Original research

Brain neuronal and glial damage during acute COVID-19 infection in absence of clinical neurological manifestations

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ABSTRACT

Background To assess whether SARS-CoV-2 infection may affect the central nervous system, specifically neurons and glia cells, even without clinical neurological involvement.

Methods In this single centre prospective study, serum levels of neurofilament light chain (sNfL) and glial fibrillar acidic protein (sGFAp) were assessed using SimoaTM assay Neurology 2-Plex B Assay Kit, in 148 hospitalised patients with COVID-19 without clinical neurological manifestations and compared them to 53 patients with interstitial pulmonary fibrosis (IPF) and 108 healthy controls (HCs).

Results Age and sex-corrected sNfL levels were higher in patients with COVID-19 (median log10-sNfL 1.41; IQR 1.04–1.83) than patients with IPF (median log10-sNfL 1.18; IQR 0.98–1.38; p<0.001) and HCs (median log10-sNfL 0.89; IQR 0.72–1.14; p<0.001). Likewise, age and sex-corrected sGFAP levels were higher in patients with COVID-19 (median log10-sGFAP 2.26; IQR 2.02–2.53) in comparison with patients with IPF (median log10-sGFAP 2.15; IQR 1.94–2.30; p<0.001) and HCs (median log10-sGFAP 1.87; IQR 0.64–2.09; p<0.001). No significant difference was found between patients with HCs and IPF (p=0.388 for sNfL and p=0.251 for sGFAp). In patients with COVID-19, a prognostic model with mortality as dependent variable (26/148 patients died during hospitalisation) and sNfl, sGFAp and age as independent variables, showed an area under curve of 0.72 (95% CI 0.59 to 0.84; negative predictive value (NPV) (%):80,positive predictive value (PPV)(%): 84; p=0.0008).

Conclusion The results of our study suggest that neuronal and glial degeneration can occur in patients with COVID-19 regardless of overt clinical neurological manifestations. With age, levels of sNfl and GFAp can predict in-hospital COVID-19-associated mortality and might be useful to assess COVID-19 patient prognostic profile.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ The elevation of neuronal and glial damage serum biomarkers has been described in patients with COVID-19 with neurological manifestations. It is interesting to know whether SARS-CoV-2 infection may induce neuronal and glial damage also in patients without signs of clinical neurological involvement.

WHAT THIS STUDY ADDS

⇒ We found increased levels of serum neurofilament light chain (sNfL) and glial fibrillar acidic protein (sGFAp), reliable biomarkers of neuronal and glial injury, in hospitalised patients with COVID-19 without clinical neurological manifestations. Moreover, a model including age, sNfL and sGFAp at hospital admission can be helpful in identifying patients who are at high risk of COVID-19-associated mortality.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE AND/OR POLICY

⇒ Our results suggest that an inflammatory-mediated neuronal and glial degeneration can occur in patients with COVID-19 regardless of overt clinical neurological manifestations, but its pathogenic mechanisms and long-term consequences are still unknown. Future researches will elucidate these mechanisms and explore the long-term consequences of this subclinical central nervous system damage. Levels of sNfl and sGFAp at hospital admission might be useful to assess COVID-19 patient prognostic profile.

INTRODUCTION

The SARS-CoV-2 is responsible for the systemic and often devastating infection causing the ongoing COVID-19 pandemic.[1] Growing evidence indicates that neurological manifestations might become evident and may persist over a long time in patients with COVID-19.[2 3] Indeed, a number of studies[4–7] have provided a detailed characterisation of the clinical neurological involvement occurring during SARS-CoV-2 infection, which added to ex vivo neuropathological studies, point out the relevant damage to the central nervous system (CNS) occurring in patients with COVID-19.[8] While this bulk of data is definitely in favour of a particular tropism of SARS-Cov-2 for the central and peripheral nervous system leading to overt clinical manifestations, it would be interesting to know whether the SARS-CoV-2 infection may damage the nervous system,

