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Cerebrospinal fluid in COVID-19 neurological complications: Neuroaxonal damage, anti-SARS-Cov2 antibodies but no evidence of cytokine storm

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ABSTRACT

Objective: To study in cerebrospinal fluid (CSF) of COVID-19 subjects if a "cytokine storm" or neuroinflammation are implicated in pathogenesis of neurological complications.
Methods: Cross-sectional study of CSF neuroinflammatory profiles from 18 COVID-19 subjects with neurological complications categorized by diagnosis (stroke, encephalopathy, headache) and illness severity. COVID-19 CSF was compared with CSF from healthy, infectious and neuroinflammatory disorders and stroke controls ($n = 82$). Cytokines (IL-6, TNFα, IFNγ, IL-10, IL-12p70, IL-17A), inflammation and coagulation markers (high-sensitivity-C Reactive Protein [hsCRP], ferritin, fibrinogen, D-dimer, Factor VIII) and neurofilament light chain (NF-L), were quantified. SARS-CoV2 RNA and SARS-CoV2 IgG and IgA antibodies in CSF were tested with RT-PCR and ELISA.
Results: CSF from COVID-19 subjects showed absence of pleocytosis or specific increases in pro-inflammatory markers (IL-6, ferritin, or D-dimer). Although pro-inflammatory cytokines (IL-6, TNFα, IL-12p70) and IL-10 were increased in CSF of stroke COVID-19 subjects, a similar increase was observed in non-COVID-19 stroke subjects. Anti-SARS-CoV2 antibodies in CSF of COVID-19 subjects (77%) were observed despite no evidence of SARS-CoV2 viral RNA. CSF-NF-L was elevated in subjects with stroke and critical COVID-19 as compared to controls and other COVID-19 severity categories. CSF-hsCRP was present in all subjects with critical stages of COVID-19 (7/18) but only in 1/82 controls.
Conclusion: The paucity of neuroinflammatory changes in CSF of COVID-19 subjects and lack of SARS-CoV2 RNA do not support the presumed neurovirulence of SARS-CoV2 or neuroinflammation in pathogenesis of neurological complications in COVID-19. The role of CSF SARS-CoV2 IgG antibodies and mechanisms of neuronal damage are still undetermined.

1. Introduction

Central and peripheral nervous system disorders can develop in patients with severe acute respiratory syndrome coronavirus 2 (SARS-CoV2) infection, during acute and/or postinfectious phases of coronavirus disease 2019 (COVID-19) [1,2]. These disorders are influenced by patient age, sex and pre-existing comorbidities and are mainly represented by cerebrovascular pathologies and encephalopathies [3–7]. The so-called "COVID-19 encephalitis" [8], acute disseminated encephalomyelitis (ADEM) [9], cranial neuropathies [10] and Guillain-Barré syndrome [11,12] have also been described as well as a high frequency of headache [13–15]. There are several unanswered questions regarding the pathogenesis of these neurological complications including concerns about the neuro-invasiveness or neurovirulence of SARS-CoV2, the role of neuroinflammation and the effects of the "cytokine storm" on the central nervous system (CNS). Studies focused on the analysis of cerebrospinal fluid (CSF) in COVID-19 infection have outlined a diversity of CSF findings that lack specific profiles associated

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with the neurological symptoms [16–21]. Interestingly, IgG antibodies against SARS-CoV2 spike protein have been found in the CSF of eight patients with encephalopathy [22], and other case reports have described changes suggestive of an inflammatory process [23,24] and neuronal damage [25,26]. Although some of the previous observational studies of CSF have suggested the potential role of neuroinflammation in the neurological complications of COVID-19, there has not been an approach to examine the immune profile of CSF of COVID-19 in a controlled study that allows a comparison with other CNS pathologies proven to be associated with infectious, autoimmune or cerebrovascular disorders. Considering the growing concerns that neuroinflammation contributes to the neurological complications of COVID-19 and/or that SARS-CoV2 may have neurovirulent capability, this study sought to identify in the CSF clues about the pathogenesis of such neurological problems by investigating for markers of neuroinflammation including those associated with cytokine storm, the presence of SARS-CoV2 RNA and antibodies to SARS-CoV2. We aimed to determine whether the CSF from patients with COVID-19 neurological complications exhibit a profile consistent with neuroinflammation or share common pathogenic immune pathways with other neurological disorders, by comparing the CSF profile of such patients with control groups including healthy, stroke in non-COVID-19 patients, and subjects with neurological infections and neuroinflammatory disorders.

2. Methods

2.1. Study design

A cross-sectional study to investigate immune and neuroinflammatory changes associated with pathogenesis of neurological involvement in COVID-19 was performed in the CSF of 18 adult COVID-19 patients with neurological manifestations and compared to those of 14 age-matched healthy and 68 non-COVID-19 neurological disease controls. The sample size of the COVID-19 group was determined by convenience and all CSF was collected prospectively from patients undergoing standard of care evaluation for COVID-19 neurological complications during the period April 1–July 31, 2020. Only patients with complete record of neurological examination by a neurologist, CSF availability after completing the required clinical tests, neuroimaging and nucleic acid amplification test (NAAT) by RT-PCR for SARS-CoV2 in nasopharyngeal swab (NS) [NS-NAAT] or demonstration of serum anti-SARS-CoV2 IgG or IgA antibodies were included. The CSF from subjects with COVID-19 was compared with CSF controls from non-COVID-19 subjects with neurological infections, neuroinflammatory disorders and stroke to establish immune profiles which may allow the recognition of common pathogenic pathways. The CSF control samples, collected before the COVID-19 pandemic, were derived from newly diagnosed, treatment naïve and well characterized subjects available at the Johns Hopkins Division of Neuroimmunology and Neuroinfectious Diseases-CSF Biorepository. The control samples were stored at −80C for variable period prior to analyses (6–72 months). CSF disease-control samples fit the criteria previously established for each of the selected disorders and were selected with the best attempt to match by age (+/− 5 years) the COVID-19 CSF samples. CSF control samples included: 1) healthy controls, derived from subjects with normal neurological examination and normal brain MRI who underwent evaluation for headaches or pseudotumor cerebri ($n = 14$); 2) acute infectious meningitis ($n = 12$), 3) acute viral encephalitis e.g., herpes simplex, varicella-zoster encephalitis ($n = 11$) [27], 4) autoimmune encephalitis ($n = 14$) [28], 5) NMO (n = 11) [29], 6) neurosarcoidosis ($n = 12$) [30], and 7) stroke ($n = 8$), which included subjects with ischemic stroke [31] preceding the COVID-19 period.

2.2. Clinical definitions for COVID-19 group

Neurological manifestations in COVID-19 were categorized in three diagnostic groups: stroke, encephalopathies and headaches/others. COVID-19 stroke cases included subjects with ischemic stroke from intracranial atherosclerosis, cardioembolic, small vessel disease and other causes [31] and/or hemorrhagic stroke including intracerebral and subarachnoid hemorrhages confirmed by clinical and neuroimaging assessment. COVID-19 encephalopathy diagnosis included subjects with diffuse neurological dysfunction with altered consciousness with change in cognition and/or with a perceptual disturbance not better accounted for by a pre-existing or evolving chronic dementia [32] or sedation without evidence of stroke. Subjects with headache without mental status changes, with or without cranial nerve involvement without evidence of stroke or other structural lesions were classified in the group of headaches/other. COVID-19 disease severity was based on National Institutes of Health (NIH) Guidelines [33] and defined as follows: 1) Critical illness: respiratory failure, septic shock, and/or multiple organ dysfunction 2) Severe illness: respiratory frequency > 30 breaths per minute, oxygen saturation (SaO2) $\leq 93\%$ on room air at sea level, a ratio of arterial partial pressure of oxygen to fraction of inspired oxygen (PaO_2/FiO_2) < 300 mm Hg or lung infiltrates $> 50\%$; 3) Moderate illness: Evidence of lower respiratory disease by clinical assessment or imaging and SaO2 $> 93\%$ on room air at sea level and 4) Mild Illness: Individuals who had any of various signs and symptoms (e.g., fever, cough, sore throat, malaise, headache, muscle pain) without shortness of breath, dyspnea, or abnormal imaging.

