LETTER

Nervous system: subclinical target of SARS-CoV-2 infection

INTRODUCTION

Neurofilament light chain (NfL) is the most abundant and soluble protein of the neuronal cytoskeleton and is released during axonal injury. An increase of cerebrospinal fluid (CSF) and serum levels of NfL has been demonstrated in patients with several inflammatory and degenerative neurological disorders of the nervous system, in correlation with clinical and radiological activity.[1] Several clinical reports showed that patients with COVID-19 suffer from neurological symptoms including non-specific manifestations as headache, hyposmia, dysgeusia and altered consciousness.[2] To date, the pathogenesis of the aforementioned symptoms and the role of inflammatory factors in the context of this severe systemic condition have not been elucidated. Different possible mechanisms of neuronal damage have been hypothesised, including direct viral nervous system invasion through hematogenous dissemination or neuronal retrograde dissemination and indirect injury mediated by inflammatory processes due to the cytokine storm.[2] The aim of this study was to explore the nervous system involvement in patients with COVID-19 by evaluating the presence of neurological symptoms and measuring serum NfL levels as biomarker of axonal damage.

PATIENTS AND METHODS

We performed a 1-week point prevalence survey and evaluated all patients with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection admitted at the COVID-19 medical and intensive care unit (ICU) areas of the University Hospital of Verona, Italy. Exclusion criteria were neurological comorbidities, which could be independently associated with neuroaxonal damage.

Clinical data and testing

The diagnosis of COVID-19 was based on the presence of SARS-CoV-2 confirmed by positive assay of nasopharyngeal samples on reverse transcriptase PCR. Demographic, anamnestic and clinical data, including presence of comorbidities, symptom onset, antecedent/current treatments, symptoms of respiratory/intestinal involvement, symptoms possibly suggesting nervous system involvement (ie, presence of altered consciousness, myalgia, fatigue, headache, vertigo, hypogeusia and hyposmia), were prospectively analysed in each included case and collected in a standardised form.

NfL analysis

Investigators blinded to clinical data measured serum NfL concentration in duplicate in patients and in a group of healthy controls (n=54) for comparison using SIMOA Nf-light kit in SR-X immunoassay analyser, Simoa (Quanterix Corporation, Billerica, Massachusetts, USA), which runs ultrasensitive paramagnetic bead-based enzyme-linked immunosorbent assays.[3]

Statistical analysis

Continuous and categorical variables were reported as mean (SD) and percentages, respectively. χ^2 test and t-test were used for comparison between the subgroup of patients with NfL concentration within the normal range and those with increased levels. Reference threshold for normal range of serum NfL levels among age groups has been extracted from a 10-year local group of healthy controls and, for age-intervals not available in our cohort (>70 years), from literature.[4] A second comparison was performed between the subgroup of patients with neurological symptoms and subjects not presenting those manifestations. Analyses were performed using STATA V.15, and statistical significance was set at <0.05.

RESULTS

Characteristics of the analysed cohort

Among the initial cohort (n=131 cases), 24 patients were excluded because of antecedent neurological comorbidities (history of cognitive impairment, n=19; recent history of ischaemic stroke, n=4; and haemorrhagic stroke, n=1) which could independently alter NfL values.

Overall, 107 patients were included in the point prevalence survey; 25 patients were women (23.4%), and the mean age was 66 years (SD 1.9). The severity of the respiratory failure required admission to ICU in 46 cases (43%). Comorbidities were observed in 91 patients (85%), mainly hypertension (69.2%), diabetes (25.2%) and cardiovascular diseases (23.6%). Respiratory failure and fever were common (91.6% and 96% of cases, respectively), while diarrhoea was more rarely observed (23.4% of cases). Neurological symptoms were recorded in 59 patients (85.5%), most frequently fatigue (68.1%), hypogeusia (31.9%), myalgia (23.2%), hyposmia (21.7%), altered consciousness (18.8%), headache (17.4%), vertigo (8.7%) and syncope (8.7%). None of the included cases presented focal symptoms, visual, language or speech dysfunctions. Detailed data are reported in table 1.

NfL analysis

The mean level of NfL was 73.3 pg/mL (SD 89.5). Among included cases, 61 patients (57%) had increased values of serum NfL compared with the reference controls. Patients with increased serum NfL levels were more likely to be admitted to ICU and to undergo orotracheal intubation ($p<0.01$). In addition, subjects with increased NfL values have had a significant longer time since COVID-19 symptom onset ($p<0.01$). There was no statistically relevant association between neurological symptoms and serum NfL levels (table 1).