Table 1 Demographic, clinical and laboratory features of healthy controls (HCs), interstitial lung disease (IPF) and patients with COVID included in the study

	HCs	IPF	COVID	P value
Number	108	53	148	
Sex (male %)	36%	75%	62%	<0.001
Age (years) (median, IQR)	52.5, 31.25–69	72, 64–78	71, 58–82	<0.001
% of patients with heart failure	NA	0%	16%	NS
% of patients with hypertension	NA	46%	35%	NS
% of patients with COPD	NA	0%	8%	NS
% of patients with type two diabetes	NA	10%	15%	NS
% of current smoker patients—% ex-smokers	NA	0%–61%	4%–35%	NS
Neutrophils/lymphocytes ratio (median, IQR)	NA	NA	5.88, 2,90–10,90	NA
Total monocytes (median, IQR)	NA	NA	0.51×10^3/mmc, 0,32–0.73×10^3/mmc	NA
Total basophils (median, IQR)	NA	NA	0.02×10^3/mmc, 0.01–0.03×10^3/mmc	NA
C reactive protein (median, IQR)	NA	NA	5.40 mg/dL, 2.09–12.36 mg/dL	NA
PO_2/FiO_2 ratio (median, IQR)	NA	NA	2.18, 1.58–3.13	NA

COPD, chronic obstructive pulmonary disease; NA, not applicable; NS, not significant.

specifically neurons and glia cells, even without signs of clinical neurological involvement.

Neurofilament light chain (NfL) and glial fibrillar acidic protein (GFAp) represent two promising markers of neuronal and glial degeneration. Indeed, the recent development of ultrasensitive digital immunoassays has enabled reliable measurements of these CNS-relevant biomarkers in serum, where they were not previously detectable.[9 10] Being NfL a major cytoskeletal intermediate filament protein in myelinated axons, increased CSF and serum levels of NfL have been associated with CNS damage in various neurological conditions[9] and, due to its high specificity for structural proteins of neurons, it has been considered a reliable biomarker of neuroaxonal damage. Thus, serum levels of NfL have been used to assess and monitor neuronal damage in many neurological disorders.[11] On the other hand, GFAp is an intermediate filament highly expressed in astrocytes[12] and serum levels of GFAp have been increasingly used as a biomarker of astrocytic activation and injury, with an ever-growing body of evidence supporting its use to detect even subtle injuries to the CNS.[10 13 14]

Recent studies have documented the elevation of serum NfL (sNfL) and GFAp (sGFAp) in patients during the acute phase of COVID-19,[15–25] but only scattered data are available regarding the elevation of these CNS biomarkers in patients with COVID-19 with no evidence of clinical neurological involvement.[17 21 26] To provide consistent evidence that SARS-CoV-2 infection can cause damage to the nervous system even when signs of definite clinical involvement are absent, we studied here hospitalised patients with COVID-19 without clinical neurological manifestations and compared them to a group of patients with interstitial pulmonary fibrosis (IPF) different from COVID-19 and to a group of healthy controls (HCs).

PATIENTS AND METHODS

This prospective study was performed at the Siena University Hospital between October 2021 and April 2022. Serum samples from patients with COVID-19 with a positive nasopharyngeal swab for SARS-COV2–PCR test and requiring hospitalisation were obtained. All serum samples were collected within 48 hours from hospital admission, before any treatment or infusion of intravenous steroids or invasive ventilation. Serum aliquots were stored at −80°C until assay. All the recruited patients had an interstitial lung involvement demonstrated at chest X-ray. COVID-19 severity was evaluated according with the WHO clinical progression scale.[27] Patients had no history of neurological diseases and they did not develop any neurological symptoms during the hospitalisation, except minor non-disabling manifestations (ie, anosmia and dysgeusia) not requiring neurological referral. During hospitalisation, a neurological assessment was performed at least weekly to confirm the absence of any significant neurological signs and symptoms and included an assessment of sensorium, cognition, cranial nerves (tolerating anosmia and dysgeusia), motor, sensory, cerebellar, reflexes, meningeal irritation and long tract signs.