CSF pleocytosis was defined as >5 leucocytes/μL, elevated CSF protein was defined as >45 mg/dL, a normal IgG index was considered to be <0.7 mg/dL, and a normal *CSF/serum albumin ratio* (Qalb) as <9 We determined the presence of COVID-19 hyperinflammatory syndrome (C-HIS), known to have the immune features of "cytokine storm", based on the clinical profile and combination of markers of systemic inflammation (e.g., ferritin, D-dimer, CRP and IL-6) [34]. To determine the effect of time to CSF sampling as related with period of infection, two groups were established: An "early" CSF collection group for samples obtained within 8 days of the first positive NS-NAAT, and a "late" CSF collection group for samples obtained 9 days or after [35].

2.3. Laboratory studies

2.3.1. SARS-CoV2 virus and anti-SARS-CoV2 antibody detection in CSF

NAAT of SARS-CoV2 RNA in CSF was performed by RT-PCR. Two regions of the nucleocapsid (N) gene (N1 and N2) were used as assay targets per the FDA Emergency Use Authorization package insert (https://www.fda.gov/media/134922/download). ddPCR was used to confirm the results on a subset of the specimens (https://www.fda.gov/media/137579/download). The human RNase P gene (RP) was the internal control for both assays [36]. Quantification of anti-SARS-CoV2 IgG and IgA antibodies used a previously validated ELISA kit (Euroimmune, Germany) [37] which identify antibodies against subunit 1 of the trimeric SARS-CoV2 spike protein. The cutoff for positivity was 1.23 units for IgG and 5 units for IgA as established previously (64).

2.3.2. Cytokine profiling

To establish the role of cytokines in pathogenesis of COVID-19 neurological complications, we determined the CSF concentrations of selected cytokines IL-6, TNFα, IFNγ, IL-10, IL12p70 and IL17A described to be involved in severe and critical COVID-19 and the so-called "cytokine storm" [38–41]. Quantification of the cytokines was performed using the Simoa™. Cytokine 6-plex panel array assay using a Quanterix HD-X ® analyzer. CSF from COVID-19 and controls subjects were tested simultaneously.

2.3.3. Assessment of neuronal injury, acute phase reactants and coagulation markers

Quantification of neurofilament light chain (NF-L) in CSF, a marker of neuroaxonal damage [42], was used as indicator of neuronal injury in COVID-19 and control subjects. CSF NFL was measured simultaneously

in both COVID-19 and control samples using the Simoa™ NF-Light Kit (Quanterix Corporation, Lexington, MA, USA) on the Quanterix HD-X® platform. Acute phase reactants such as ferritin, C-reactive protein (CRP) and coagulation markers including D-dimer, fibrinogen and factor VIII, markers associated with disease severity in COVID-19 [43–46] were also evaluated in CSF of COVID-19 and control subjects. Ferritin and hsCRP were measured on Roche Diagnostics Cobas c 701 and e 801 analyzers, respectively. Fibrinogen quantification used a clot-based assay (Siemens, Marburg Germany). D-dimer was measured by an immunoturbidimetric assay (Innovance D-Dimer, Siemens, Marburg, Germany). Factor VIII assessment used a chromogenic Assay (Chromogenix, Bedford, MA).

2.4. Statistics

Continuous variables were described using medians and interquartile ranges, while categorical features with percentages. Planned comparisons between COVID-19 and control groups were performed using Mann-Whitney test. All 3 COVID-19 diagnostic categories were compared with each control group and with each other. For analysis of cytokines, values with a coefficient of variation higher than 30% were disregarded. Missing concentration values below the lower limit of detection were calculated by dividing the lower limit of quantification (LLOQ) corresponding for each cytokine by the square root of 2. Spearman's correlation coefficient (Rho; ρ) was evaluated as well for relating NF-light concentrations with the other immunomarkers. Significant p values were set below 0.05. We specified our primary analyses as global tests comparing COVID-19 groups versus healthy and neurologic disease controls, and we considered our study to be exploratory in nature. As a result, we did not adjust for multiple comparisons. Analytes other than cytokines were analyzed with the obtained raw data. Statistical analysis was performed in Stata v.14. (StataCorp, Texas, USA).

2.5. Study approval

This study was approved by the Johns Hopkins Institutional Review Board (IRB) for longitudinal acquisition of clinical and biological samples in patients with neurological disorders. An informed consent was obtained from each patient or next-of-kin representative.

2.6. Data availability

All data reported within the article are available anonymized on reasonable request by qualified investigators.

3. Results

3.1. Patient clinical characteristics

Eighteen subjects with COVID-19 and neurological symptoms were included in this study. The diagnosis of COVID-19 was established by NS-NAAT in 16 patients, and two patients were diagnosed based on positive serum anti-SARS-CoV2 IgG and IgA antibodies. The NS-NAAT CT value, a presumptive indicator of the magnitude of viral infection [47,48], was established in 12 patients, 7 of them with CT < 30. Of the 18 COVID-19 subjects, 7 were categorized as stroke (39%), 6 as encephalopathy (33%) and 5 as headaches/others (28%). The clinical and neuroimaging features, and systemic inflammatory markers for all COVID-19 subjects are described in Table 1. The temporal profile of infection and neurological symptoms, the time of diagnosis by NS-NAAT, CSF sampling as related with onset of systemic and neurological symptoms and clinical events are outlined in Fig. 1. The median age of the patients was 56 years (IQR 32–69). Ten patients were male (56%). Eight (44%) patients were classified with critical illness, 5 (28%) with severe illness, 4 (22%) with moderate illness and 1 with mild illness. Overall, the median time from onset of COVID-19 to neurological symptoms was 0.5 days (IQR 0–10.5). In nine patients (50%), neurological manifestations were part of the initial clinical presentation of COVID-19 symptoms (3 stroke, 4 encephalopathy and 2 headache). Of the 18 patients included in our cohort of COVID-19 subjects, only 2 subjects (1 and 2, Table 1) had received steroids during the 5 days preceding the lumbar puncture. None of the COVID-19 patients received any experimental drugs, antivirals or neutralizing antibodies during the period prior to CSF collection. Four (22%) of the 18 patients died, while 13 (72%) improved. Six of 18 (33%) patients had 3 or more comorbidities while 9 (66%) had at least one comorbidity. Fourteen healthy controls were included. The mean age for the healthy controls ($N = 14$) was 67.5 years, 57% were male. Disease controls included acute infectious meningitis ($n = 12$), acute viral encephalitis ($n = 11$), autoimmune encephalitis ($n = 14$), NMO (n = 11), neurosarcoidosis (n = 12) and stroke ($n = 8$). The age and sex distribution and CSF features for all control groups are described in Table A1.

