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ORIGINAL COMMUNICATION

Serum neurofilament light chain levels in Covid-19 patients without major neurological manifestations

Federico Verde[1,2] · Ilaria Milone[1] · Ilaria Bulgarelli[3] · Silvia Peverelli[1] · Claudia Colombrita[1] · Alessio Maranzano[1,4] · Narghes Calcagno[1,4] · Nicola Ticozzi[1,2] · Giovanni Battista Perego[5] · Gianfranco Parati[5,6] · Erminio Torresani[3] · Antonia Ratti[1,7] · Vincenzo Silani[1,2]

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Abstract

Background Increased serum levels of neurofilament light chain (sNFL), a biomarker of neuroaxonal damage, have been reported in patients with Covid-19. We aimed at investigating whether sNFL is increased in Covid-19 patients without major neurological manifestations, is associated with disease severity, respiratory and routine blood parameters, and changes longitudinally in the short term.

Methods sNFL levels were measured with single molecule array (Simoa) technology in 57 hospitalized Covid-19 patients without major neurological manifestations and in 30 neurologically healthy controls. Patients were evaluated for PaO2/FiO2 ratio on arterial blood gas, Brescia Respiratory Covid Severity Scale (BRCSS), white blood cell counts, serum C-reactive protein (CRP), plasma D-dimer, plasma fibrinogen, and serum creatinine at admission. In 20 patients, NFL was also measured on serum samples obtained at a later timepoint during the hospital stay.

Results Covid-19 patients had higher baseline sNFL levels compared to controls, regardless of disease severity. Baseline sNFL correlated with serum CRP and plasma D-dimer in patients with mild disease, but was not associated with measures of respiratory impairment. Longitudinal sNFL levels tended to be higher than baseline ones, albeit not significantly, and correlated with serum CRP and plasma D-dimer. The PaO2/FiO2 ratio was not associated with longitudinal sNFL, whereas BRCSS only correlated with longitudinal sNFL variation.

Conclusions We provide neurochemical evidence of subclinical axonal damage in Covid-19 also in the absence of major neurological manifestations. This is apparently not fully explained by hypoxic injury; rather, systemic inflammation might promote this damage. However, a direct neurotoxic effect of SARS-CoV-2 cannot be excluded.

Keywords Covid-19 · Neurofilament light chain (NFL) · Biomarker · Serum · Neuroaxonal damage

✉ Federico Verde
f.verde@auxologico.it

[1] Department of Neurology and Laboratory of Neuroscience, IRCCS Istituto Auxologico Italiano, Piazzale Brescia, 20, 20149 Milan, Italy

[2] Department of Pathophysiology and Transplantation and "Dino Ferrari" Center, Università degli Studi di Milano, Milan, Italy

[3] Department of Laboratory Medicine, Laboratory of Clinical Chemistry, IRCCS Istituto Auxologico Italiano, Milan, Italy

[4] Università degli Studi di Milano, Neurology Residency Program, Milan, Italy

[5] Department of Cardiology, IRCCS Istituto Auxologico Italiano, Milan, Italy

[6] Department of Medicine and Surgery, Università degli Studi di Milano-Bicocca, Milan, Italy

[7] Department of Medical Biotechnologies and Translational Medicine, Università degli Studi di Milano, Milan, Italy

Introduction

Neurological manifestations occur in up to one-third of patients with Covid-19, the disease caused by SARS-CoV-2 infection, the most common being fatigue, myalgia, smell and taste alterations, headache, and confusion/delirium [1]. Neurological syndromes in Covid-19 can also occur in the absence of prominent respiratory disturbances [2], whereas subtle cognitive dysfunction is frequently reported by patients up to several months after recovery [3]. The mechanisms of neural damage in Covid-19 are not fully understood; hypotheses include direct invasion of the central nervous system (CNS), endothelial dysfunction, hypercoagulability, systemic inflammation, and hypoxia [4].

Previous studies have investigated neurochemical biomarkers in Covid-19, mostly focusing on neurofilament light chain (NFL), the main cerebrospinal fluid (CSF) and blood biomarker of neuroaxonal injury [5]. Kanberg et al. [6] and Ameres et al. [7] observed increased NFL levels in plasma and serum samples, respectively, of Covid-19 patients compared to healthy controls. In Covid-19 patients admitted to an intensive care unit (ICU), serum NFL (sNFL) levels are higher compared to those of non-Covid-19 ICU patients and are associated with an unfavorable outcome [8, 9]. In hospitalized Covid-19 patients, higher blood NFL levels may portend a worse disease course and increased disability at discharge [10–13]. Elevated CSF NFL levels have been observed in the majority of Covid-19 patients with neurological symptoms [14]; however, a study reported increased CSF NFL concentrations in Covid-19 patients with CNS syndromes, but not in those with peripheral nervous system (PNS) involvement, whereas sNFL levels were similarly augmented in all hospitalized Covid-19 patients, suggesting—together with the lack of correlation between CSF and serum NFL levels—that increases of NFL in the blood are partly determined by peripheral mechanisms [15]. Notably, blood NFL levels have been reported to normalize within 6 months after acute Covid-19 in a large majority of patients [16, 17].

Here, we measured sNFL in hospitalized Covid-19 patients without major neurological manifestations and in neurologically healthy controls, aiming at investigating whether sNFL levels: (1) are elevated in Covid-19; (2) are influenced by disease severity; (3) are associated with alterations of respiratory and routine blood parameters; (4) change longitudinally during the in-hospital disease course.

