

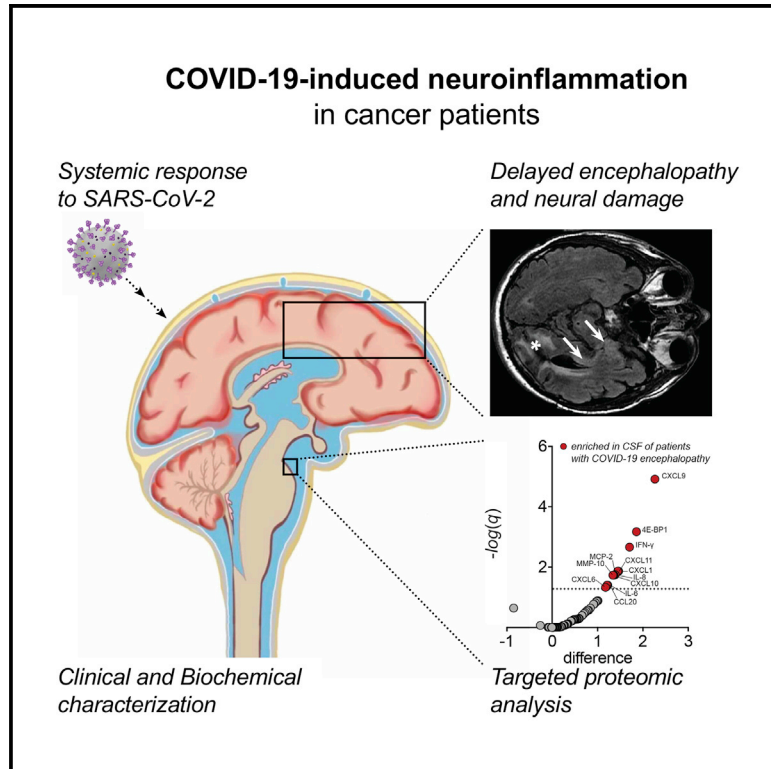


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Inflammatory Leptomeningeal Cytokines Mediate COVID-19 Neurologic Symptoms in Cancer Patients

Graphical abstract



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In Brief

Remsik et al. analyze the cerebrospinal fluid of cancer patients with neurologic symptoms of COVID-19 and detect unique markers of inflammation and neurodegeneration, present weeks after initial SARS-CoV-2 infection. Cytokine storming, both systemically and intracranially, likely contribute to neurologic dysfunction, indicating a potential therapeutic target for investigation.

Highlights

- Inflammatory cytokines are detected in the CSF weeks after SARS-CoV-2 infection
- Levels of IFN-β and IL-8 are specifically enriched in the CSF compared with plasma
- CSF markers of senescence and neurodegeneration are consistent with neuronal injury
- Intracranial levels of MMP-10 correlate with the degree of neurologic disability



Article

Inflammatory Leptomeningeal Cytokines Mediate COVID-19 Neurologic Symptoms in Cancer Patients

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SUMMARY

SARS-CoV-2 infection induces a wide spectrum of neurologic dysfunction that emerges weeks after the acute respiratory infection. To better understand this pathology, we prospectively analyzed a cohort of cancer patients with neurologic manifestations of COVID-19, including a targeted proteomics analysis of the cerebrospinal fluid. We find that cancer patients with neurologic sequelae of COVID-19 harbor leptomeningeal inflammatory cytokines in the absence of viral neuroinvasion. The majority of these inflammatory mediators are driven by type II interferon and are known to induce neuronal injury in other disease states. In these patients, levels of matrix metalloproteinase-10 within the spinal fluid correlate with the degree of neurologic dysfunction. Furthermore, this neuroinflammatory process persists weeks after convalescence from acute respiratory infection. These prolonged neurologic sequelae following systemic cytokine release syndrome lead to long-term neurocognitive dysfunction. Our findings suggest a role for anti-inflammatory treatment(s) in the management of neurologic complications of COVID-19 infection.