3.2. CSF characteristics

CSF features for the COVID-19 subjects are summarized in Table 2. The CSF was collected within 8 days of the first positive NS-NAAT ("early" CSF collection group) in 8 patients (44%, median 4 (IQR 1–6) while other 10 patients had a CSF collection 9 days or more after COVID-19 diagnosis ("late" CSF collection group (56%, median 20 (IQR 13–27). There was no evidence of CSF pleocytosis except in 4 subjects. In 8 subjects, the CSF protein was elevated. Four of the subjects with increase protein and pleocytosis had blood contamination in the CSF (Table 2). The CSF IgG index and CSF/albumin ratio (Qalb) were within normal range in 7 patients where these indexes were tested. No evidence of oligoclonal bands (OCBs) in CSF or corresponding serum was found in 5 subjects where this test was obtained.

3.3. SARS-CoV2 testing in CSF

None of the 18 CSF samples from COVID-19 was positive for SARS-CoV2 RNA by RT-PCR. A subset of 7 CSF samples were also tested with Reverse Transcription Droplet Digital PCR (ddPCR) with negative results. We also determined the presence of anti-SARS-CoV2 IgG and IgA antibodies against subunit 1 of the trimeric SARS-CoV2 spike protein. IgG antibodies to SARS-CoV2 spike protein were detected in 13 of 17 (77%) CSF tested while the anti-SARS-CoV2 IgA antibody was detected in 4 of those CSF samples (Table 2, appendix table A-2 and fig. A-1). The titer of the IgG antibody did not correlate with the period between the onset of COVID-19 symptoms and CSF sampling ($p = 0.53$) or period between NS-NAAT diagnosis and CSF sampling ($p = 0.45$). The presence of IgG antibodies to SARS-CoV2 in CSF was observed in all COVID-19 diagnostic groups or disease severity categories. In 10/13 subjects with positive IgG antibodies there was no pleocytosis and in 7/13 the protein levels were normal. The 4 subjects with pleocytosis and 5 of the subjects with elevated protein had RBC > 50. None of the 20 control samples tested for COVID-19 antibodies were positive.

3.4. Laboratory findings in CSF of COVID-19 and control groups

The description of CSF analytes in the COVID-19 diagnostic groups and control groups are included in Table 3. Comparative analysis and statistical outcomes for all CSF analytes is shown in Fig. 2. A representative heat map of the P value significance for all analyte comparisons between the COVID-19 diagnostic categories, disease severity and timing of CSF collection with the control groups are described in Fig. 3. No significant differences in the CSF WCC and protein concentrations between the three diagnostic categories of the COVID-19 neurological problems were found. As compared with healthy controls, there were no significant differences in the WCC and protein concentration with exception of the COVID-19 headache group which had a significantly lower protein concentration. Overall, the WCC and protein

Table 1
Clinical characteristics of subjects with COVID-19 neurological manifestations.

ID #	Age range Sex	COVID Severity/C/HIS[A]	Co-morbidities	Neuro symptoms onset[B] days	Initial neuro symptoms	Neuroimaging findings by MRI /HCT scan	Neurological diagnosis	NS RT-PCR CT value	Serum CRP[C] mg/dL	Serum ferritin[C] ng/mL	Serum D-dimer[C] mg/L	Serum IL-6 [C] pg/mL	Outcome
1	40–49 M	Critical	Hypertension	0	AMS	SAH ACA aneurysm	Stroke-SAH	Negative[D]	NA	NA	NA	NA	Death
2	20–29 F	Critical	Obesity	0	AMS Seizure	Multiple ischemic strokes	Stroke- Ischemic Hypoxic brain injury	Positive[E]	12.5	392	4.2	41	Improved
3	50–59 M	Critical	Hypertension	0	AMS Headache	SAH/ DSA: Normal	Stroke-SAH	Negative[D]	5.6	431	3.3	135	Improved
4	70–79 F	Critical/C-HIS	Hypertension Diabetes Stroke Epilepsy Dementia	0	AMS Seizure	Old occipital stroke	Encephalopathy Known Epilepsy	31.43	11.2	1364	4.5	108	Death
5	50–59 M	Critical/C-HIS	Hypertension Diabetes Obesity	15	AMS	Cerebellar stroke	Stroke-Ischemic	28.1	7.4	1338	4.9	86	Improved
6	60–69 M	Critical/C-HIS	Hypertension Diabetes Parkinson dis. Neurosyphilis	4	AMS	Normal	Encephalopathy	Positive[E]	33.7	1159	5.6	503	Death
7	60–69 M	Critical/C-HIS	Hypertension	8	Headache Tremor	Stroke MCA, MCA stenosis	Stroke- Ischemic	25.1	34.2	1616	4.7	944	Death
8	30–39 F	Critical	Obesity	10	Headache Diplopia Anosmia Ageusia	Normal	Pseudotumor cerebri	38.2	8.0	115	5.2	144	Improved
9	60–69 M	Severe	Hypertension Diabetes Atrial fibrillation	1	Headache AMS Seizure Ophthalmo-paresis	Multiple ischemic strokes ICH	Stroke- Ischemic ICH	31.1	10.0	831	1.7	10.5	Improved
10	20–29 F	Severe	Ovarian teratoma	0	Headache Anosmia Facial palsy	Normal	Bell's palsy	Positive[E]	11.7	123	0.6	4.5	Improved
11	30–39 M	Severe	Hepatitis B	0	Headache	Normal	Headache of systemic illness	17.7	9.75	887	0.7	37	Improved
12	70–79 F	Severe/C-HIS	None	0	AMS Apraxia	Global brain Atrophy	Encephalopathy	27.4	20.8	1074	3.0	112	Improved
13	60–69 M	Severe/C-HIS	Dementia Sickle cell disease Renal transplant	0	AMS Anosmia Ageusia	Normal	Encephalopathy	30.1	246.9	7879	9.8	490	Improved
14	30–39 F	Moderate	Obesity	30	Headache Blurry vision Anosmia	Non-specific white matter changes	Pseudotumor cerebri	29.4	NA	NA	NA	NA	Improved
15	70–79 M	Moderate	Hypertension Prostate Cancer	0	AMS Cognitive decline	Bilateral pontine and thalamic T2W hyperintensities	Encephalopathy	Positive[E]	0.1	132	5.3	4.1	Cognitive sequela
16	30–39 F	Moderate	Obesity	12	Delusions Paranoia	Normal	Encephalopathy Bipolar disorder	30.6	1.6	75	1.0	NA	Improved
17	70–79 M	Moderate	Hypertension Diabetes Stroke Dementia	3	AMS	Stroke, putamen	Stroke- Ischemic Encephalopathy	19.9	6.0	551	7.2	163	Improved

(*continued on next page*)

Table 1 (*continued*)

ID #	Age range Sex	COVID Severity/C/HIS[A]	Co-morbidities	Neuro symptoms onset[B] days	Initial neuro symptoms	Neuroimaging findings by MRI /HCT scan	Neurological diagnosis	NS RT-PCR CT value	Serum CRP[C] mg/dL	Serum ferritin[C] ng/mL	Serum D-dimer[C] mg/L	Serum IL-6 [C] pg/mL	Outcome
18	20–29 F	Mild	None	14	Headache Anosmia Ageusia	Normal	Migraine	19.0	NA	NA	NA	NA	Improved

Abbreviations: AMS: altered mental status. HCT: head computed tomography. ICH: intracerebral hemorrhage. MRI: magnetic resonance imaging. ACA: anterior cerebral artery. MCA: middle cerebral artery. SAH: subarachnoid hemorrhage.

[A] COVID-19 severity based on NIH guidelines [33]. COVID-19-hyperinflammatory syndrome (C-HIS) classification was based on clinical and laboratory criteria [34].