Methods

Patients

We evaluated 57 patients with Covid-19 and 30 neurologically healthy controls. The former were consecutive patients admitted to the Covid Units of our Institute between March 16th and April 30th, 2020 and fulfilling the following criteria: (1) age ≥ 18 years; (2) demonstration of SARS-CoV-2 infection by RT-PCR on a nasopharyngeal swab specimen; (3) absence of pre-existing chronic or recent acute neurological diseases associated with CNS or PNS tissue damage and/or with known elevation of CSF and/or blood NFL levels; (4) absence of a diagnosis of a major neurological manifestation during the in-hospital disease course (e.g., stroke, encephalitis, seizures, critical illness polyneuropathy or myopathy, Guillain–Barré syndrome); (5) blood sampling and biobanking of serum performed mostly at admission or within few days and always within 30 days after symptom onset. Patients were subdivided into 3 categories based on the clinical severity of Covid-19: mild, i.e., patients not requiring oxygen therapy or only treated with low-flow oxygen therapy; moderate, i.e., patients necessitating continuous positive airway pressure (CPAP) or noninvasive ventilation (NIV) at some point during the hospital stay; and severe, i.e., patients undergoing invasive mechanical ventilation and/or dying at hospital. As proxies for the degree of respiratory impairment, we considered the Brescia Respiratory Covid Severity Scale (BRCSS), a semi-quantitative score mainly based on the intensity of intervention needed to treat respiratory insufficiency [18], and the PaO2/FiO2 ratio, i.e., the ratio between the partial pressure of oxygen measured by arterial blood gas sampling and the inspiratory fraction of oxygen [19]. The PaO2/FiO2 ratio was available for all patients except for one with very mild disease. All patients had available routine blood panels at admission, from which we considered the following parameters: serum C-reactive protein (CRP), plasma D-dimer, plasma fibrinogen, serum creatinine, and total white blood cell (WBC), neutrophil, and lymphocyte counts. For a subgroup of patients, a longitudinal serum sample from the same hospital stay was available. We included longitudinal samples which were taken between 7 and 30 days after the first blood draw.

Neurologically healthy controls, recruited in the period between May 27th and December 17th, 2020, were ≥ 18 years old and were either physicians and other healthcare professionals working in our Institute ($n = 11$) or relatives and caregivers of patients evaluated in the neurological outpatient clinics of the Institute ($n = 19$). Controls had neither symptoms of Covid-19 at blood sampling

nor a personal history of Covid-19. Physicians recruited as controls underwent regular SARS-CoV-2 screening (RT-PCR on nasopharyngeal swab specimen and serum anti-nucleocapsid (anti-N) antibodies) and all tested negative at the time of serum sampling.

The study protocol was approved by the Ethics Committee of our Institute and all patients and controls gave their informed consent for the study. The study conforms with World Medical Association Declaration of Helsinki.

Measurement of sNFL

Serum samples were biobanked according to current guidelines [20]. After collection, blood was kept at room temperature for 30 min to allow coagulation and then at 4 °C until centrifugation at 2000×*g* for 10 min, which was performed within 4 h from sampling. Serum samples were then aliquoted in polypropylene tubes and stored at − 80 °C until analysis. Measurement of NFL was performed in duplicates by single molecule array (Simoa) technology on the SR-X analyzer from Quanterix (Billerica, MA, USA), using the commercially available NF-Light kit (catalogue number, 103400) according to the manufacturer's instructions. Duplicates with a coefficient of variation (CV) > 20% were rerun. Mean intra-assay CVs were between 6.7% and 16.4%.

Statistical analysis

Outliers in baseline sNFL values of Covid-19 patients were defined as those below (Q1–3*IQR) or above (Q3 + 3*IQR) (Q1, first quartile; Q3, third quartile; IQR, interquartile range). Whereas no outliers were found at the lower extreme of sNFL levels, 4 high outliers were identified. These values were capped and substituted by the limit value itself (Q3 + 3*IQR). The only patient with an outlier baseline sNFL value and a follow-up sNFL value was excluded from the longitudinal analysis of sNFL levels. The control cohort did not contain any outliers of sNFL levels. Comparisons of quantitative variables between two groups were made with the Mann–Whitney *U* test, whereas for comparisons between more than two groups the Kruskal–Wallis test was used, followed, in case of statistically significant differences, by Dunn's post hoc analysis. Comparison of the distributions of categorical variables between different groups was made with the Chi-square test. The correlations between quantitative variables were assessed by means of Spearman's rank correlation. The level of statistical significance for all tests was set at $p < 0.05$. The Prism 9 program (GraphPad Software, San Diego, CA, USA) was used for statistical analyses.

Results

Demographic and clinical characteristics of study participants

The demographic features of the 57 Covid-19 patients (41 males and 16 females) and of the 30 controls (19 males and 11 females) are summarized in Table 1. Among Covid-19 patients, 20 had mild, 18 moderate, and 19 severe disease. All patients, with the exception of one individual with mild disease, had radiological evidence of interstitial pneumonia on chest radiography and/or computed tomography. Fourteen patients died during the hospital stay because of respiratory insufficiency caused by SARS-CoV-2 pneumonia or of other complications of Covid-19. The age and sex distributions did not differ significantly between patients with Covid-19 and controls ($p = 0.924$ and $p = 0.410$, respectively), nor were they different between the three categories of clinical severity of Covid-19 ($p = 0.353$ and $p = 0.688$, respectively). Among both Covid-19 patients and controls, females had an older median age than males (Covid-19 patients: 70.5 vs 58 years, $p = 0.0107$; controls: 76 vs 58 years, $p = 0.0003$). The time interval between onset of Covid-19 symptoms and baseline serum sampling for sNFL measurement differed between the three Covid-19 categories ($p = 0.0092$), with a longer time interval in moderate Covid-19 (median, 16 days) compared to mild (median, 12.5 days; $p = 0.0467$) and severe disease (median, 13 days; $p = 0.0127$).