INTRODUCTION

Emerging hospital series demonstrate that acute respiratory infection with SARS-CoV-2 is frequently associated with neurologic dysfunction. Mild neurologic symptoms, including headaches, early anosmia, and dysgeusia, occur in a large portion of the infected population and typically resolve (Dell'Era et al., 2020). More serious complications, such as protracted delirium, seizures, and meningoencephalitis, appear to afflict more critically ill patients with hypoxic respiratory failure and may be devastating to highly susceptible individuals (Mao et al., 2020). Cancer patients, in particular, are at heightened risk of severe infections from COVID-19 due to their baseline immunocompromised state and poor functional reserve (Kuderer et al., 2020). The mechanism by which COVID-19 impacts the central nervous system (CNS) is unclear, with hypotheses including direct viral neuroinvasion, neurologic toxicity from the systemic cytokine release syndrome (CRS), or a combination of both. Investigations to date lack consensus regarding the neuroinvasion potential of SARS-

CoV-2, with detectable virus present within the cerebrospinal fluid (CSF) in only a small number of patients with neurologic toxicity (Farhadian et al., 2020; Helms et al., 2020; Mao et al., 2020; Moriguchi et al., 2020). Small case reports of 1–3 patients have reported an elevation in pro-inflammatory cytokines, such as interleukin (IL)-6, C-X-C motif chemokine ligand (CXCL)-10 (also known as interferon-induced protein-10), and C-C motif chemokine ligand (CCL)-2, in the spinal fluid of acutely infected individuals (Benameur et al., 2020; Bodro et al., 2020; Farhadian et al., 2020). No available literature has characterized the full extent and duration of the neuroinflammatory response to COVID-19 in a large sample of patients, or compared the degree of neuroinflammation with the severity of neurologic dysfunction.

Here, we present the clinical neurologic characterization of cancer patients with neurologic toxicity after SARS-CoV-2 infection correlated with biochemical analysis of CSF. As comparators, we analyzed CSF collected from cancer patients with an array of neuroinflammatory conditions, including autoimmune encephalitis and chimeric-antigen-receptor T cell (CAR T)-associated



Table 1. Pathologic Findings in Cancer Patients with Neurologic Manifestations of COVID-19

	Median or n	Range or %
Patient characteristics		
Active cancer or history of cancer	18/18	100
Time from respiratory illness to onset of neurologic symptoms (days)	19.0	0–77
Baseline Karnofsky performance score (KPS)	85	60–100
KPS at lumbar puncture	50	20–70
DRS at lumbar puncture	13	1–26
Neurologic syndrome (n = 18)		
Prolonged delirium	10/18	55.6%
Limbic encephalitis	4/18	22.2%
Refractory headaches	2/18	11.1%
Rhombencephalitis	1/18	5.6%
Ischemic infarcts	1/18	5.6%
Electroencephalography (n = 14)		
Posterior dominant rhythm (PDR)	7.5	5–9
Absent PDR	6/14	42.9%
Sedatives	4/14	28.6%
EEG abnormalities:		
Diffuse slowing	12/14	85.7%
Focal slowing	5/14	35.7%
Triphasic waves	4/14	28.6%
Seizures	1/14	10.0%
Generalized rhythmic delta activity (GRDA)	1/14	10.0%
Neurologic imaging (MRI, n = 14; CT, n = 4)		
Increased T2-signal in focal cortical and white matter structures (MRI):	3/14	21.4%
Limbic regions	2/14	14.3%
Cerebellum	1/14	7.1%
New diffuse microhemorrhages (MRI)	1/14	7.1%
Large territory infarcts (MRI + CT)	1/18	5.6%
Increased white matter disease from prior MRI (COVID-19 non-related)	3/14	36.4%
Abnormal contrast enhancement, excluding brain metastasis (MRI)	0/14	0.0%
See also Tables S1–S4 , S6 , and Figure S1 .		

neurotoxicity. We correlated CSF composition with degree of neurologic dysfunction. In doing so, we have found evidence for a sustained neuroinflammation and subsequent neuronal damage as a key pathway in the pathogenesis of COVID-19-associated encephalopathy.

RESULTS

Clinical Characterization of COVID-19-Related Neurologic Symptoms in Cancer patients

Between May and July 2020, we prospectively evaluated 18 cancer patients with confirmed SARS-CoV-2 respiratory infection who subsequently developed moderate to severe neurologic

symptoms ([Tables 1](#) and [S1](#); [Figure S1](#)). Primary cancer type included a wide range of solid-tumor and hematologic malignancies. Thirteen (72.2%) of our patients received tumor-directed treatment within 30 days of COVID-19 onset, and 7 (38.9%) were baseline immunocompromised before infection (median absolute lymphocyte count, 0.9; range 0.2–4.3). Medical comorbidities were common in this population: hypertension in 55.6%, former smoker in 44.4%, hyperlipidemia in 33.3%, diabetes mellitus in 27.8%, and previous ischemic infarct in 5.6%.