Serum samples of patients affected by IPF were also collected at the time of diagnosis. IPF has been included as a control group because it represents a non-infectious chronic progressive fibrotic lung disease characterised by the same radiological and histopathologic pattern of usual interstitial pneumonia,[28] limited to the lungs and without any systemic involvement.[29]

No patient had psychiatric or neurologic comorbidity nor was on specific treatment for IPF at the time of sample collection. Moreover, no patients with IPF had been infected by SARS-CoV-2.

Finally, serum samples from HCs were collected. They had no history of autoimmune, psychiatric or neurologic diseases and had never been infected by SARS-CoV-2. All the available demographic, laboratory and clinical data of patients were recorded in an electronic case record form. Laboratory data included the absolute lymphocyte and neutrophil counts, neutrophil to lymphocyte ratio and C-reactive protein at admission. The ratio of arterial oxygen partial pressure (PaO2) in mm Hg to fractional inspired oxygen (FiO2) expressed as a fraction (PaO2/FiO2) at hospital admission was also recorded for all patients with COVID-19 as a marker of the degree of hypoxia. Previous medical history and hospital outcomes, including mortality, were also recorded.

sNfL and sGFAP single molecular array (SimoaTM) assay

sNfL and sGFAP concentrations were measured using SimoaTM assay Neurology 2-Plex B (GFAP, NfL) Assay Kit (Catalog #103520; Quanterix, Billerica, MA, USA) run on the semiautomated ultrasensitive SR-X Biomarker Detection System (Quanterix). Samples were diluted at 1:4 and randomly distributed on 96-well plates. Quality control (QC)

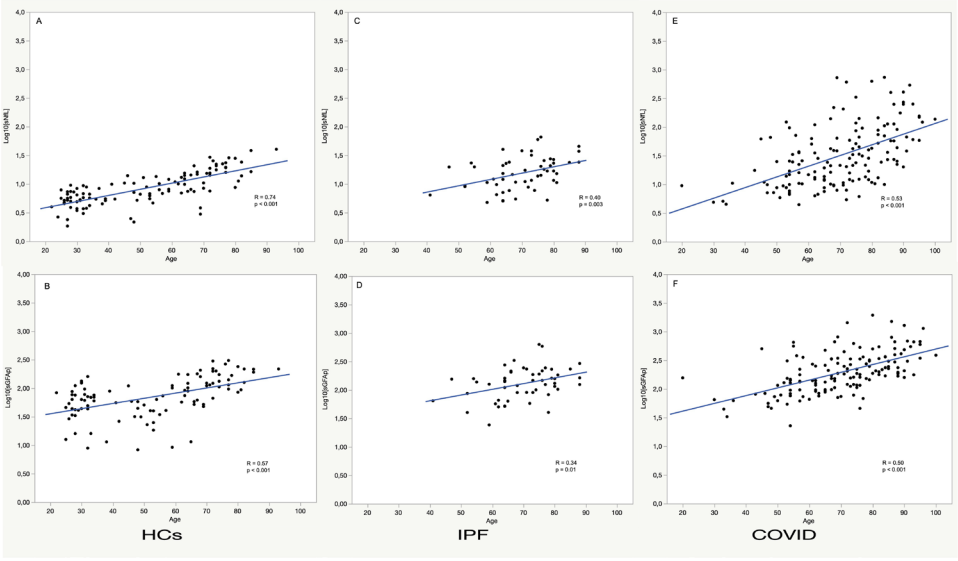

Figure 1 Correlation between age and values of log10-sNfl (A, C, E) and log10 glial fibrillar acidic protein (sGFAp) values (B, D, F) in healthy controls (HCs), patients with interstitial pulmonary fibrosis (IPF) and patients with COVID-19. Tied p and rho values obtained by the Spearman correlation test are indicated.

samples provided with the kit had concentrations within the predefined range and the coefficient of variance across the plates was <10%. All samples were analysed blindly under alpha-numeric codes. The diagnostic codes were broken only after QC-verified NfL and GFAp concentrations were reported to the database manager.