We next examined whether patients of the three categories of Covid-19 severity differed pertaining to respiratory and routine laboratory parameters (Table 2). As expected, the score on the BRCSS differed significantly among patients with mild, moderate, and severe disease ($p < 0.0001$), with mild cases having a lower median score (1) than moderate (2.5) and severe (5) cases ($p = 0.0032$ and $p < 0.0001$, respectively), and moderate cases having a lower median score than severe cases ($p = 0.0421$). Accordingly, the PaO2/FiO2 ratio ($n = 56$) differed significantly among the three categories ($p < 0.0001$), with highest median value (302) in mild, intermediate (221) in moderate, and lowest (112) in severe disease, whereby in post hoc analysis statistical significance was reached only for the comparisons between mild and severe Covid-19 cases ($p < 0.0001$) and between moderate and severe cases ($p = 0.0065$) (Table 2). Regarding laboratory parameters, a significant difference was observed between serum CRP levels of the three groups ($p = 0.0430$), with severe cases having a higher median level (13.5 mg/dL) compared to mild ones (7.5 mg/dL; $p = 0.0400$). Plasma D-dimer levels also significantly differed between the three categories of Covid-19 severity ($p = 0.0137$), whereby severe

Table 1 Demographic features of Covid-19 patients and controls

	Covid-19, all	Covid-19, mild	Covid-19, moderate	Covid-19, severe	Controls	*p*
Number	57	20	18	19	30	N/A
M, F	41 (71.9%), 16 (28.1%)	14 (70%), 6 (30%)	12 (66.7%), 6 (33.3%)	15 (78.9%), 4 (21.1%)	19 (66.3%), 11 (36.7%)	> 0.05
Age (years; median, IQR)	64 (54–72)	59.5 (49–73)	60 (55–67)	69 (56–75)	62.5 (55–72.5)	> 0.05
Time interval between symptom onset and baseline blood sampling for sNFL (days; median, IQR)	13.5 (11.5–18)	**12.5** (9–15)	**16** (14–20)	**13** (11.5–19)	N/A	0.0092 (mild vs moderate: $p = 0.0467$; moderate vs severe: $p = 0.0127$)
Patients with longitudinal sNFL values (n)	20	4	4	12	N/A	N/A
Time interval between baseline and longitudinal blood sampling for sNFL (median, IQR)	12.5 (8–15)	7.5 (7–8)	14 (8–22)	14 (12–15)	N/A	> 0.05

In case of statistically significant differences between the considered groups ($p < 0.05$), the involved values are written in bold, followed by significant differences in post hoc comparisons reported in brackets

F, female. IQR, interquartile range. M, male. N/A, not applicable/not applied. sNFL, serum neurofilament light chain

cases had higher levels (median, 3779 ng/mL) compared to mild (median, 1327 ng/mL; $p = 0.0342$) and moderate ones (median, 1109 ng/mL; $p = 0.0330$). On the contrary, plasma fibrinogen and serum creatinine levels did not differ between the three categories ($p = 0.523$ and $p = 0.3447$, respectively). Total WBC counts were significantly different between patients with mild, moderate, and severe disease ($p = 0.0350$), with severe cases having higher counts (median, $8.8 \times 10^3/\mu L$) than mild ones (median, $6.1 \times 10^3/\mu L$) ($p = 0.0500$). Also blood neutrophil counts differed between the three categories of Covid-19 severity ($p = 0.0051$), with lowest levels in mild (median, $3.7 \times 10^3/\mu L$), intermediate in moderate (median, $4.5 \times 10^3/\mu L$), and highest in severe disease (median, $7 \times 10^3/\mu L$), whereby only the difference between mild and severe cases reached statistical significance ($p = 0.0043$). An opposite trend was observed for blood lymphocyte counts ($p = 0.0352$), with highest levels in mild (median, $1.4 \times 10^3/\mu L$), intermediate in moderate (median, $1 \times 10^3/\mu L$), and lowest in severe disease (median, $0.8 \times 10^3/\mu L$), and a statistically significant difference between mild and severe cases in post hoc analysis ($p = 0.0322$) (Table 2).

Analysis of baseline sNFL levels

When we measured sNFL, a positive correlation with age was observed both in controls ($r_S = 0.6738$; 95% CI 0.4047–0.8355; $p < 0.0001$) and in Covid-19 patients ($r_S = 0.5174$; 95% CI, 0.2897–0.6897; $p < 0.0001$) (Table 3). In agreement with the older median age in females compared to males in both Covid-19 patients and controls, in both groups females had higher median sNFL levels than males, but the difference was statistically significant only in controls (Covid-19 patients: 36.0 pg/mL vs 31.7 pg/mL, $p = 0.722$; controls: 20.0 pg/mL vs 10.2 pg/mL, $p = 0.0033$).

Covid-19 patients had significantly higher sNFL levels compared to controls (median, 33.9 pg/mL vs 13.1 pg/mL; $p < 0.0001$) (Table 2). When stratifying patients according to their clinical severity, each category showed significantly higher sNFL values compared to controls (mild Covid-19: median sNFL, 31.0 pg/mL; $p = 0.0082$; moderate: median, 21.0 pg/mL; $p = 0.0171$; severe: median, 47.6 pg/mL; $p < 0.0001$), whereas no significant differences were observed between the three categories of Covid-19 severity ($p > 0.05$ for all comparisons) (Fig. 1). However, Covid-19 patients who died during the hospital