SARS-CoV-2 nasopharyngeal swab was positive in 16 patients; 2 additional patients were included on the basis of appropriately timed positive serum SARS-CoV-2 antibody screen and a recent respiratory illness consistent with COVID-19. All patients presented with classic features of a SARS-CoV-2 respiratory infection, including dyspnea and cough ([Table S2](#)). A large proportion displayed elevated ferritin, D-dimer, IL-6, and C-reactive protein ([Table S3](#)). Eleven patients experienced severe hypoxic respiratory failure and systemic inflammatory response syndrome requiring mechanical ventilatory support ([Table S2](#)). Patients displayed a wide range of neurologic manifestations, with all patients demonstrating at least a mild dysexecutive syndrome consistent with global encephalopathy. Additional neurologic diagnoses included prolonged hyperactive or hypoactive critical care delirium (n = 10), limbic encephalitis (n = 4), refractory headaches (n = 2), rhombencephalitis (n = 1), and large territory infarctions (n = 1). All critically ill patients underwent sedation holidays to confirm that the persistent neurologic impairment was out of proportion to that expected of critical care delirium. There was a delay of 19 days (median; range, 0–77 days) between the onset of respiratory infection and clinical documentation of neurological symptoms.

In addition to bedside neurologic examination, standard neurologic testing included neuroimaging in the form of brain magnetic resonance imaging (n = 14) or head computed tomography (n = 4) ([Table 1](#); [Figure 1](#)). Three patients demonstrated encephalitic changes in the form of non-enhancing T2-hyperintense white matter and cortical changes afflicting the limbic or cerebellar structures. Large territory infarcts, diffuse microhemorrhages, and increased subcortical or periventricular white matter disease compared with pre-COVID-19 comparison imaging were also evident.

Electroencephalography ([Table 1](#)) was completed in 14 patients. Diffuse bihemispheric slowing was the most common finding observed (n = 12), and was irrespective of the use of pharmacologic sedation. A single patient with limbic encephalitis developed temporal lobe seizures.

CSF Composition in the Setting of COVID-19

Thirteen patients underwent spinal fluid analysis at a median of 57 days (range, 5–142) from the onset of respiratory symptoms and 37 days (range, 1–117) from the onset of neurologic symptoms ([Tables S3](#) and [S4](#)). Spinal fluid collection was undertaken when neurologic symptoms could not be attributed to alternative non-infectious cause(s) and when the benefits of CSF analysis outweighed the procedural risk associated with holding anticoagulation. These clinical concerns limited the feasibility of CSF testing at earlier time points. In addition, the delayed onset of neurologic symptoms after initial respiratory presentation

