%)
Obstructive Sleep Apnea	1 (4.2%)
NIH severity	
Mild	0 (0.0%)
Moderate	4 (16.7%)
Severe	6 (25.0%)
Critical	14 (58.3%)
Neurological syndromes	
Encephalopathy	17 (70.8%)
Meningoencephalitis	7 (29.2%)
Additional movement disorders	8 (33.3%)
Additional stroke	5 (20.8%)
Time between first infectious symptoms and neurological manifestation (in days)	8 [1-17]
ICU hospitalization	13 (54.2%)
Duration of ICU stay (in days)	28 [17-33]
Mechanical ventilation	13 (54.2%)
Duration (in days)	22 [16-29]

¹Median [IQR]; n (%).

Abbreviations: NIH: National Institutes of Health; ICU: intensive care unit.

Table 3 - CSF findings in 24 COVID-19 patients with neurological manifestations.

	Total n=24 ¹	Encephalopathy n=17 ¹	Meningoencephalitis n=7 ¹	<i>p</i>
Time between first neurological symptoms and LP (in days) (n=23)	5 [3-12]	5 [3-13]	4 [2-4]	0.121
WCC (cell/ μ l)	2 [0-5]	1 [0-2]	8 [6-28]	<0.001
Abnormal WCC (>4/ μ l)	7 (29%)	0 (0%)	7 (100%)	<0.001
Protein (g/L)	0.42 [0.29-0.60]	0.34 [0.22-0.44]	0.62 [0.52-0.97]	0.003
Abnormal protein (>0.4 g/l)	12 (50%)	5 (29%)	7 (100%)	0.005
Isoelectric focusing of CSF* (n=23)				0.199
Type 1 pattern	10 (43%)	6 (35%)	4 (67%)	
Type 2 pattern	2 (8.7%)	1 (5.9%)	1 (17%)	
Type 3 pattern	11 (48%)	10 (59%)	1 (17%)	
IgG index (n=23)	0.62 [0.47-0.71]	0.62 [0.49-0.65]	0.60 [0.42-0.74]	0.972
Abnormal IgG index (>0.7)	6 (26%)	3 (18%)	3 (50%)	0.279
Albumin ratio (CSF/serum) (n=23)	0.006 [0.004-0.010]	0.005 [0.004-0.007]	0.012 [0.008-0.015]	0.020
Abnormal albumin ratio (\geq 0.0075)	7 (30%)	3 (18%)	4 (67%)	0.045
Neopterin (nmol/l) (n=24)	9.1 [5.6-22.1]	9.1 [6.6-22.9]	6.4 [5.3-20.4]	0.525
Abnormal neopterin (>5 nmol/l)	18 (75%)	13 (76%)	5 (71%)	1.000
Positive anti-SARS-CoV-2 IgM (n=15)	2 (13%)	0 (0%)	2 (50%)	0.057
Positive anti-SARS-CoV-2 IgG (n=17)	6 (35%)	4 (33%)	2 (40%)	1.000
NfL (ng/l) (n=19)	2 905 [1 428-7 124]	3 177 [2 017-5 846]	1 428 [918-7 766]	0.368
Total-tau (ng/l) (n=21)	256 [199-394]	265 [206-417]	199 [170-294]	0.407
Abnormal T-tau (>400)	5 (24%)	4 (25%)	1 (20%)	1.000
Phosphorylated-tau 181 (ng/L) (n=21)	32 [20-37]	33 [21-43]	22 [20-32]	0.363
Abnormal P-tau (>60)	1 (4.8%)	1 (6.2%)	0 (0%)	1.000
A β 1-42 peptide (ng/l) (n=21)	536 [432-904]	516 [423-838]	808 [475-904]	0.660
Abnormal A β 1-42 (<550)	11 (52%)	9 (56%)	2 (40%)	0.635
A β 1-42 / A β 1-40 ratio (n=21)	0.082 [0.060-0.096]	0.082 [0.059-0.091]	0.097 [0.073-0.101]	0.130
Abnormal A β 1-42 / A β 1-40 (<0.055)	0 (0%)	0 (0%)	0 (0%)	
14-3-3 protein (n=23)				0.526
Intermediate	2 (8.7%)	1 (6.2%)	1 (14%)	
Negative	21 (91%)	15 (94%)	6 (86%)	

Abbreviations: CSF: cerebrospinal fluid; IgG: immunoglobulin G; IgM: immunoglobulin M; NfL: neurofilament light chain; P-tau: phosphorylated-tau; T-tau: total-tau; WCC: white cell count.

¹Median [IQR]; n (%).

* Type 1: no specific band in CSF and serum (normal); Type 2: specific oligoclonal IgG bands in the CSF and no corresponding band in serum (intrathecal IgG synthesis); Type 3: IgG oligoclonal bands in CSF and additional identical bands in the CSF and serum (intrathecal IgG synthesis); Type 4: similar oligoclonal bands in the CSF and serum (systemic, no intrathecal IgG synthesis); Type 5: monoclonal bands in CSF and serum (no IgG synthesis in CNS).

Legend of the figure

Fig. 1 - Level of CSF NfL in NeuroCOVID group and controls.

Boxplots of cerebrospinal fluid (CSF) neurofilament light chain (NfL) in NeuroCOVID (n=19, 13 encephalopathies in blue and six meningoencephalitis in red) and controls of non-COVID patients with psychiatric illnesses (n=20, in black)