Table 2 Clinical and laboratory features of Covid-19 patients and controls

	Covid-19, all	Covid-19, mild	Covid-19, moderate	Covid-19, severe	Controls	*p*
BRCSS (median, IQR)	2 (1–3)	1 (1–1)	2.5 (2–3)	5 (3–7)	N/A	< 0.0001 (mild vs moderate: $p = 0.0032$; moderate vs severe: $p = 0.0421$; mild vs severe: $p < 0.0001$)
PaO2/FiO2 (median, IQR)	213 (121–291) (n = 56)	**302** (263–355) (n = 19)	**221** (172–250) (n = 18)	**112** (85–140) (n = 19)	N/A	< 0.0001 (mild vs severe: $p < 0.0001$; moderate vs severe: $p = 0.0065$)
Serum CRP (mg/dL; median, IQR)	9.9 (4.6–14.9)	**7.5** (2.3–10.8)	**9.9** (7.7–14.6)	**13.5** (6.1–18.0)	N/A	0.0430 (mild vs severe: $p = 0.0400$)
Plasma D-dimer (ng/mL; median, IQR)	1591 (880–3826)	**1327** (705–2357)	**1109** (885–1918)	**3779** (1536–13,480)	N/A	0.0137 (mild vs severe: $p = 0.0342$; moderate vs severe: $p = 0.0330$)
Plasma fibrinogen (mg/dL; median, IQR)	592 (472–709)	532 (487–709)	647 (491–727)	592 (462–751)	N/A	> 0.05
Serum creatinine (mg/dL; median, IQR)	0.87 (0.74–0.99)	0.94 (0.79–1.07)	0.88 (0.73–0.95)	0.79 (0.71–0.93)	N/A	> 0.05
WBC count (*10^3/μL; median, IQR)	6.7 (5.5–9.3)	**6.1** (4.9–8.0)	**6.1** (5.3–8.2)	**8.8** (6.9–13.1)	N/A	0.0350 (mild vs severe: $p = 0.0500$)
Blood neutrophil count (*10^3/μL; median, IQR)	5.2 (3.5–7.3)	**3.7** (3.2–5.3)	**4.5** (3.8–6.3)	**7** (5.4–11.1)	N/A	0.0051 (mild vs severe: $p = 0.0043$)
Blood lymphocyte count (*10^3/μL; median, IQR)	1 (0.8–1.4)	**1.4** (1.0–1.8)	**1.0** (0.8–1.3)	**0.8** (0.7–1.2)	N/A	0.0352 (mild vs severe: $p = 0.0322$)
Baseline sNFL (pg/mL; median, IQR)	33.9 (17.6–69.8)	**31.0** (16.7–50.7)	**21.0** (17.0–37.9)	**47.6** (22.5–89.2)	**13.1** (9.4–18.9)	< 0.0001 (mild vs controls, $p = 0.0082$; moderate vs controls, $p = 0.0171$; severe vs controls, $p < 0.0001$); moreover, all Covid-19 vs controls: $p < 0.0001$
Baseline sNFL of Covid-19 patients with longitudinal sNFL measurements (pg/mL; median, IQR)	34.8 (15.3–85.5)	46.3 (6.8–95.6)	33.5 (13.6–61.2)	34.8 (15.3–85.5)	N/A	N/A
Longitudinal sNFL (pg/mL; median, IQR)	68.1 (28.9–138.3)	56.6 (6.3–122.4)	43.7 (22.7–155.3)	69 (53.6–164.4)	N/A	> 0.05
Absolute difference between longitudinal and baseline sNFL (pg/mL; median, IQR)	20.6 (2.3–84.1)	6.2 (0.8–15.7)	10.6 (-12.4–107.7)	47.5 (15.4–133.6)	N/A	> 0.05
Percent difference between longitudinal and baseline sNFL (median, IQR)	131.8 (7.6–255.5	16.5 (3.5–37.2)	108.8 (10.2–240.6)	220.2 (36.5–293.3)	N/A	> 0.05

In case of statistically significant differences between the considered groups ($p < 0.05$), the involved values are written in bold, followed by significant differences in post hoc comparisons reported in brackets. In the case of baseline sNFL levels of Covid-19 patients compared to controls, the *p* value of the comparison between the whole Covid-19 cohort and controls is added after those of the comparisons between single categories of disease severity and controls. BRCSS, PaO2/FiO2, serum CRP, plasma D-dimer, plasma fibrinogen, serum creatinine, and WBC, neutrophil, and lymphocyte counts refer to values at admission

BRCSS, Brescia Respiratory Covid Severity Scale; CRP, C-reactive protein; IQR, interquartile range; N/A, not applicable/not applied; PaO2/FiO2, ratio between arterial partial pressure of oxygen and inspiratory fraction of oxygen; sNFL, serum neurofilament light chain; WBC, white blood cell

Table 3 Correlations between clinical and laboratory features and sNFL levels in Covid-19 patients

Patient group	Clinical or laboratory feature	sNFL	r_S	95% CI	p
Covid-19, all	Age	Baseline sNFL	0.5174	0.2897 to 0.6897	< 0.0001
Mild Covid-19	Serum CRP	Baseline sNFL	0.6586	0.2922 to 0.8564	0.0016
Mild Covid-19	Plasma D-dimer	Baseline sNFL	0.5774	0.1676 to 0.8171	0.0077
Covid-19, all	Blood lymphocyte count	Baseline sNFL	− 0.2703	− 0.5018 to − 0.002544	0.0420
Mild Covid-19	Blood lymphocyte count	Baseline sNFL	− 0.6868	− 0.8696 to − 0.3385	0.0008
Covid-19, all	Serum CRP	Longitudinal sNFL	0.4962	0.05484 to 0.7754	0.0261
Covid-19, all	Plasma D-dimer	Longitudinal sNFL	0.5744	0.1632 to 0.8156	0.0081
Covid-19, all	BRCSS	Absolute longitudinal variation of sNFL	0.5131	0.07736 to 0.7843	0.0207
Covid-19, all	BRCSS	Percent longitudinal variation of sNFL	0.5744	0.1631 to 0.8156	0.0081
Covid-19, all	Serum CRP	Absolute longitudinal variation of sNFL	0.6391	0.2610 to 0.8472	0.0024
Covid-19, all	Serum CRP	Percent longitudinal variation of sNFL	0.5955	0.1942 to 0.8260	0.0056
Covid-19, all	Plasma D-dimer	Absolute longitudinal variation of sNFL	0.5504	0.1288 to 0.8035	0.0119
Covid-19, all	Plasma D-dimer	Percent longitudinal variation of sNFL	0.4977	0.05683 to 0.7762	0.0255

BRCSS, serum CRP, plasma D-dimer, and blood lymphocyte count refer to values at admission; BRCSS, Brescia Respiratory Covid Severity Scale; CI, confidence interval; CRP, C-reactive protein; sNFL, serum neurofilament light chain

