Intrathecal inflammatory responses in the absence of SARS-CoV-2 nucleic acid in the CSF of COVID-19 hospitalized patients

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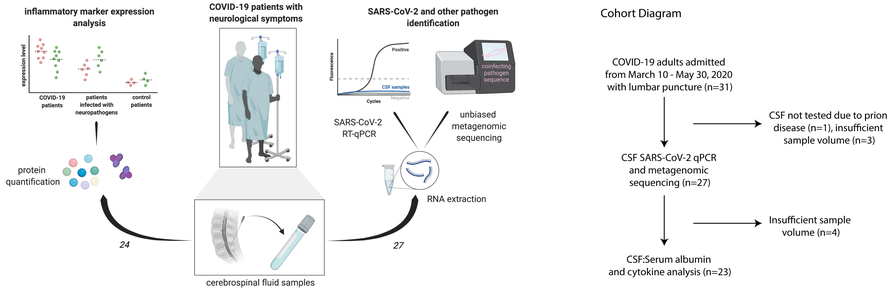
INTRODUCTION

Background: Neurologic symptoms have been described in 30-60% of hospitalized patients with Coronavirus Disease 2019 (COVID-19), but little is known about CSF profiles in these patients.
Objectives: To evaluate hospitalized patients with COVID-19 and neurologic symptoms by determining the CSF viral burden of SARS-CoV-2 and comparing intrathecal inflammatory responses to healthy controls and patients with established neurotropic pathogens

METHODS

Design: A multisite, prospective observational cohort study (MGH and BWH)
Inclusion criteria: Hospitalized COVID-19 patients who underwent a lumbar puncture for neurological symptoms between 03/10/2020 – 05/30/2020



Cohort Diagram

COVID-19 adults admitted from March 10 - May 30, 2020 with lumbar puncture (n=31)

CSF not tested due to prion disease (n=1), insufficient sample volume (n=3)

CSF SARS-CoV-2 qPCR and metagenomic sequencing (n=27)

Insufficient sample volume (n=4)

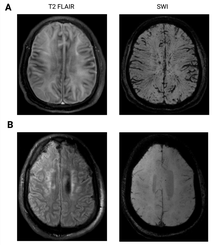
CSF:Serum albumin and cytokine analysis (n=23)

RESULTS

Table 1: Clinical Characteristics of 27 Hospitalized Patients with COVID-19

	Median or n	Range or %
Age	56	24-82
Male:Female	21:6	n/a
WHO Severity Scale at lumbar puncture	3	3-7
Neurologic Syndrome prompting CSF (n=27)		
Encephalopathy / Delirium	16/27	59%
Seizure	5/27	19%
Weakness / Sensory Disturbance	6/27	22%
Anosmia	1/27	4%
Neurological Imaging (MRI, n=25)		
Acute ischemic infarct(s)	3/25	12%
White matter changes likely due to small vessel disease	2/25	8%
Leukoencephalopathy and microhemorrhages	3/25	12%
Enhancing white matter lesions (ADEM)	1/25	4%
Lumbar root enhancement	1/25	4%
SARS-CoV-2 or Immunomodulatory Treatments (n=10)		
Tocilizumab	3/10	30%
Hydroxychloroquine	2/10	20%
Remdesivir	1/10	10%
Inhaled Nitric Oxide	1/10	10%
IVIG/Steroids	5/10	50%

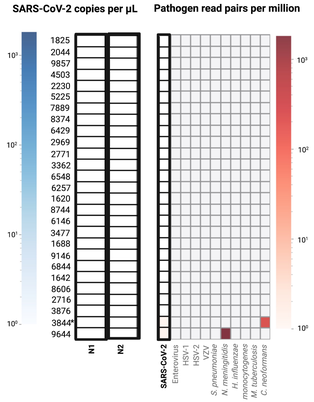
Neuroimaging Findings in Patients with Neurologic Manifestations of COVID-19



A.) 57 year old man with asthma and obesity with anisocoria and loss of brainstem reflexes after COVID-19 hypoxic respiratory failure. Representative images demonstrate extensive FLAIR hyperintense signal abnormalities and numerous microhemmorhages in the supratentorial white matter, including the corpus callosum.

B) 63 year old man with diabetes and obstructive sleep apnea with COVID-19 hypoxic respiratory failure with prolonged encephalopathy. Representative images show targetoid lesions in the supratentorial white matter related to cavitating necrotizing changes.

Detection of pathogens in CSF from patients with COVID-19.

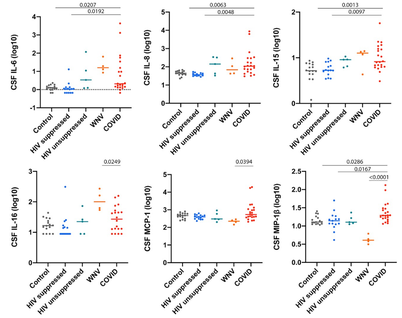


Point 1: Detectable CSF SARS-CoV-2 RNA was absent in all cases despite a range of clinical neurological presentations and evidence of neuroimaging abnormalities, including leukoencephalopathy.