Statistical analysis

Data were summarised as number of patients (percentage/frequency) and median (IQR). Group differences for normally distributed data were assessed using analysis of variance. Quantitative data were compared with the Fisher exact test. Kolmogorov-Smirnov test was performed for the demonstration of normal distribution. Since, sNFL and sGFAp values were skewed, sGFAP and sNfL levels were log10 transformed. Analysis of covariance was performed by analysing log10 sNfL and sGFAP levels as dependent variables, groups (COVID-19, IPFs and HCs) as fixed variables, and age and sex as covariates, to examine differences between sNfL and sGFAP levels among the groups. Correlations between sNfL and sGFAp levels and demographic measures and other laboratory values were assessed using two-tailed Spearman's rank correlation coefficients.

Logistic regression models were built to assess the best discriminatory variables between patients with COVID-19 who survived and those who died. We assessed the validity of the variables used to distinguish patients with COVID-19 and HCs by areas under curves (AUC) in the receiver operating characteristic. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated for cut-offs of the different variables.

A value of $p<0.05$ was considered significant. Analysis results and graphs were generated with SPSS statistics (IBM SPSS V.26, Chicago, Illinois), JMP, (V.15. SAS Institute, Cary, North Carolina, 1989–2022) and GraphPad Prism V.9.2 software.

RESULTS

A total of 309 subjects (148 hospitalised patients with COVID-19, 53 patients with IPF and 108 HCs) were included in the study.

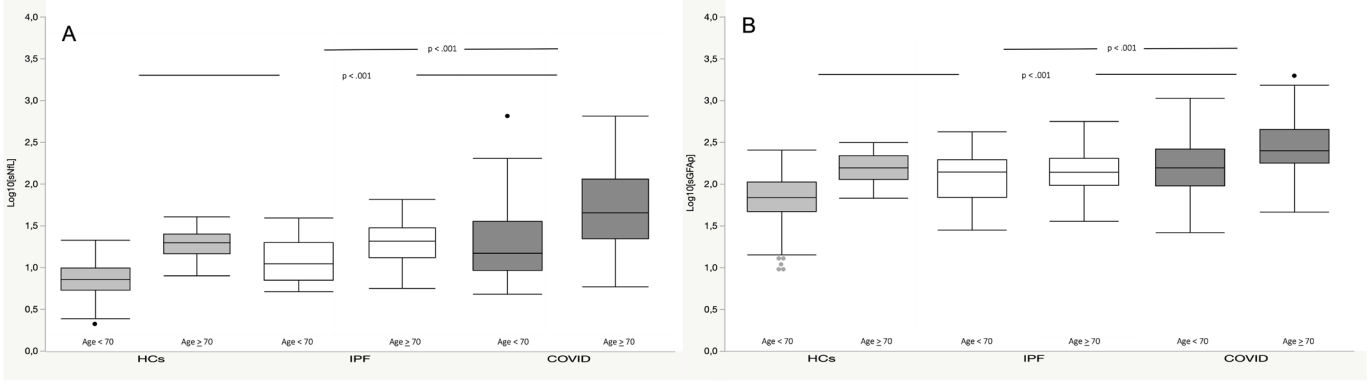

Figure 2 log10-serum levels of neurofilament light chain (sNfl) values (A) and log10-glial fibrillar acidic protein (sGFAp) values (B) in<70 year old and>70 year old healthy controls (HCs), patients with interstitial pulmonary fibrosis (IPF) and patients with COVID-19. Box plots express the first (Q1) and third (Q3) quartiles by the upper and lower horizontal lines in a rectangular box, in which there is a horizontal line showing the median. The whiskers extend upwards and downwards to the highest or lowest observation within the upper (Q3+1.5 × IQR) and lower (Q1 - 1.5×IQR) limits. P values indicate statistical significance between the different groups.