[B] Time of onset of neurological symptoms as related with the presumed onset of systemic COVID-19 symptoms. 0 days denotes neurological symptoms were part of onset of COVID-19 symptoms.

[C] Maximum serum value during hospitalization.

[D] COVID-19 diagnosis was based on positive serum SARS-CoV2 IgG/ IgA antibody.

[E] CT value not available.

concentrations were lower in COVID-19 cases as compared with neuroinflammatory controls. No significant differences were seen between COVID-19 and non-COVID-19 stroke cases.

3.4.1. *Markers of neuroaxonal degeneration*

When comparing based on severity, CSF NF-L levels were significantly elevated in the critical COVID-19 group compared to the other severity categories. CSF concentrations of NF-L were also markedly elevated in the COVID-19 stroke group as compared with healthy controls ($P \leq 0.001$) and the COVID-19 headache group ($P \leq 0.01$). Although the COVID-19 encephalopathy group had an elevated median NF-L concentration of 8408 pg/mL, compared to 1105 pg/mL of the healthy control group, this difference was not statistically significant. As expected, NF-L concentrations were significantly elevated in the acute encephalitis, autoimmune encephalitis and NMO controls groups as compared with COVID-19 encephalopathy. However, the concentrations of NF-L were equivalent in the COVID-19 stroke group and the control stroke group.

3.4.2. *Cytokine profiles*

The concentrations, comparative analysis and statistical significance between COVID-19 groups and controls for all 6 cytokines analyzed are described in Table 3, and Figs. 2 and 3. Analysis of cytokines levels within the COVID-19 diagnostic categories showed that only TNFα in the COVID-19 stroke group was significantly increased compared to the headache group ($P = 0.03$). When the COVID-19 diagnostic groups were compared with controls, the COVID-19 stroke group had significant elevated concentrations of IL-6, TNFα, IL-10 and IL-12p70 as compared with the healthy control group. None of the COVID-19 diagnostic groups, including the stroke group, showed any significant increase of cytokines as compared with neuroinflammatory or non-COVID-19 stroke control groups. Instead, significant increased concentrations of selected cytokines such as TNFα and IFNγ were noted in neuroinflammatory groups such as acute meningitis and encephalitis as compared with the COVID-19 stroke and encephalopathy groups. IFNγ and IL-17A concentrations were reduced in COVID-19CSF as compared with inflammatory control groups such as acute meningitis, encephalitis and neurosarcoidosis (Fig. 3A). To determine whether such pattern of cytokines was specific to the COVID-19 stroke group, we analyzed separately the cytokine profiles of the non-COVID-19 stroke group with other control groups. We found the non-COVID-19 stroke group had significant increases in the concentrations of IL-6, TNFα, IL-10 and IL-12p70 as compared with the healthy control group, and reduced levels of IFNγ and IL-17A as compared with acute meningitis groups (Fig. 3D), a profile similar to the one observed in the COVID-19 stroke group. Cytokines profiles as related with COVID-19 disease severity and timing of CSF collection are summarized in the statistical significance heatmap illustrated in Fig. 3. When COVID-19 subjects were categorized by disease severity, the critical illness group ($n = 8$) had significantly increased levels of IL-10 and IL-12p70 when compared with healthy controls (Fig. 3B). The timing of CSF collection did not show any significant effect on the profile of COVID-19 cytokines with exception of an increased IL-12p70 in the "early" COVID-19 CSF sampling group as compared with healthy controls (Fig. 3C). Six patients (33%) were diagnosed with systemic features of C-HIS [34] of whom four had encephalopathy. Analysis of the COVID-19 subjects with, which is characterized by marked systemic inflammatory response or "cytokine storm" [34], showed that CSF cytokine levels did not have any significant differences with the healthy control group. However, an analysis within the COVID-19 group showed levels of IL-6 and IL-10 were significantly elevated ($P = 0.02$ and 0.01 respectively) while concentrations of INFγ and IL-12p70 were significantly lower ($P = 0.04$ for both cytokines) in the COVID-19 C-HIS cases ($n = 6$) when compared with other non-H-CIS COVID-19 cases ($n = 12$). The concentration of IL-6 in the CSF of COVID-19 cases did not correlate with the corresponding serum IL-6 ($P = 0.27$). The effect of specific treatments (e.g., steroids,

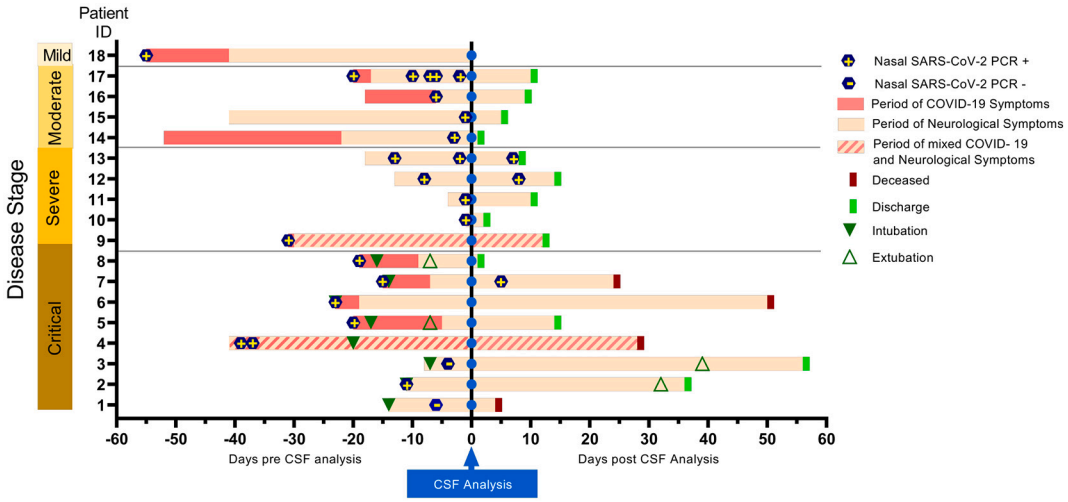

Fig. 1. Timeline of clinical features in patients with COVID-19 with neurological complications.
Temporal profile of COVID-19 and neurological symptoms as related with the time of CSF analysis (vertical blue line) for the 18 subjects included in the study. Patients were classified based on the NIH disease severity classification [33]. Eight subjects presented with neurological symptoms as the first manifestation of COVID-19 (light pink bar), eight exhibited systemic illness symptoms preceding neurological symptoms (dark pink bar) and two presented with mixed neurological and systemic symptoms (diagonal stripes).

antivirals) on CSF inflammatory markers was also evaluated. Of the 18 patients included in our cohort of COVID-19 subjects, only 2 subjects (1 and 2, Table 1) had received steroids during the 5 days preceding the lumbar puncture. None of the COVID-19 patients received any of the experimental drugs used during the period prior to CSF collection.