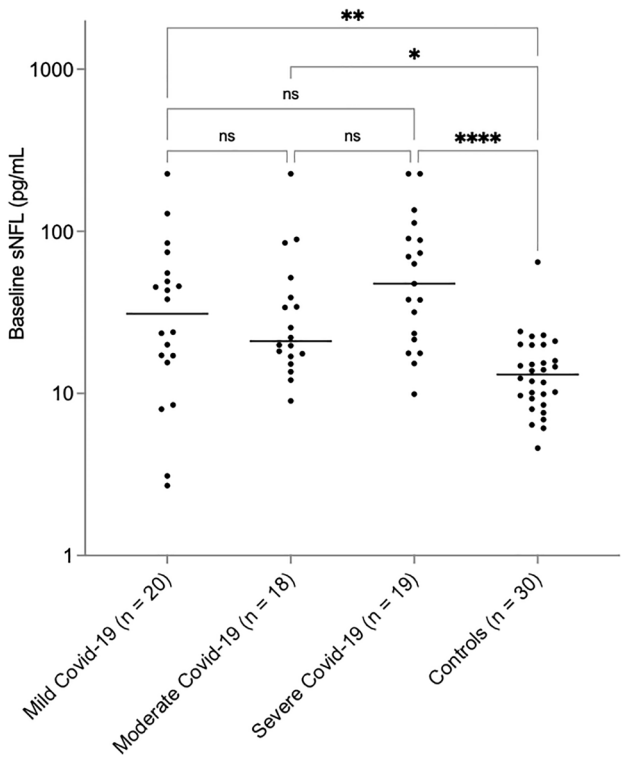

Fig. 1 Baseline sNFL levels in patients with mild, moderate, and severe Covid-19 and controls. Horizontal bars in each group represent median values. *$p < 0.05$. **$p < 0.01$. ****$p < 0.0001$. ns, non-significant; sNFL, serum neurofilament light chain

stay ($n = 14$) had significantly higher baseline sNFL levels (median, 55.3 pg/mL) compared to surviving ones ($n = 43$; median, 23.5 pg/mL; $p = 0.0300$).

sNFL levels did not correlate with the time interval between onset of symptoms of SARS-CoV-2 infection and baseline blood sampling for the biomarker ($p = 0.285$). Regarding respiratory parameters, sNFL did not correlate with BRCSS ($p = 0.195$) nor with PaO2/FiO2 ratio ($p = 0.924$). As for routine laboratory parameters, serum CRP and plasma D-dimer had a positive correlation with sNFL only in patients with mild Covid-19 (serum CRP: $r_S = 0.6586$; 95% CI 0.2922–0.8564; $p = 0.0016$; plasma D-dimer: $r_S = 0.5774$; 95% CI 0.1676–0.8171; $p = 0.0077$) (Table 3, Fig. 2A, B). Plasma fibrinogen and WBC and neutrophil counts did not correlate with sNFL levels in the whole Covid-19 cohort ($p = 0.358$, $p = 0.233$, and $p = 0.060$, respectively) nor within any category of disease severity ($p > 0.05$ for all correlations). On the contrary, a negative correlation emerged between blood lymphocyte count and sNFL in the whole Covid-19 cohort ($r_S = -0.2703$; 95% CI − 0.5018 to − 0.002544; $p = 0.0420$), which was stronger when limiting the analysis to patients with mild Covid-19 ($r_S = -0.6868$; 95% CI − 0.8696 to − 0.3385; $p = 0.0008$; Fig. 2C). Finally, we found no correlation between serum creatinine and sNFL ($p = 0.1725$).

Longitudinal analysis of sNFL levels in Covid-19 patients

Longitudinal analysis of sNFL levels was performed in 20 patients (mild Covid-19, $n = 4$; moderate, $n = 4$; severe, $n = 12$). Longitudinal variations in sNFL levels are depicted in Fig. 3. The median interval between baseline and longitudinal blood sampling for sNFL was 12.5 days (range, 7–29 days) (Table 1). There was a strong positive correlation between baseline and longitudinal sNFL levels