Due to limited sample availability, sNfL was not tested in one patient with COVID-19, whereas sGFAp levels were not tested in two patients with COVID-19 and seven HCs. The median age of patients with COVID-19 was 71 years (IQR 58–82) and 62% were men, compared with 72 years (range 64–78) and 75% of men among patients with IPF, and 52.5 years (IQR 31, 25–69) and 36% men among HCs (table 1).

According to the WHO clinical progression scale,[27] 63 patients with COVID-19 (median age 68, IQR 56.5–76. 38% F) had moderate diseases (namely, hospitalised patients not requiring oxygen therapy or requiring oxygen by mask or nasal prongs) and 85 patients (median age 74, IQR 62–85, 38% F) had severe disease (namely, hospitalised patients requiring oxygen by non-invasive ventilation or high flow or patients requiring intubation and mechanical ventilation). Twenty-six patients died during their hospitalisation for COVID-19 (17.5%, median age of patients who died was 84.5; IQR 68–90, 50% were women; the median age of patients who survived was 69; IQR 57–78, 36% were women; p<0.001 for age). All dead COVID-19 patients experienced a severe disease course during their hospitalisation.

sNfL and sGFAP levels in COVID-19, IPF and HCs

As expected, log10 sNfL levels and log10 sGFAp levels showed a positive correlation with age in all three groups (log10 sNfL: R=0.55 in COVID-19, 0.40 in IPF and 0.74 in HC; log10 sGFAP: R=0.40 in COVID-19, 0.34 in IPF and 0.50 in HCs; p<0.001 for all) (figure 1). Age and sex-corrected levels of sNfL were higher in patients with COVID-19 without clinical neurological manifestations (median log10 sNfL 1.41; IQR 1.04–1.83) than in patients with IPF (median log10 sNfL 1.18; IQR 0.98–1.38; p<0.001) and HCs (median log10 sNfL 0.89; IQR 0.72–1.14; p<0.001). These values were not different comparing patients with IPF and HCs (p=0.31) (figure 2A). Similarly, age and sex-corrected levels of sGFAP levels were higher in patients with COVID-19 (median log10 sGFAP 2.26; IQR 2.02–2.53) than in patients with IPF (median log10 sGFAP 2.15; IQR 1.94–2.30; p<0.001) and HCs (median log10 sGFAP 187; IQR 0.64–2.09; p<0.001). These values were not different comparing patients with IPF and HCs (p=0.22) (figure 2B).

sNfL and sGFAP levels in patients with COVID-19 grouped for disease severity

When our cohort of patients with COVID-19 was grouped according to disease severity as moderate or severe, we did not find any significant difference in sNfL and sGFAP levels between these subgroups of patients with COVID-19 (moderate COVID-19: median log10 sNfL 1.39; IQR 1.08–1.65; severe COVID-19: median log10 sNfL 1.44; IQR 1.09–1.98 ; p=0.16; moderate COVID-19: median log10 sGFAp 2.20; IQR 1.98–2.47; severe COVID-19: median log10 sGFAp 2.30; IQR 2.06–2.56; p=0.96) and both subgroups of patients with COVID-19 showed higher levels of sNfL and sGFAP than patients with IPF (p<0.001).

Levels of sNfL and sGFAP and COVID-19-associated mortality

After stratification of patients with COVID-19 based on their in-hospital mortality, different logistic regression models were built to find the most accurate model able to discriminate patients who survived from those who did not survive. By using in-hospital mortality as dependent variable, and log10-sNfl and log10-sGFAp as independent variables, the model showed a good performance with an AUC 0.67 (95% CI 0.54 to 0.80;NPV (%): 75, PPV (%): 82; p=0.008). The performance of the model increased when age was added to this model as the third independent variable (AUC 0.72 (95% CI 0.59 to 0.84; NPV (%): 80, PPV(%): 84; p=0.0008) (figure 3).