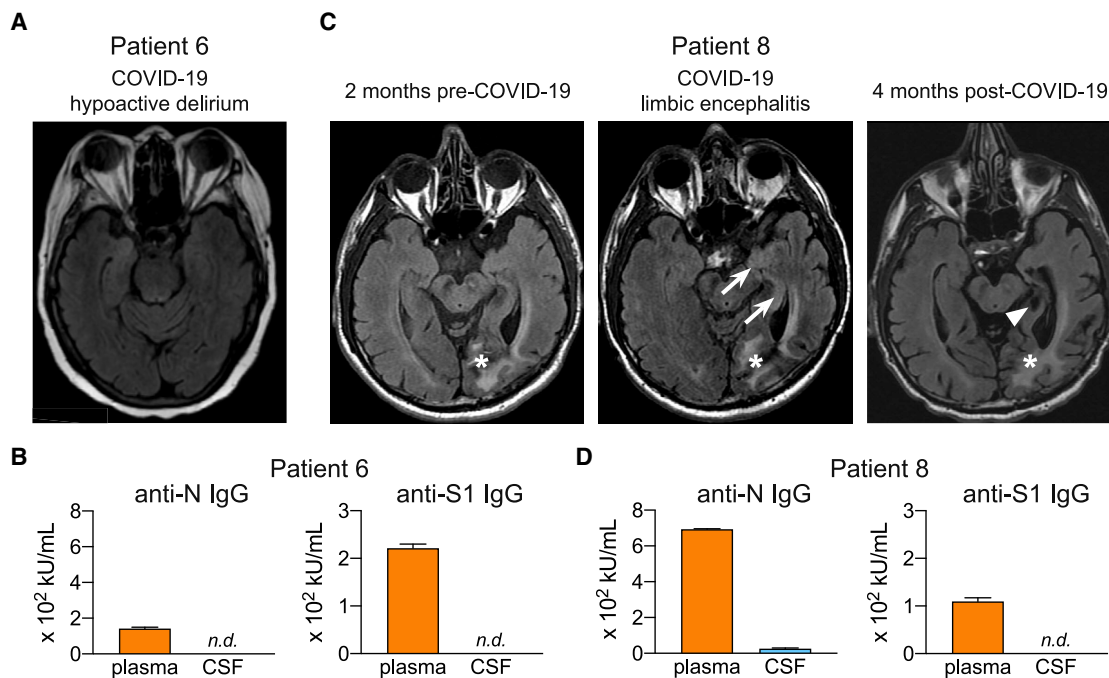


Figure 1. Radiographic Findings and CSF COVID-19 Antibodies in Patients with Neurologic Manifestations of COVID-19

(A) Patient 6 is a 71-year-old woman with NSCLC (non-small cell lung cancer) and prolonged hypocoactive delirium after COVID-19 hypoxic respiratory failure. Representative axial FLAIR image from MRI brain obtained during hypocoactive delirium is pictured.

(B) Anti-N and anti-S1 COVID-19 IgG assayed by enzyme-linked immunosorbent assay (ELISA) of patient 6 plasma and CSF.

(C) Patient 8 is a 61-year old man with NSCLC and COVID-19 limbic encephalitis. Axial FLAIR images obtained 2 months before COVID-19 infection show baseline post-operative encephalomalacia (arrowheads, left). At the time of acute COVID-19 infection, during refractory left temporal lobe seizures, axial FLAIR images demonstrate new T2 signal abnormality within the left medial temporal lobe (arrows, middle). Following resolution of COVID-19 limbic encephalitis and after treatment with high-dose corticosteroids and antiepileptics, axial FLAIR images demonstrate improvement in medial temporal FLAIR abnormality, and new left hippocampal and parahippocampal gyrus atrophy (arrowhead, right).

(D) Anti-N and anti-S1 COVID-19 IgG assayed by ELISA of patient 8 plasma and CSF. n.d., not detected. Data shown as mean \pm SD (B and D).

See also [Figures S1](#) and [S2](#).

obviated the need for CSF testing during the acute phase of infection in several patients.

Notably, cell count, protein, and glucose levels were normal. Two patients had measurable pleocytosis; both of these patients had known CNS metastases. CSF immune cell differentials were lymphocyte predominant in 76.9% and monocyte predominant in 23.1% of patients. Oligoclonal bands were detected in both the serum and CSF in 83% of tested patients, indicating systemic rather than intracerebral production of gamma globulins. Only two patients had elevated intracranial pressure and abnormal protein levels, sequelae more easily ascribed to active brain metastases rather than COVID-19 neurologic injury.

To characterize SARS-CoV-2 neuroinvasion within this cancer population, we optimized a polymerase chain reaction (PCR)-based assay for the CSF and used a commercially available ELISA to test for SARS-CoV-2 viral RNA and N and S structural proteins in the CSF. No patients demonstrated detectable levels of viral PCR or structural proteins in the CSF. We also tested the levels of angiotensin-converting enzyme 2 (ACE2), the SARS-CoV-2 receptor, within the CSF-brain and CSF-blood barriers in banked pre-pandemic cerebral tissues. We detected low levels of ACE2 in the intravascular monocytes and capillary endothelium within both the choroid plexus and meninges, representing potential means of viral entry into the leptomeninges

([Figure S2](#)). Anti-N COVID-19 IgG were detected in the CSF of only one patient with limbic encephalitis and temporal lobe seizures, indicating blood-brain barrier breakdown ([Andriuta et al., 2020](#)) ([Figure 1](#); [Table S5](#)).