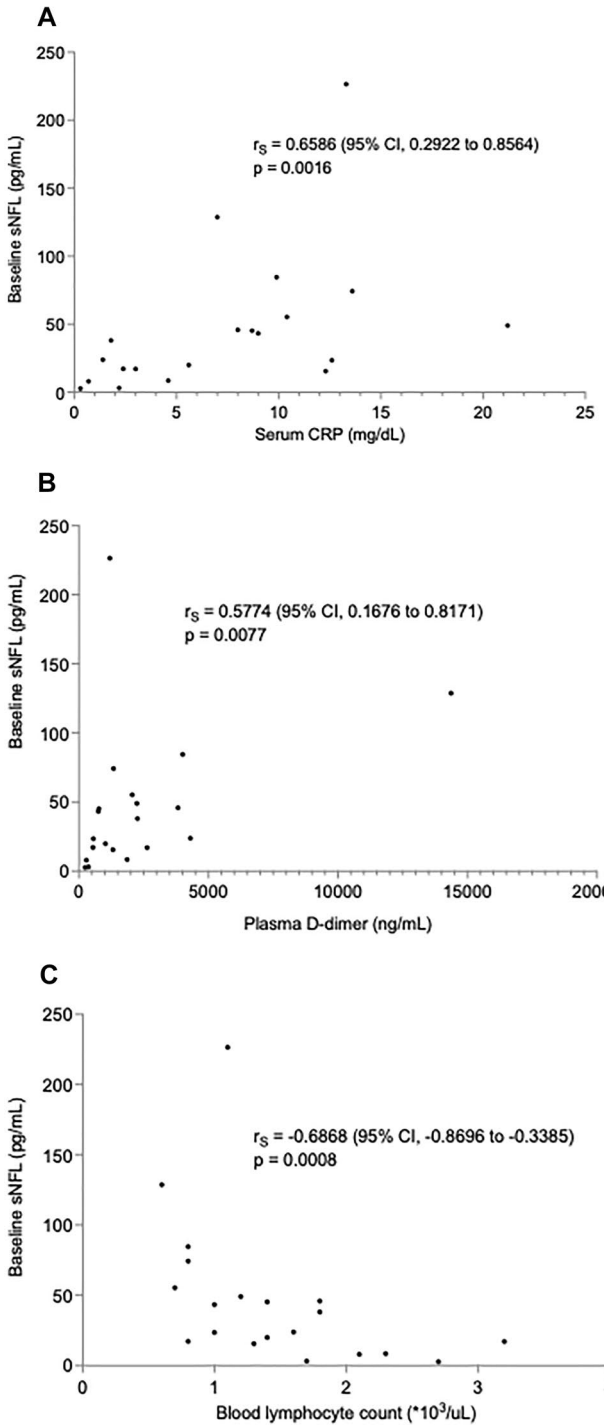

Fig. 2 Correlations of baseline sNFL levels with serum CRP, plasma D-dimer, and blood lymphocyte count at admission in patients with mild Covid-19. **A** Positive correlation between serum CRP at admission and baseline sNFL. **B** Positive correlation between plasma D-dimer at admission and baseline sNFL. **C** Negative correlation between blood lymphocyte count at admission and baseline sNFL. CI, confidence interval; CRP, C-reactive protein; sNFL, serum neurofilament light chain

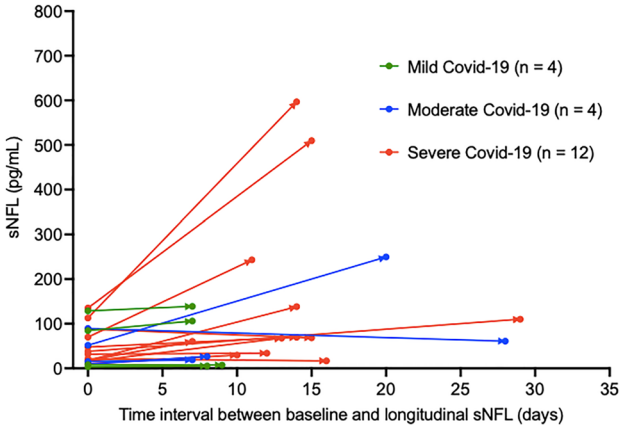

Fig. 3 Longitudinal variations in sNFL levels in patients with mild, moderate, and severe Covid-19. sNFL, serum neurofilament light chain

of individual patients ($r_S = 0.7920$; 95% CI 0.5280–0.9164; $p < 0.0001$). The median longitudinal sNFL level of all 20 Covid-19 patients (68.1 pg/mL) was higher than the baseline median level of the same patients (34.8 pg/mL), without, however, reaching the threshold of statistical significance ($p = 0.0898$) (Table 2). Similar, not statistically significant, differences were observed also between longitudinal and baseline sNFL levels within the three categories of disease severity. The median difference between longitudinal and baseline sNFL levels was 20.6 pg/mL (Table 2). Sixteen/twenty patients showed an increase of sNFL levels from baseline to longitudinal samples, whereas for only 4/20 patients a reduction was observed. Longitudinal sNFL levels did not significantly differ between the 3 categories of Covid-19 severity ($p = 0.477$). As one patient who underwent longitudinal blood sampling for sNFL developed a clinically and electromyographically diagnosed mild damage of the left brachial plexus which was attributed to prolonged prone positioning for mechanical ventilation, we cannot exclude that this affected sNFL levels of the second blood sample, although the motor deficit was observed > 2 weeks after the second sampling. Nevertheless, also when excluding this pair of sNFL levels from the analysis, the median follow-up sNFL level was still higher than the baseline one (67.8 pg/mL vs 31.7 pg/mL; $p = 0.0949$).

While longitudinal sNFL levels did not correlate with BRCSS score or PaO2/FiO2 ratio at admission ($p = 0.085$ and $p = 0.533$, respectively), both serum CRP and plasma D-dimer at admission positively correlated with longitudinal sNFL (serum CRP: $r_S = 0.4962$; 95% CI 0.05484–0.7754; $p = 0.0261$; plasma D-dimer: $r_S = 0.5744$; 95% CI 0.1632–0.8156; $p = 0.0081$) (Table 3, Fig. 4A, B). On the contrary, no correlations were observed between longitudinal sNFL and plasma fibrinogen ($p = 0.363$), serum

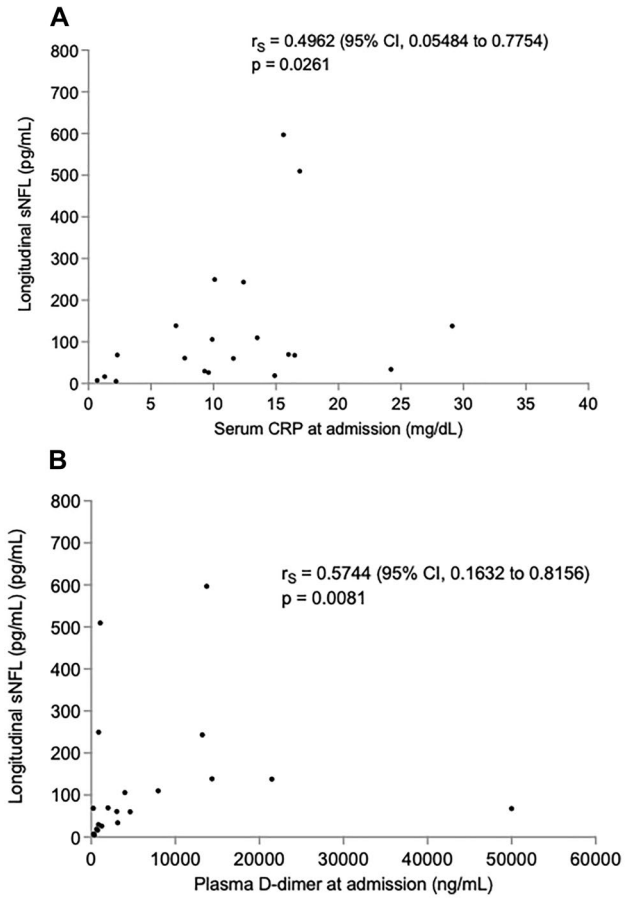

Fig. 4 Correlations of longitudinal sNFL with serum CRP and plasma D-dimer at admission. **A** Correlation of longitudinal sNFL with serum CRP at admission. **B** Correlation of longitudinal sNFL with plasma D-dimer at admission. CI, confidence interval; CRP, C-reactive protein; sNFL, serum neurofilament light chain