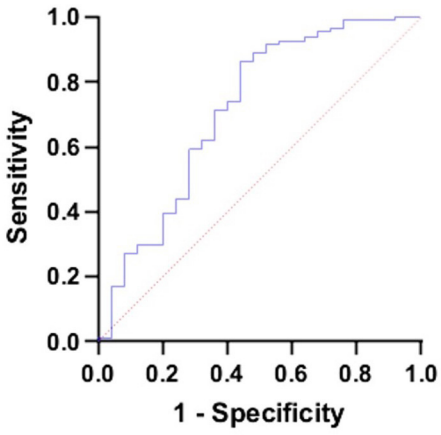

Figure 3 ROC curve showing specificity and sensitivity of the predictive model using in-hospital mortality as a dependent variable, and age, log10sNfl, and log10sGFAp as independent variables. Area under the curve: 0.72; predictive positive value: 84%; predictive negative value: 80%; p=0.0008. sGFAp, glial fibrillar acidic protein; sNfL, serum levels of neurofilament light chain.

DISCUSSION

In this prospective study, we showed increased levels of sNfL and sGFAp in acute hospitalised patients with no evidence of clinical neurological involvement in comparison to other non-COVID-19 patients with IPF and a group of HCs. Elevated levels of both sNfL and sGFAp in hospitalised[15 17 19 20 22 23] and non-hospitalised[21] patients with COVID-19 have been documented, providing compelling evidence of the CNS damage related to SARS-CoV-2 infection. Our results add further support to this notion by showing that CNS damage can be seen in COVID-19 patients even without overt clinical neurological manifestations. In addition, we compared levels of sNfL and sGFAp of patients with COVID-19 with those of patients with IPF and found that these values were higher in COVID-19 than in patients with IPF, who showed similar values to HCs. Interestingly, in our study, sNfL and sGFAp values were higher even in patients with COVID-19 with moderate disease severity than in patients with IPF.

Two peculiar mechanisms differentiate the pathophysiology of SARS-CoV-2 infection: the neuronal and glial viral invasion and the CNS parenchymal injury caused by the viral-induced immune response. Angiotensin-converting enzyme 2 is expressed in both neurons, glial and endothelial cells throughout the brain, including the hypothalamus, cortex, striatum and brainstem,[30 31] making CNS parenchyma vulnerable to the SARS-CoV-2 invasion mediated by its binding to spike glycoprotein. SARS-CoV-2 induces a neuroinflammatory response characterised by elevated oxidative stress, upregulation of several genes, including IL1B, IL6, IFITM, MX1 and OAS2 and an increased level of the interleukins (IL-1β, IL- 6, IL-10) and tumour necrosis factor-α, which promote the degradation of endothelial tight junction proteins, particularly claudin-5 and zonula occludens-1, increasing the blood-brain barrier permeability.[32] These events have been hypothesised to induce reactive astrocytosis and microglia activation, which might ultimately be responsible for neuronal damage and death in patients with COVID-19.[33] Specifically, astrocyte reactive subtype A1 acts as a potent direct killer of the neurons,[34] and the elevation of sGFAP can be seen as a marker of

astrogliosis. It should also be considered here that the systemic inflammatory response itself is a driver for neuronal damage as already demonstrated for patients with septic shock and no evidence of CNS infection.[35] Alongside, IPF is a chronic progressive fibrotic lung disease characterised by the same radiological and histopathologic pattern of usual interstitial pneumonia,[28] but the contribution of the immune response to the tissue damage in IPF[36] is generally considered scarce and the disease is viewed as a consequence of fibroblasts dysfunction and senescence rather than of dysregulated inflammation.[37] High levels of IL-6 and IL-8 have been described only in those patients with IPF experiencing acute exacerbations of the disease (and not in stable ones as our IPF cohort) and transforming growth factor-β (TGF-β), IL-10, IL-4 and IL-13 were not increased.[38] Our results also suggest that the pattern of interstitial pneumonia, with the consequent alterations of alveolar gas exchanges, does not represent a mechanism responsible for neuronal and glial damage and gives additional support to the notion that CNS damage during COVID-19 is independent and unrelated to respiratory insufficiency.[39]