Proteomic Analysis of Neuroinflammation

As direct viral invasion could not be found to explain prolonged neurologic dysfunction at the time point of our patients' CSF analyses, we then subjected the CSF from 10 patients (CoV+) to an extensive proteomics assay to elucidate patterns of inflammation and neuronal damage. Cancer is an inherently immunocompromised state. We therefore compared CSF from COVID-19-positive cancer patients with three distinct cancer patient cohorts from the pre-pandemic era ([Table S6](#)): to control for alternative cancer-related causes of CNS inflammation, the first group consisted of patients matched for age, cancer type, and presence of brain metastases (CoV-). To compare with other well-characterized neuroinflammatory syndromes in cancer patients, the second group consisted of patients with CAR T cell-associated neurotoxicity, also known as immune effector cell-associated neurotoxicity syndrome (ICANS) ([Lee et al., 2019](#)); the third group comprised patients with autoimmune encephalitis (AIE) (idiopathic $n = 3$, and suspected immune checkpoint inhibitor-associated $n = 3$) ([Graus et al., 2016](#); [Santomasso](#)

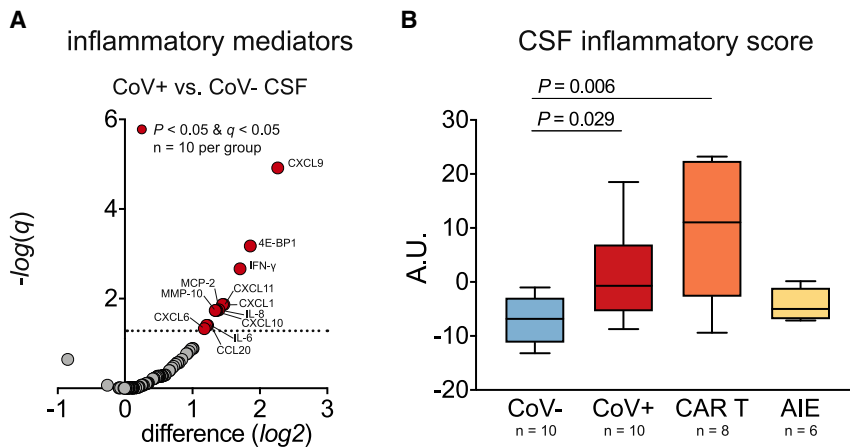


Figure 2. Accumulation of Inflammatory Mediators in CSF of Patients with Neurologic Manifestations of COVID-19

(A) Targeted proteomic analysis of cytokines and chemokines in CSF of cancer patients by proximity extension assay (O-link). Enrichment plot of 12 individual cytokines and chemokines in the CSF of cancer patients with COVID-19 (CoV+, n = 10) compared with their cancer-matched controls without COVID-19 (CoV-, n = 10; multiple t tests), as determined with targeted proteomic analysis (full line, median; dotted line, quartiles; Whitney-Mann U test p reported within the plot; ANOVA Kruskal-Wallis test p = 0.015).

(B) CSF inflammatory mediators measured by O-link in four cohorts of cancer patients: COVID-19-positive patients (CoV+, n = 10), COVID-19-negative patients (CoV-, n = 10), patients with CAR T cell neurotoxicity (CAR T, n = 8), and patients

with autoimmune encephalitis (AIE) (n = 6). Composite score of 12 inflammatory cytokines and chemokines as identified in (A). A.U., arbitrary units. Error bars indicate range, box indicates the 25th–75th percentiles, middle line indicates median.

See also [Table S5](#) and [Figure S3](#).

et al., 2018). Correlative analysis across these 4 cohorts identified a significant accumulation of 12 inflammatory mediators in the CSF of COVID-19 patients, at levels approaching those of patients with severe ICANS (Figures 2A and S3A). These included IL-6 and -8, interferon (IFN)- γ , CXCL-1, -6, -9, -10, and -11, CCL-8 and -20, matrix metalloproteinase-10 (MMP-10), and eukaryotic translation initiation factor 4E binding protein 1 (4E-BP1).