3.4.3. Acute phase reactants and coagulation markers

Using a high-sensitive CRP (hsCRP) assay, we found that CRP was present almost exclusively in the CSF of COVID-19 subjects as it was detected in 7/18 subjects, 4 COVID-19 encephalopathy and 3 COVID-19 stroke, while only one CSF (from an autoimmune encephalitis subject) of the 82 CSF controls had detectable hsCRP ($P = 0.001$). CSF hsCRP levels strongly correlated with CRP serum levels (P = 0.001, Spearman's ρ). CSF hsCRP was present only in critical or severe COVID-19 subjects and was elevated in 5 of 6 subjects with COVID-19C-HIS. In contrast, while the CSF ferritin had a 100% detection rate in the CSF of all COVID-19 and comparison groups (Table 3), there was not significant difference between the concentrations of CSF ferritin when COVID-19 diagnostic groups were compared with healthy, neuroinflammatory or stroke controls. However, an analysis within the COVID-19 diagnostic categories showed the stroke group had significantly elevated levels of CSF ferritin as compared with the headache group ($P = 0.04$). Analysis of COVID-19 categorized by disease severity showed that CSF ferritin levels in severe COVID-19 subjects were significantly increased as compared with healthy controls and NMO cases (Fig. 3). Similar findings were observed in the "late" CSF collection group. However, those observations were likely biased by the inclusion of cases of subarachnoid hemorrhage and ICH (e.g., cases 1,3 and 9), clinical situations well known for an increased CSF ferritin [49]. Importantly, CSF ferritin levels did not parallel serum ferritin levels (r 0.206, $P = 0.46$), one of the most important markers of systemic immune activation in COVID-19 [46,50]. Among markers of coagulation, CSF D-dimer was present in 4 COVID-19 stroke subjects and in 25 of the CSF controls ($P = 0.06$). CSF D-dimer was not significantly different between COVID-19 groups and healthy controls. Instead, CSF D-dimer in cases of acute meningitis and neurosarcoidosis was significantly increased as compared with COVID-19 encephalopathy cases. Markers of coagulation such as fibrinogen and Factor VIII were undetectable in the CSF of COVID-19 subjects and comparison controls.

4. Discussion

Our study reveals a paucity of neuroinflammatory changes in the CSF of COVID-19 patients with neurological complications as reflected by the lack of specific increases in CSF pro-inflammatory cytokines or markers of systemic inflammation such as IL-6, ferritin, or D-dimer as typically seen in serum of COVID-19 patients. These findings paralleled a lack of cellular responses like pleocytosis or other markers of immunological activity within the CNS such as increase in IgG index or OCBs. In 4 subjects, pleocytosis and elevated protein was seen likely due to cross contamination with blood rather than a primary neuroinflammatory process. The absence of meaningful neuroinflammatory changes in the CSF of COVID-19 cases is further demonstrated when it is compared to control CSF from acute infectious or autoimmune neuroinflammatory pathologies that show significantly greater inflammatory changes. The lack of CSF pleocytosis in COVID-19 subjects, normal protein, and absence of abnormalities in IgG index or Q(Alb), concurs with other studies [16,18–20]. Furthermore, our case-control study approach of CSF immune markers showed that in subjects with COVID-19 who experienced complications such as stroke or encephalopathy, most of the CSF changes appear to be determined by other pathologies such as ischemic or hypoxic disease likely driven by systemic or vascular factors that influence the development of such brain pathologies rather than primary neuroimmune mediated processes. An important caveat is that we did not test the full spectrum of reported neurologic complications in COVID-19 such as multiple cranial neuropathies, ADEM, or GBS.

We failed to detect SARS-CoV2 viral RNA in the CSF of all COVID-19 subjects examined, concurring with other studies [16,18–21]. The lack of SARS-CoV2 RNA in the CSF may be interpreted as lack of neuroinvasiveness, absence of active viral replication or simply a relatively low viral trafficking into the CNS. Although the detection of RNA viruses in CSF has been historically challenging in some viral disorders of the CNS [51], the absence of viral RNA along with the lack of pleocytosis and other inflammatory changes in the CSF of COVID-19 patients supports the conclusion that there is not an active trafficking of SARS-CoV2 into the CNS causing neuroinflammation. This distinguishes it from other RNA viruses like poliomyelitis, enterovirus, West-Nile virus that are difficult to detect but produce blatant signs of neuroinflammation in the CSF [51,52]. Interestingly, a noteworthy observation in our study is a high frequency (77%) of SARS-CoV2 spike IgG antibodies in the CSF of COVID-19 cases. Given the absence of viral RNA in the CSF, the lack of

Table 2
Cerebrospinal fluid neuroinflammatory markers in COVID-19 subjects.

ID #	COVID-19 diagnostic group	+NS NAAT to LP days	CSF WCC cell/mL	CSF RBC cell/mL	CSF Protein mg/dL	IgG index	Q Alb	Anti-SARS-Cov2 IgG	Anti-SARS-Cov2 IgA	IL-6 pg/mL	IFNγ pg/mL	TNFα pg/mL	IL10 pg/mL	IL12-p70 pg/mL	IL17A pg/mL	NF-L ng/mL	hsCRP mg/L	Ferritin ng/mL	D-Dimer mg/L
1	Stroke/SAH	NA	313	152,000	56	NA	NA	Pos	Pos	0.19	0.20	0.28	0.12	0.14	0.10	81,933	1.1	3104	2.44
2	Stroke/ Ischemic	11	0	15	5.5	NA	NA	Neg	Neg	17.85	0.04	0.39	0.35	0.12	0.01	22,478	0.2	20.1	0.55
3	Stroke/SAH	NA	5	2000	85	NA	NA	NA	NA	1065.57	0.26	1.12	1.02	0.57	0.09	14,674	0.2	516.0	5.91
5	Stroke/ Ischemic[A]	20	18	6000	277	NA	NA	Pos	Pos	17.92	NA	18.65	0.63	0.15	0.13	8774	NA	NA	5.03
7	Stroke/ Ischemic[A]	15	1	0	26	0.55[B]	3.65	Pos	Neg	139.06	0.11	0.40	0.59	0.45	0.02	80,296	0.8	11.3	0.19
9	Stroke/ Ischemic and ICH	21	56	2000	63	NA	NA	Pos	Neg	41.80	0.07	0.66	NA	NA	0.01	142,179	0.6	88.9	0.16
4	Encephalopathy[A]	9	1	9	54	NA	NA	Pos	Neg	14.29	0.02	0.32	0.37	0.16	0.01	18,333	0.7	14.6	0.19
6	Encephalopathy[A]	23	25	1000	31	NA	NA	Pos	Neg	9.80	NA	0.14	NA	NA	0.02	13,361	0.3	11.7	0.19
12	Encephalopathy[A]	8	0	0	52	NA	NA	Pos	Neg	31.86	0.02	0.34	0.54	0.17	0.01	3456	0.3	16.0	0.19
13	Encephalopathy[A]	2	1	99	56	0.62[B]	7.95	Pos	Neg	7.55	0.01	0.30	0.22	0.07	0.00	18,303	0.5	26.2	0.19
15	Encephalopathy	1	1	0	31	0.43[B]	3.84	Pos	Neg	2.41	0.01	0.12	0.07	0.03	0.00	1400	0.2	7.4	0.19
16	Encephalopathy	6	1	160	62	0.72[B]	8.88	Pos	Pos	2.24	0.20	0.09	0.07	0.04	0.01	446	0.2	6.3	0.19
17	Encephalopathy	2	3	0	30	0.38	4.53	Pos	Neg	6.69	0.01	0.24	0.17	NA	0.00	2333	0.2	5.2	0.19
8	Headache/other	19	1	109	23	NA	NA	Pos	Pos	3.21	0.20	0.15	NA	0.02	0.10	1596	0.2	6.3	0.19
10	Headache/ other	1	3	0	33	NA	NA	Neg	Neg	6.89	0.08	0.28	0.24	NA	0.00	293	0.2	8.0	0.19
11	Headache/ other	1	0	1	35	NA	NA	Neg	Neg	3.10	0.20	NA	0.10	0.02	0.10	178	0.2	8.4	0.19
14	Headache/ other	3	3	0	27	0.52[B]	5.06	Pos	Neg	2.87	0.02	0.08	0.08	NA	0.10	8541	0.2	4.4	0.19
18	Headache/ other	55	1	27	27	0.50	4.02	Neg	Neg	1.44	0.20	0.28	0.05	0.14	0.10	428	0.2	10.9	0.19

[A] Diagnostic group associated with COVID-19 hyperinflammatory syndrome (C-HIS) [34].