The increase in sNfl and sGFAP levels seen in patients with COVID-19 cannot be regarded as specific to this disease. Taking into account the other infectious diseases, blood NfL and GFAp values have been demonstrated to increase in patients with sepsis-associated encephalopathy[35 40] and in septic patients admitted to an intensive care unit.[41] CSF and plasma NfL levels have been found elevated in other viral infections. Patients with HIV, especially those with HIV-associated dementia, have increased levels of CSF and plasma NfL.[42–44] Similarly, patients with Varicella-zoster virus encephalitis and patients with herpes zoster without signs of CNS involvement show increased levels of CSF and plasma NfL, too.[45]

Finally, we found here a close correlation between age and sNfL and sGFAp levels. The age dependency of these biomarkers of neuronal[46] and glial[10] degeneration is known and probably reflects an age-related increase in oxidative stress, metabolic dysfunction, reduced DNA stability, ion homeostasis dysregulation in neurons and changes in glial cell homeostasis towards reactive inflammatory phenotypes.[47] Indeed, our results add to those of previous studies in suggesting a prognostic role of sGFAp[23] and sNfL[22–25] by showing that a model including age and levels of sNfL and sGFAp at hospital admission can help identify patients who are at high risk of COVID-19-associated mortality and combined with other prognostic markers that are routinely measured in intensive care might be useful to assess COVID-19 patient prognostic profile.

We can, therefore, conclude that increased levels of sNfL and sGFAp suggest that an inflammatory-mediated neuronal and glial degeneration can occur in patients with COVID-19 regardless of overt clinical neurological manifestations and that sNfL and sGFAp increased levels, with age, can be relevant to predict the outcome. Further and larger studies are needed to better elucidate the pathogenic mechanisms underlying the nervous system damage during acute COVID-19 and to explore the long-term consequences of the subclinical CNS damage revealed by the elevation of sNfL and sGFAp in patients with COVID-19.

Contributors Conceptualisation and design: DP, SL, LB, EB, NDS. Data analysis: DP, SL, CM, LB, NDS. Methodology: DP, SL, Md'A. Interpretation: DP, SL, LB, CM, RC, MM, DC, Md'A, EB, NDS. Data collection: DP, SL, LB, CM, MM, DC. First draft: DP, SL, CM, LB, EB, NDS. Supervision: EB, NDS. Guarantor: NDS.

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Competing interests DP, SL, LB, CM: none declared. Unrelated to this work, RC received speaker honoraria from Roche and Merck. She was awarded a MAGNIMS-ECTRIMS fellowship in 2019. MM, DC, Md'A: none declared. Competing interests unrelated to this work, EB provides consultancy to GSK, BI and Chiesi. Unrelated to this work, NDS is a consultant for Biogen, Merck, Novartis, Sanofi-Genzyme, Roche, and Teva and is on the speakers' bureaus of Biogen, Merck, Novartis, Roche, Sanofi-Genzyme, and Teva. He has received travel funds from Merck, Novartis, Roche, Sanofi-Genzyme, and Teva and has grants pending from FISM. He is co-founder of Siena-Imaging.

Patient consent for publication Consent obtained directly from patient(s)

Ethics approval The study was approved by our local ethics committee—University of Siena (C.E.A.V.S.E. Markerlung 17431). Participants gave informed consent to participate in the study before taking part.

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Data availability statement Data are available upon reasonable request.

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