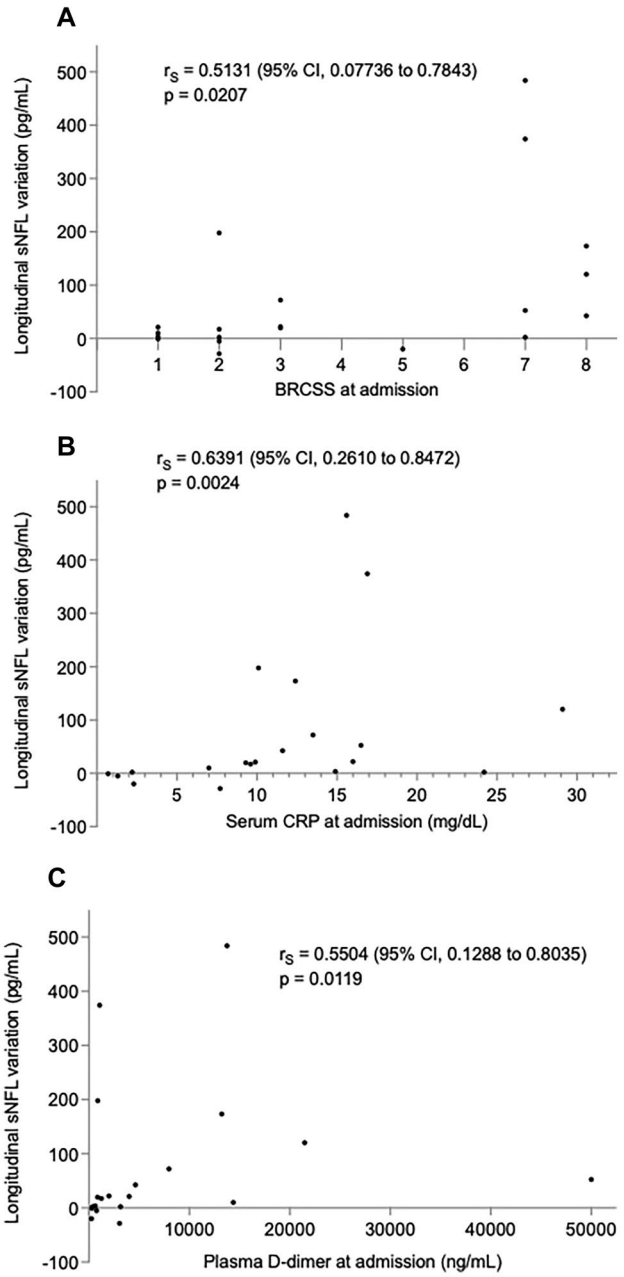

Fig. 5 Correlations of absolute longitudinal variations in sNFL levels with BRCSS score, serum CRP, and plasma D-dimer at admission. **A** Correlation of absolute longitudinal variations of sNFL with BRCSS at admission. **B** Correlation of absolute longitudinal variations of sNFL with serum CRP at admission. **C** Correlation of absolute longitudinal variations of sNFL with plasma D-dimer at admission. BRCSS, Brescia Respiratory Covid Severity Scale; CI, confidence interval; CRP, C-reactive protein; sNFL, serum neurofilament light chain

creatinine ($p = 0.4710$) or WBC ($p = 0.075$), neutrophil ($p = 0.266$), or lymphocyte counts ($p = 0.942$).

Neither absolute nor percent longitudinal variations of sNFL levels differed between the 3 categories of Covid-19 severity ($p = 0.302$ and $p = 0.341$, respectively) (Table 2), nor did they correlate with the length of the time interval between the two corresponding blood samples ($p = 0.596$ and $p = 0.884$, respectively). Pertaining to respiratory parameters, no correlation was found between absolute or percent longitudinal sNFL variation and PaO2/FiO2 at admission ($p = 0.093$ and $p = 0.067$, respectively). However, both absolute and percent longitudinal variation of sNFL positively correlated with BRCSS score at admission (absolute variation: $r_S = 0.5131$; 95% CI 0.07736–0.7843; $p = 0.0207$; percent variation: $r_S = 0.5744$; 95% CI 0.1631–0.8156; $p = 0.0081$) (Fig. 5A). Among routine laboratory parameters, serum CRP at admission had a significant positive correlation with both absolute and percent longitudinal variation of sNFL levels (absolute variation: $r_S = 0.6391$; 95% CI 0.2610–0.8472; $p = 0.0024$; percent variation: $r_S = 0.5955$; 95% CI 0.1942–0.8260; $p = 0.0056$) (Fig. 5B). Similarly, a significant positive correlation was found between plasma D-dimer at admission and longitudinal

variation of sNFL, both absolute ($r_S = 0.5504$; 95% CI 0.1288–0.8035; $p = 0.0119$) and percent ($r_S = 0.4977$; 95% CI 0.05683–0.7762; $p = 0.0255$) (Fig. 5C). On the contrary, no correlations were found between absolute or percent sNFL variation and plasma fibrinogen, serum creatinine, or WBC, neutrophil, or lymphocyte counts at admission (all $p > 0.05$).