[B] Oligoclonal bands tested negative.

pleocytosis which may facilitate B-cells trafficking into the CNS, and absence of intrathecal IgG production (e.g., IgG index, OCBs), CSF antibodies to SARS-CoV2 likely originate from serum and then transfer into the CNS despite an otherwise intact blood-CSF barrier, as occurs in other CNS pathologies [53]. Alternatively, stroke or ischemic changes may have altered the CSF-blood brain barrier to facilitate permeability of IgG antibodies. Presence of SARS-CoV2 antibodies in CSF has been also reported by previous studies which raises the possibility that they are directly pathogenic in the neurological complications of COVID-19 [22,54]. The role of SARS-CoV2 antibodies in the CSF remains uncertain but future studies looking for sites of SARS-CoV2 antibody cross reactivity in the CNS, potential long-term neurological effects such as the so-called "long term haulers" [55], post-COVID-19 conditions or pathological consequences in animal models, would be helpful to clarify this question.

Notably, our study showed an impressive lack of pro-inflammatory cytokines in the CSF of subjects with COVID-19 neurological problems. With the exception of COVID-19 stroke cases, COVID-19 encephalopathy or headache cases did not show a noticeable pro-inflammatory cytokine response in the CSF as compared with controls. This observation suggests that local increases of pro-inflammatory cytokines are unlikely the pathogenic factors associated with the neurological symptoms observed in COVID-19 encephalopathy or headache. Only the CSF of the COVID-19 stroke group appeared to have significant increase in IL-6, α, IL10 and IL-12p70 as compared with the healthy control group and the COVID-19 headache group. However, these cytokine increases were largely equivalent in the non-COVID-19 stroke controls, suggesting that the cytokine increases in the brain of COVID-19 stroke subjects are likely driven by stroke and ischemic pathology [56] rather than specific neuroinflammatory changes associated with COVID-19. Furthermore, CSF from subjects with C-HIS, a condition characterized by marked systemic rise in pro-inflammatory mediators or "cytokine storm" [34], showed not increases in proinflammatory cytokines as compared with healthy controls although increases in IL-6 and IL-10 and lower IFNγ and IL12p70 differentiate them from non-C-HIS subjects. The findings of our study are in contrast with recent studies which suggest neuroinflammation and "cytokine storms" are central to the pathogenesis of some of the neurological complications in COVID-19. A larger study from a Brazil showed that CSF from a subgroup of "inflammatory neurological disease" comprised by 9 subjects with ADEM, encephalitis, meningitis and myelitis exhibited increase of subsets of cytokines including IL-6, IL10 and IL12 as well as chemokines such as CXCL8 (IL8) and CXCL10 [57]. Similarly, a study of 13 "COVID-19 encephalitis" cases found increases in CXCL8 as well as markers of glial activation such as GFAP [58]. Another study of CSF in 18 subjects with cancer who exhibited a variety of neurological manifestations after COVID-19 used target proteomic assays to identify relative increases in subsets of chemokines such as CXCL8, CXCL10, as well as IFNγ and MMP-10, a metalloproteinase associated with neuronal dysfunction [59]. Although suggestive of activation of inflammatory markers, such findings are not necessarily indicative of "cytokine storms" or specific adaptive immune responses within the CNS but rather reflect the pattern of activation and homeostatic neuroglial responses to pathogenic processes such as ischemia, hypoxemia and sepsis [56,60–62].

Our study also demonstrated absent parallel increase in CSF of markers such as IL-6, ferritin, D-dimer or coagulation factors as it has been observed in the serum of COVID-19 patients. A notable exception was the presence of detectable levels of CSF hsCRP in a subset of subjects with critical and severe COVID-19 illness, stroke and encephalopathy, which correlated with the magnitude of corresponding serum increase. It is uncertain if CRP in CSF is actively or passively transported from serum, due to brain endothelial pathology or from brain disease processes, as neurons may have capability to produce such pentraxin [63]. Future studies should focus on determining the role of CRP in CSF, and potential long-term implications in mechanisms of neurodegeneration [63]. Surprisingly, levels of CSF ferritin and D-dimer, showed no

Table 3
Cerebrospinal fluid quantification of neuroinflammatory biomarkers in COVID-19 and control groups.

Analytes Median (IQR)	COVID-19				Comparison control groups						
	All	Stroke	Encephalopathy	Headache	Healthy controls	Acute meningitis	Acute encephalitis	Autoimmune encephalitis	NMO	Neuro-Sarcoidosis	Stroke
	N = 18	*N* = 7	N = 6	*N* = 5	N = 14	N = 12	N = 11	N = 14	N = 11	*N* = 12	N = 8
WCC cell/μL	2 (1–5)	5 (1–56)	1	2 (1–4)	1 (0–2)	65 (20−203)	7 (1−21)	3 (2–7)	9 (2–74)	26 (2−32)	3 (1–27)
Protein mg/dL	34 (29.2–56.8)	56.4 (26.2–85.3)	53 (31.3–56.8)	29.2 (27.5–33)	41.1 (33–2-57)	80.3 (49.5–108.5)	50.8 (34–73)	34.5 (21–43)	38 (27–63)	76.5 (43.8–137.8)	50.5 (30.2–73.2)
NF-L pg/mL	8657 (1400–18,333)	22,477 (8773–81,933)	8408 (1400–18,302)	428 (292–1595)	1105 (750–2291)	6860 (2334–16,425)	20,560 (11243–89,277)	20,914 (2594–29,769)	8812 (1867–11,979)	2697 (1168–9380)	4330 (898–35,388)
IL-6 pg/mL	7.22 (2.8–17.92)	17.92 (6.69–139.06)	8.67 (2.41–14.29)	3.09 (2.86–3.20)	3.31 (2.32–4.67)	44.57 (3.38–165.17)	6.51 (2.75–28.33)	3.26 (2.62–23.52)	4.05 (2.8–61.93)	22.12 (2.35–188.8)	37.13 (6.45–98.51)
TNFα pg/mL	0.27 (0.14–0.39)	0.4 (0.28–1.20)	0.22 (0.12–0.32)	0.21 (0.11–0.28)	0.13 (0.11–0.20)	1.47 (0.77–3.11)	0.72 (0.46–0.82)	0.38 (0.17–0.85)	0.27 (0.13–0.30)	1.68 (0.21–7.23)	0.52 (0.27–0.75)
IFNγ pg/mL	0.08 (0.01–0.20)	0.09 (0.04–0.20)	0.02 (0.01–0.02)	0.20 (0.08–0.20)	0.18 (0.02–0.20)	0.73 (0.20–4.22)	0.25 (0.08–2.06)	0.20 (0.08–0.20)	0.18 (0.02–0.20)	3.04 (0.05–55.49)	0.08 (0.02–0.20)
IL-10 pg/mL	0.21 (0.07–0.54)	0.47 (0.16–0.62)	0.21 (0.07–0.37)	0.09 (0.06–0.17)	0.08 (0.05–0.16)	0.58 (0.45–1.03)	0.30 (0.07–0.87)	0.13 (0.08–0.45)	0.19 (0.07–0.35)	0.44 (0.08–1.29)	0.64 (0.34–0.78)
IL-12p70 pg/mL	0.13 (0.03–0.15)	0.15 (0.14–0.45	0.07 (0.03–0.16)	0.03 (0.02–0.14)	0.03 (0.02–0.04)	0.23 (0.13–0.68)	0.08 (0.02–0.44)	0.08 (0.03–0.20)	0.06 (0.04–0.09)	0.19 (0.05–0.59)	0.24 (0.06–0.25)
IL-17A pg/mL	0.02 (0.01–0.10)	0.02 (0.01–0.10)	0.01 (0.00–0.01)	0.10 (0.10–0.10)	0.10 (0.00–0.10)	0.16 (0.09–1.71)	0.09 (0.02–0.14)	0.10 (0.03–0.10)	0.08 (0.03–0.10)	0.10 (0.05–0.17)	0.03 (0.01–0.07)
Ferritin ng/mL	11.3 (7.4–20.1)	54.5 (11.3–516)	13.1 (7.4–16)	8 (6.3–8.4)	8.8 (7.8–11)	9.4 (6.9–14)	10.7 (5.9–35)	8.4 (4.3–10.7)	7.4 (6.2–9.6)	7.9 (5.5–14)	9 (4.7–46.7)
D-dimer mg/L	0.19	0.55 (0.19–2.44)	0.19	0.19	0.19 (0.19–0.37)	0.24 (0.19–1.4)	0.3 (0.19–1.14)	0.19	0.19 (0.19–0.65)	1.2 (0.33–4.01)	0.32 (0.19–1.44)
D-dimer* >0.19 mg/L	4	4	0	0	3	5	3	1	2	7	4
hsCRP* >0.2 mg/L	7	3	4	0	0	0	0	1	0	0	0