Figure 1: *A) Heatmap depicting the amount of SARS-CoV-2 RNA detected by RT-qPCR (blue) and RNA from SARS-CoV-2 and 10 common respiratory pathogens detected in metagenomic sequencing (red) White boxes represents measured zero while grey boxes represent no measurement*

RESULTS

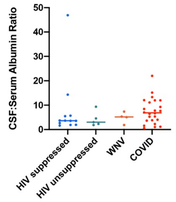
Cytokine and chemokine expression in patients with COVID-19 and other infections and healthy controls.



Point 2: COVID-19 patients had significantly elevated median concentrations of IL-6, IL-15, and MIP-1β, compared to healthy controls. COVID-19 patients had significantly increased levels of IL-6, IL-8, IL-15, and MIP-1β compared to people with suppressed HIV, but not when compared to unsuppressed HIV infection

Figure 2: *Healthy controls (n=16), patients diagnosed with suppressed HIV (n=15), unsuppressed HIV (n=5), WNV (n=4), and COVID-19 (n=21; two patients with confirmed CNS coinfections were omitted). Each figure panel characterizes a unique cytokine. Data points represent measurements for individual patients (horizontal line = mean fluorescent signal).*

QAlb in patients with COVID-19, HIV, and WNV



Point 3: One-third of COVID-19 patients examined (8/23) had high Q-Alb (Q-Alb >9), a marker of blood brain barrier (BBB) breakdown, while BBB breakdown was observed in only 3 patients with established neurotropic infections (HIV-suppressed (n=2) and HIV unsuppressed (n=1)). No WNV patients had Q-Alb >9.

Figure 3: *= For four cohorts (x-axis), including patients with suppressed HIV (n=15), unsuppressed HIV (n=5), WNV (n=4), and COVID-19 (n=23), the CSF:serum albumin ratio (Q-Alb) (y-axis) for each patient displayed. One patient with suppressed HIV (Q-Alb = 47) had VZV coinfection. The mean Q-alb for each cohort is represented by a horizontal line.*

Point 4: Correlations between all cytokines and chemokines and Q-Alb was moderate to strong, and intrathecal inflammatory profiles were intercorrelated within patients and clustered among those with potential BBB breakdown.

Correlation between Q-Alb and cytokine or chemokine expression in COVID-19 patients

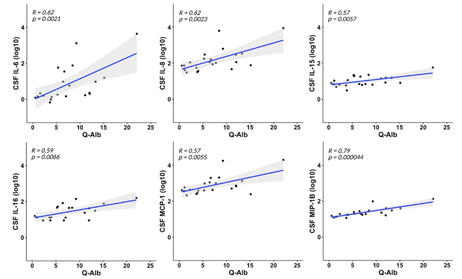


Figure 4: *Each figure panel characterizes a unique cytokine, with fluorescent signal (log base 10) representing cytokine expression. Data points represent cytokine expression (y-axis) and Q-alb (x-axis) measurements for individual patients. For each unique cytokine, the regression line is shown in blue and 95% confidence interval is shaded in grey; Pearson correlation coefficient (R) and significance (p) are also displayed.*

Interrelationships between BBB permeability and CSF cytokines in COVID-19 patients.

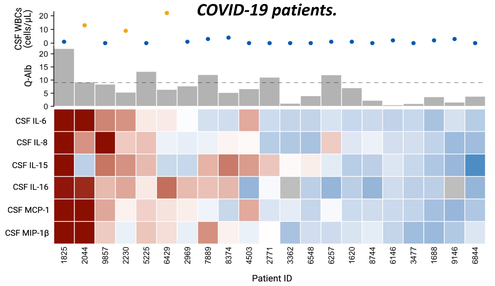


Figure 5 *COVID-19 patient (n=21; two patients with confirmed CNS coinfections were omitted), cytokine and chemokine expression levels were quantified (log base 10), and relative expression levels were z-score transformed across all samples and represented by color. Patients and cytokines were ordered by unsupervised hierarchical clustering analyses of the cohort. Columns represent individual patients; rows, cytokine or chemokine. The CSF:serum albumin ratio (Q-Alb) is displayed by the height of the bars above each patient, where the dotted line (at Q-Alb = 9) represents the boundary between normal and abnormal. The data points displayed above the Q-Alb ratio show the CSF white blood cell count (WBC) for each patient where the orange indicates pleocytosis defined as CSF WBC > 5 cells/ul and blue indicates normal cell count.*

Conclusions

- There was no quantifiable CSF SARS-CoV-2 RNA in 27 prospectively enrolled neurosymptomatic hospitalized COVID-19 patients using qPCR and metagenomic sequencing, which is consistent with majority of case reports and smaller case series in the literature.
- A proinflammatory CSF profile was observed in a subset of COVID-19 patients despite minimal pleocytosis and absent viral nucleic acid. The CSF profile was atypical for neurotropic pathogens, suggesting further study is required to determine the role of CSF inflammation and BBB breakdown in COVID-19-associated neurological disease.