Discussion

In our work, we found increased sNFL levels in hospitalized Covid-19 patients without major neurological manifestations, with no significant differences between subgroups with mild, moderate, and severe disease. In patients with mild Covid-19, sNFL correlated positively with serum CRP and plasma D-dimer at admission and negatively with blood lymphocyte count at admission. Short-term longitudinal sNFL levels were nominally, but not significantly, higher than corresponding baseline ones. Levels of serum CRP and plasma D-dimer at admission correlated with longitudinal sNFL levels. While no correlation was found between PaO2/FiO2 ratio and sNFL, the BRCSS score at admission only correlated with longitudinal sNFL variation.

Our results confirm previous findings of increased blood NFL levels in patients with Covid-19 [6, 7, 13], as well as of higher levels in Covid-19 patients dying at hospital [10–12], thus further indicating that neuroaxonal damage, as reflected by NFL elevation in the blood, may be a feature of Covid-19. We acknowledge that this neurochemical alteration could reflect a nonspecific effect of respiratory infections, as suggested by the observation of increased plasma NFL in bacterial pneumonia [21]. The underlying mechanism common to these different disease conditions might be represented by hypoxic damage due to respiratory insufficiency, thus resembling NFL increase following hypoxic-ischemic injury after cardiac arrest [22]. This interpretation is supported by the recently published evidence of a correlation between sNFL levels and parameters of respiratory impairment in a large series of hospitalized Covid-19 patients [12]. However, in our cohort sNFL levels or their longitudinal variations had no correlation with the PaO2/FiO2 ratio, a recognized measure of the oxygenation ability of the lung parenchyma, which is impaired in SARS-CoV-2 pneumonia [23]. Moreover, the BRCSS score at admission only showed a moderate correlation with longitudinal variation of sNFL. Most importantly, we did not observe a difference of sNFL levels between categories of varying severity of Covid-19, whereby the degree of respiratory insufficiency is indeed the main determinant of clinical severity itself. These data suggest that sNFL elevation in Covid-19 could be at least partly determined by other mechanisms than hypoxic neuronal injury. One such plausible pathophysiological determinant might be systemic inflammation, in turn promoting sepsis-associated encephalopathy [24, 25]. The putative systemic, non-brain-specific, inflammatory mechanisms could have, however, a Covid-19-specific component or at least be particularly pronounced in SARS-CoV-2 infection, as suggested by the observation of higher sNFL levels in Covid-19 patients hospitalized in the ICU compared to non-Covid-19 ICU patients [9]. Notably, according to our data, the increase of NFL does not seem to depend on major impairment of the nervous system, suggesting on the contrary the possible occurrence of a certain degree of subclinical neuroaxonal damage in SARS-CoV-2 infection. This issue is particularly relevant with reference to hypothetical medium- and long-term neurological sequelae of SARS-CoV-2 infection [26–28]. In fact, deficits in cognitive processing speed have been reported in 42.1% of Covid-19 patients 5 months after hospital discharge [29]. While our finding of nominally, but not significantly, increased sNFL levels at short-term longitudinal evaluation, which is quite in agreement with previous observations [7], could be compatible with both hypoxic and inflammatory injury, we cannot rule out a third possible mechanism of Covid-19-induced neuroaxonal impairment, namely a direct damage to the nervous tissue by SARS-CoV-2 [30, 31].

Our study has the following main strengths: (1) Covid-19 patients were thoroughly characterized from a respiratory and blood biochemistry perspective; (2) they were devoid of chronic or recent acute neurological diseases affecting sNFL levels; (3) patients with varying degrees of Covid-19 severity were included; (4) blood sampling for sNFL measurement was performed relatively early in the disease course; (5) a part of the cohort underwent a longitudinal neurochemical evaluation. At the same time, we acknowledge the following limitations: (1) the study cohort is relatively small, especially pertaining to longitudinal sNFL measurements and to analyses conducted on each of the three subcategories of disease severity; (2) we did not include asymptomatic individuals with SARS-CoV-2 infection or patients with very mild disease not requiring hospitalization; (3) we did not perform a long-term longitudinal investigation of sNFL levels; (4) detailed neurological evaluations aimed at identifying minor neurological manifestations (e.g., smell and taste alterations, headache, myalgia) were not systematically performed, and therefore, the prevalence of such disturbances cannot be determined in our cohort; (5) the patients did not undergo formal neuropsychological testing; (6) we did not perform systematic neurophysiological investigations with nerve conduction studies and needle electromyography.

We have measured NFL in the serum but not in the CSF. While the levels in the two biological fluids generally correlate both in physiological and in neurological disease conditions [32], the study of Paterson et al. [15] reported a lack of correlation between serum and CSF NFL in Covid-19 patients with PNS manifestations, suggesting that sNFL

could also reflect damage to peripheral nervous structures. Therefore, the differential changes of NFL levels in the two fluid compartments in Covid-19 deserve further investigation. Likewise, other aspects in the emerging field of neurochemical biomarkers in SARS-CoV-2 infection should be further explored, including the relationships of NFL with neuroimaging and neurophysiological features, the association of NFL levels with long-term neurological (e.g., cognitive) complaints, the possible changes of NFL levels as a result of antiviral treatments, and the dynamics of other neurochemical biomarkers—namely $A\beta_{1\text{-}42}$, $A\beta_{1\text{-}40}$, total tau, phosphorylated tau, and glial fibrillary acidic protein (GFAP)—during and after infection with SARS-CoV-2.

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Author contributions FV and VS were involved in conceptualization. IM and FV helped in data curation and formal analysis. FV, IM, IB, SP, CC, AM, NC, NT, GBP, GP, and ET contributed to investigation. VS was involved in project administration. VS, AR, and FV helped in supervision. FV was involved in writing—original draft preparation. AR, NT, FV, and VS were involved in writing—review and editing.

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Data availability statement The data of the present study will be made available upon reasonable request.

Declarations

Conflicts of interest The authors have no conflicts of interest to declare.

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