DISCUSSION

Our findings suggest that a concomitant axonal damage can occur in patients with COVID-19, particularly in those with a more severe clinical course and even in patients with no evidence of specific neurological symptoms/signs. The mechanisms of neuroaxonal damage in the course of COVID-19 remain to be established; however, it is possible to hypothesise that hypercoagulability, hypoxia and the cytokine storm, which seem to be associated with disease severity,[2] might be the principal cause of nervous system damage. Perfusion abnormalities and subclinical ischaemic stroke recently described in the course of COVID-19 reinforce the possible role of deranged coagulation functions in inducing neurological complications.[5]

The neurological nature of some non-specific manifestations (ie, headache, fatigue, vertigo, hypogeusia and hyposmia) remains on open question. We did not observe a significant increase of serum NfL in patients reporting these symptoms, which could possibly reflect a consequence of the systemic disease. However, our findings that NfL levels are increased in a high number of patients with SARS-CoV-2 infection and are significantly associated with longer

Table 1 Demographic and clinical characteristics of the analysed cohort

Variable	Patients			
	All (n=107)	Normal NfL (n=46)	Increased NfL (n=61)	P value
Sex, female, n (%)	25 (23.4)	15 (32.6)	10 (16.4)	0.05
Age (years), mean (SD)	65.8 (11.9)	65.2 (12.6)	66.4 (11.6)	0.60
ICU stay, n (%)	46 (43)	10 (21.7)	36 (59)	**<0.001**
Illness onset (days), mean (SD)	15.8 (7.9)	11.3 (7.2)	19.5 (6.4)	**<0.001**
Comorbidity, n (%)				
Any	91 (85)	37 (80)	54 (88.5)	0.24
Diabetes	27 (25.2)	11 (24)	16 (26.2)	0.78
Hypertension	74 (69.2)	34 (73.9)	40 (65.6)	0.36
Ischaemic cardiopathy	25 (23.6)	32 (23.9)	50 (82)	0.13
Neoplasm	13 (12.2)	6 (13)	7 (11.5)	0.81
Respiratory chronic diseases (COPD and asthma)	12 (11.2)	4 (8.7)	8 (13.1)	0.47
BMI>30	12 (11.2)	2 (4.3)	10 (16.4)	**0.04**
Neurological symptoms, n (%)*				
Any	59 (85.5)	36 (94.7)	23 (74.2)	0.21
Hyposmia	15 (21.7)	9 (23.7)	6 (19.3)	0.85
Hypogeusia	22 (31.9)	14 (36.8)	8 (25.8)	0.51
Vertigo	6 (8.7)	5 (13.2)	1 (3.2)	0.19
Fatigue	47 (68.1)	25 (65.8)	22 (71)	0.24
Headache	12 (17.4)	9 (23.7)	3 (9.7)	0.19
Myalgia	16 (23.2)	11 (28.9)	5 (16.1)	0.32
Altered consciousness	13 (18.8)	5 (13.1)	8 (25.8)	0.12
Syncope	6 (8.7)	2 (5.3)	4 (12.9)	0.22
Other symptoms/signs, n (%)				
Respiratory failure	98 (91.6)	38 (82.6)	60 (98.4)	**0.003**
Diarrhoea	25 (23.4)	15 (32.6)	10 (16.4)	0.05
Fever	103 (96)	43 (93.5)	60 (98.4)	0.19
O_2 support, n (%)	82 (76.6)	27 (58.7)	55 (90.2)	**<0.001**
Orotracheal intubation	50 (46.7)	9 (19.6)	41 (67.2)	**<0.001**

*Questionnaire on neurological symptoms could not be administered in 38 patients.
BMI, body mass index; COPD, chronic obstructive pulmonary disease; ICU, intensive care unit; NfL, neurofilament light chain.

and more severe disease indicate that the nervous system is a possible target of COVID-19, also in patients with no evident neurological symptoms. These observations indeed lead to considering ongoing axonal damage and therefore nervous system involvement in the course of COVID-19, determining a significant impact on the clinical management and monitoring of patients with SARS-CoV-2 in the acute stage and during the follow-up. To our knowledge, this is the first study that systematically evaluates a measurable and well-defined biomarker of axonal damage in a cohort of patients with COVID-19 and can therefore represent a pivotal point for future investigations.

Our study has some limitations, including the exclusive evaluation of hospitalised and therefore more severe cases and the inability of NfL to identify muscle damage, which could occur during coronavirus infection. Despite none of our patients had symptoms/signs suggestive for peripheral nervous system involvement, when analysing NfL levels, it has to be considered that both central and peripheral nervous system damage might influence their levels. In addition, lumbar puncture was not indicated in our cohort of patients so that we were unable to compare serum and CSF NfL levels, which, however, are highly correlated in different inflammatory, degenerative and infectious-related neurological conditions.[1] The difficult pandemic situation also prevented brain MRI analysis, which could be useful to confirm brain involvement in patients with increased NfL levels. Follow-up studies with extensive clinical and paraclinical investigations will demonstrate if a persistent neuronal injury occurs in patients with COVID-19 and will help to clarify the occurrence of long-term neurological sequelae and the prognostic value of nervous system involvement.

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