Using an inflammatory signature comprising these 12 proteins, we also found a marked cumulative increase in inflammatory signaling relative to the non-COVID-19 cancer controls (p = 0.029, Figure 2B). These 12 factors associate with cytokine and chemokine signaling, immune cell function, senescence, and neuroinflammation (Figures S3B and S3C). Analysis of matched plasma and CSF from 12 COVID-19 patients with commercial bead arrays further revealed that the levels of IFN- α , - γ , and - λ , and chemokines CXCL-9 and CXCL-11, were enriched in plasma, while IFN- β and IL-8 appeared to be produced predominantly within leptomeninges (Figure 3).

Levels of these 12 inflammatory mediators in the CSF were compared with clinical scores of neurologic dysfunction at the time of spinal tap, as measured by the Karnofsky performance status (KPS) and disability rating scale (DRS) (Rappaport et al., 1982). We found that the CSF levels of MMP-10, a marker of neurodegeneration, in both COVID-19-positive patients and controls correlated with the degree of neurologic insult by KPS and approached significance with the DRS (Figures S3D and S3E). More extensive proteomic analysis of CSF from COVID-19 patients showed, besides elevation of senescence marker 4E-BP1, enrichment of 10 additional neuronal biomarkers, including well-known marker of virus- and autoimmunity-induced neurodegeneration NEFL (neurofilament light chain) (Figure 4) (Bridel et al., 2019).

DISCUSSION

Cancer patients are at a heightened risk for severe COVID-19 infection (Robilotti et al., 2020). Encephalopathy is a significant contributor to morbidity in COVID-19 patients (East-Seletsky et al., 2016; Liotta et al., 2020). Large population-based studies

suggest that neurologic complications of COVID-19 are far more widespread than originally appreciated, occurring in 7%–69% of patients with severe infection (East-Seletsky et al., 2016; Ellul et al., 2020). Understanding the pathophysiology of the neurologic consequences of COVID-19 in the cancer patient population is therefore of paramount importance.

In our COVID-19-positive patient cohort, an increase in IFN- γ and its downstream effectors, CXCL-9, -10, and -11, is demonstrated in the CSF nearly 2 months after onset of SARS-CoV-2 infection. As the major mediator against viral defenses in both intracranial and systemic processes, the systemic upregulation of the IFN- γ response is not unexpected in patients with a viral infection. However, IFN- γ demonstrates opposing roles in other neuroinflammatory conditions, regulating both pro-inflammatory and neuroprotectant properties in oligodendroglial cells, microglia, and astrocytes (Ottum et al., 2015). Increased levels of intracranial CXCL chemokines have been described in a wide array of infectious and non-infectious CNS pathologies. The subsequent intracerebral accumulation of chemokines, monocyte-macrophages, and T lymphocytes downstream of IFN- γ propagates further neurologic injury (Koper et al., 2018; Sui et al., 2004; Xu et al., 2005; Zhang et al., 2013).

Similarly, a report following the 2003 SARS outbreak revealed that systemic response during an acute respiratory infection induced glial expression of chemokines downstream of interferon, including CXCL-9 and -10, further propagating the destructive process (Xu et al., 2005).

Neurologic toxicity has been reported in other conditions with CNS cytokine “storming”; the lag between systemic cytokine peak and onset of neurologic manifestations is also not unique to COVID-19 (Dantzer et al., 2008). This biphasic pattern is well characterized in ICANS, for example, in which case severe neurotoxicity develops a median of 8 days after plasma CRS, and may not even occur until the CRS has completely resolved (Santomasso et al., 2018, 2019). Severe ICANS is also linked to a substantial elevation in cytokines within the CSF. These increased CSF cytokines are likely the result of both increased blood barrier permeability and local production by cells in the CNS. The disproportionate rise in CCL-2, CXCL-10, IL-6, and