* denotes Categoral variable, number of cases positive above the reference range.

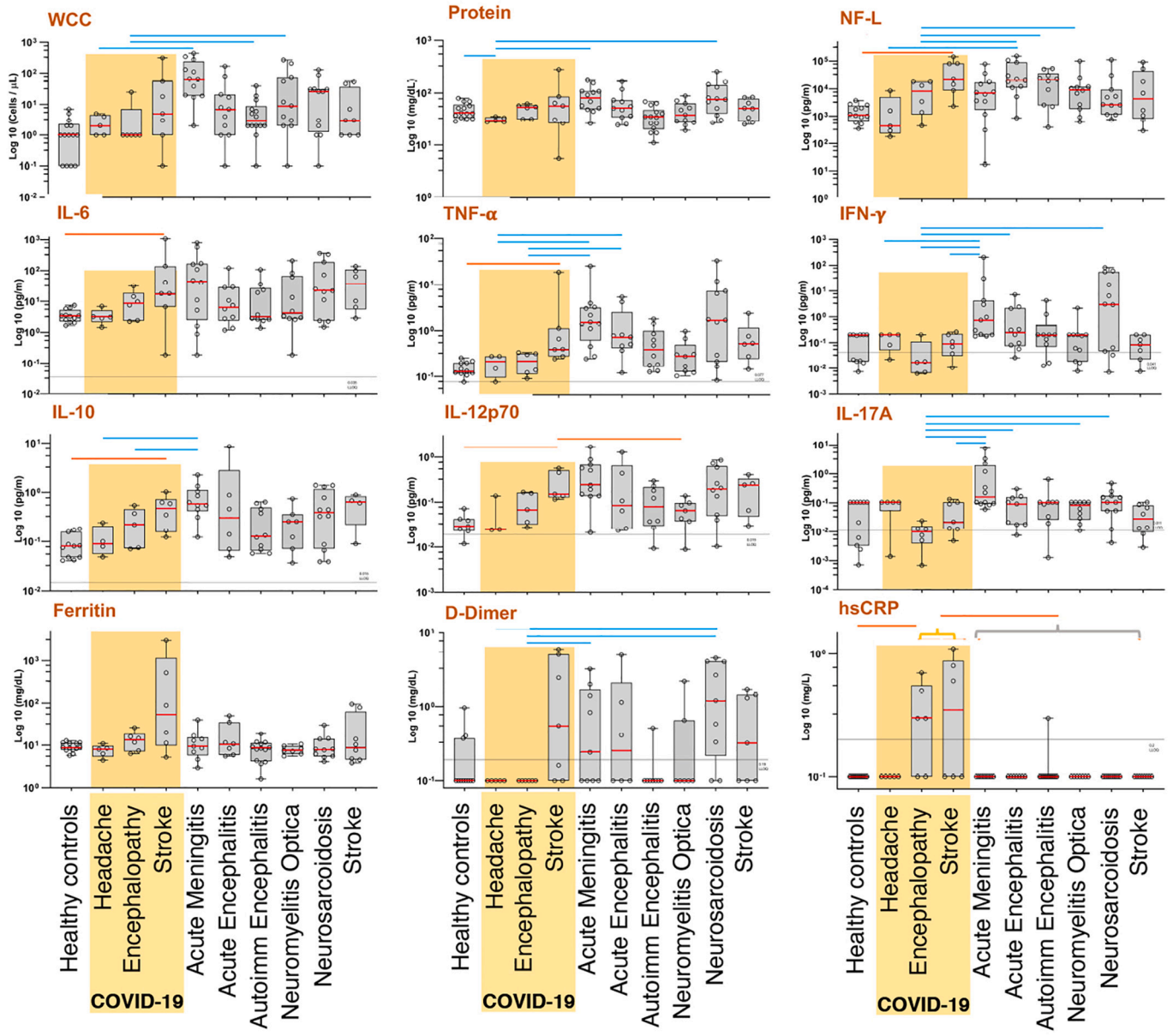

Fig. 2. Profile of CSF inflammatory markers in COVID-19 diagnostic groups and controls.
Profiles of inflammatory markers in the CSF from 18 COVID-19 subjects categorized by diagnosis (yellow box) as compared with healthy controls ($n = 14$), acute meningitis ($n = 12$) and encephalitis (n = 14), autoimmune encephalitis (n = 12), neuromyelitis optica ($n = 11$) and neurosarcoidosis (n = 14) and non-COVID-19 strokes ($n = 8$). Boxes indicate the interquartile range and whiskers show the minimum and maximum values for analytes in each group, and median (red line in box plot). A significant difference ($P < 0.05$) in which the COVID-19 diagnostic group was significantly higher than the control group is denoted as an orange line. A significant difference ($P < 0.05$) when the disease control group was significantly higher than the COVID-19 diagnostic group is denoted by the blue line. Significance for D-dimer and hsCRP was obtained by categorical analysis, present or absent. The significance for hsCRP was represented by the COVID-19 stroke and encephalopathy groups (yellow bracket) vs. the healthy controls and the overall disease control groups (gray bracket).

significant increase and/or did not mirror the marked elevation observed in the serum levels in COVID-19 subjects. Remarkably, standard assays for quantification of fibrinogen and factor VIII in CSF failed to detect such analytes in both COVID-19 and control cases including stroke cases, findings that suggest either the absence of such molecules in the CSF or the lack of sensitivity of the assay for their detection. Importantly, NF-L, a marker of neuroaxonal injury, was increased in stroke and critical COVID-19 cases, and within the COVID-19 cases, it was elevated in stroke and encephalopathy compared to the headache group. Such observation emphasizes the fact that in critical and severe COVID-19 encephalopathy cases, a process of neuronal damage occurs even in absence of neuroimaging evidence of cerebrovascular disease and may suggest that such neuronal damage is associated with ischemia and microvascular disease as it has been demonstrated in neuropathological studies. Such finding also concurs with previous observations of elevation of neuronal and glial proteins in the CSF in critical COVID-19 illness and support the approach for using such proteins as potential biomarkers of disease [64].

5. Study limitations

Although strengths of our study include a comprehensive analysis of markers of disease immunopathogenesis in the CSF from the most common neurological complications in COVID-19 as compared with CSF from controls, few limitations are important to mention. First, we mainly evaluated cytokines and immune factors which were selected based on

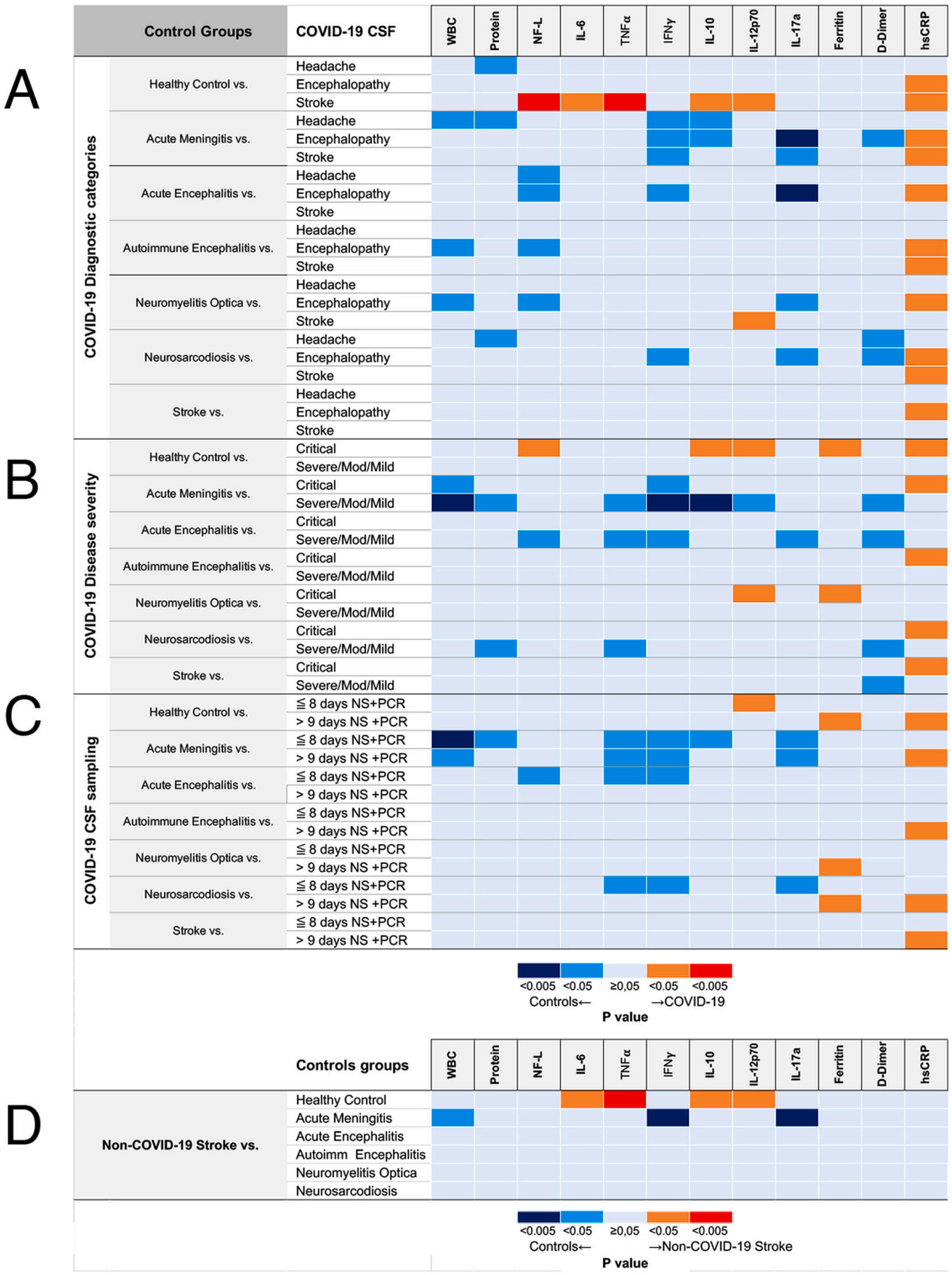

Fig. 3. Heatmap of significance analysis of COVID-19 cases vs. controls.
Heatmap description of the significance $P < 0.05$ and $P < 0.005$ after comparative analysis of (A) COVID-19 CSF diagnostic groups vs. healthy and disease controls, (B) COVID-19 disease severity groups vs. healthy and disease controls, and (C) timing of the CSF sampling in COVID-19 group vs. healthy and disease controls. (D) Heatmap description after exclusion of the COVID-19 group comparing the non-COVID-19 stroke group with other control groups. Analytes that are significantly higher in the COVID-19 group are denoted as orange ($P < 0.05$) or red ($P < 0.005$). Analytes that are significantly higher in the control groups are denoted as light blue ($P < 0.05$) or dark blue ($P < 0.005$) (A, B and C). A heat map in D shows analytes the were significantly elevated in the non-COVID-19 stroke group as compared with controls are shown as orange ($P < 0.05$) or red ($P < 0.005$). Analytes that were significantly higher in the control groups vs. non-COVID-19 stroke group were denoted light blue ($P < 0.05$) or dark blue ($P < 0.005$). Significance for D-dimer and hsCRP was obtained by categorical analysis, present or absent.

their relevance to COVID-19. There was a limitation in studying paired CSF-serum samples for cytokine and antibody profiling. This was a necessary impediment because the limited availability of CSF and blood samples for research purposes during the emergency situation. Second, this study is limited to the clinical experience in a tertiary referral center, a relatively small cohort of patients accrued during a short period of time and a small sample size for the relatively high number of comparisons.

6. Conclusions

Although the prospect of a consensus for common CSF signatures in patients with neurological manifestations of COVID-19 is challenged by the diversity of clinical presentations, patient heterogeneity, overlapping risk factors and co-morbidities, our study has further implications for the understanding of the neuropathogenesis of these neurological complications. The paucity of inflammatory changes in COVID-19 CSF undermines the hypothesis that conventional neuroinflammation, encephalitic processes or SARS-CoV2 neurovirulence play major roles in the pathogenesis of the most common neurological complications in COVID-19 that were studied here. The previously identified "neuroinflammatory" processes in the CSF of COVID-19 [23,24,59,65] or changes described in the so-called "COVID-19 encephalitis" [58] could be derived from homeostatic neuroglial responses by microglia and astroglia to systemic pathology such as ischemia, hypoxia or systemic critical illnesses [56,66–68] rather than adaptive immune mediated, "cytokine storm" or inflammation driven by neurovirulence. Evidence from our study of increases in NF-L further supports the evidence of injury of neuronal cell populations in severe cases of COVID-19. Increase in CSF-CRP in severe cases of COVID-19 neurological complication may suggest other mechanisms including vascular injury may be part of the neuropathogenic processes.

Author contributions

CAP conceived, designed and supervised the overall study. CAP, MAG and PVB designed the overall study, acquired, organized and analyzed data. MAG, GP and KCF analyzed the data and performed statistical analysis. AM, AL, LS, TK, HM and MC conducted laboratory analysis, acquired and analyzed data. All other members of the Hopkins Neuro-COVID-19 Group (appendix A-1) contributed to the acquisition of clinical data by evaluating patients and acquiring biological samples. CAP, MAG and PVB drafted the manuscript, and all authors contributed to the discussion of results, revised and edited the manuscript.

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Declaration of Competing Interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jns.2021.117517.

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