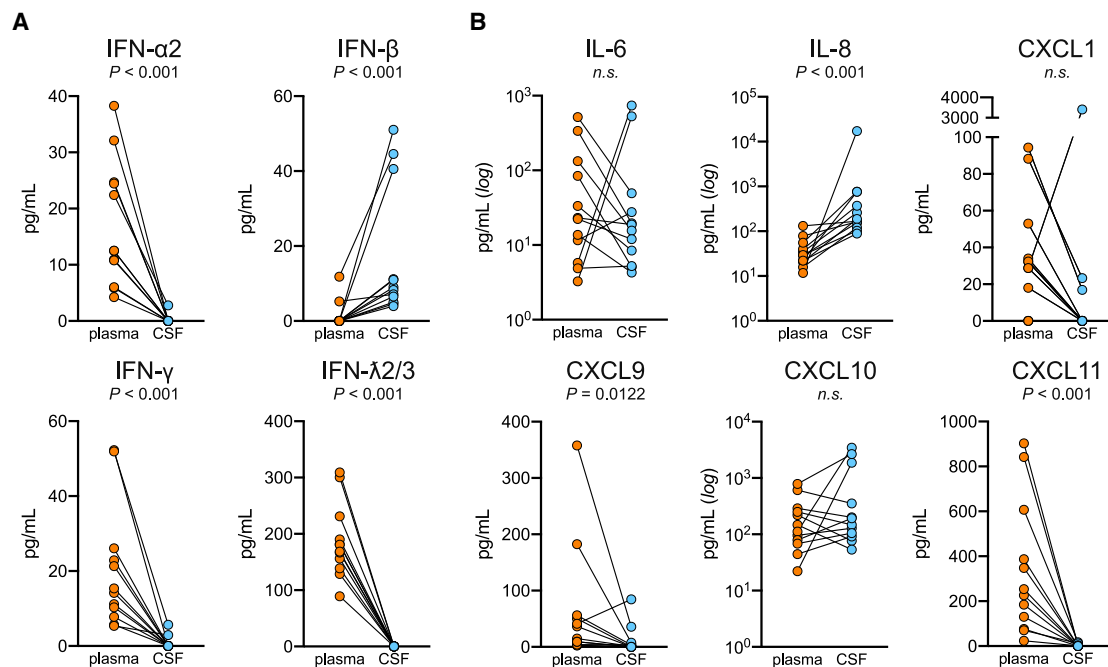


Figure 3. Analysis of Systemic and Leptomeningeal Inflammatory Response in Paired Plasma and CSF from Patients with Neurologic Manifestations of COVID-19

(A) Levels of type I, II, and III interferons as determined by cytometric bead array in matched plasma and CSF ($n = 12$, Wilcoxon signed rank test).

(B) Levels of inflammatory cytokines and chemokines determined by cytometric bead array in matched plasma and CSF ($n = 12$, Wilcoxon signed rank test). Matched, individual values are plotted.

IL-8 within the CNS of such patients points to production by activated myeloid, astrocyte, and/or endothelial cells within the CNS (Santomasso et al., 2019). Another commonality between the COVID-19-positive and CAR T cell neurotoxicity cytokine cohorts is the relative enrichment in intensive care patients among these two groups (60% and 100%, respectively) compared with the additional controls. This potential confounder should be considered when interpreting the relative rise in CSF inflammatory mediators among these two cohorts, with the question as to whether more mildly affected COVID-19-positive patients also harbor a neuroinflammatory response.

Significant accumulation of cytokines within the leptomeninges of patients with COVID-19 is congruent with the mounting evidence of immune infiltration within the brains of such patients on autopsy studies (Matschke et al., 2020; Solomon et al., 2020; von Weyhern et al., 2020). A striking predilection for brainstem and cranial nerve infiltration of both myeloid and lymphoid-derived cells has been observed. The largest autopsy series to date of 43 patients reported a high degree of diffuse astrogliosis, microglial activation in the brainstem and cerebellum, and prominent cytotoxic T lymphocyte infiltration in the brainstem, with more scattered involvement of the frontal cortex and basal ganglia (Matschke et al., 2020). The microanatomic localization of cytotoxic T lymphocytes was most concentrated in the perivascular regions, but also in close approximation to IBA-1-positive cells both in the parenchyma and meninges. This perivascular and meningeal localization of T lymphocytes is also supported by other smaller autopsy series (Solomon et al., 2020; von Weyhern et al., 2020).

Interestingly, these findings of CNS immune infiltrate do not uniformly correlate with detection of SARS-CoV-2 within the autopsy specimens, supporting secondary immune activation as the hypothesized insult. Our findings support this pathophysiologic hypothesis. There was no evidence of SARS-CoV-2 viral RNA detection, nucleocapsid protein, or spike protein in the CSF of our cancer population, which is reassuring given the baseline immunocompromised state and high degree of medical comorbidities afflicting our patients. Only one patient had detectable SARS-CoV-2 antibodies in the CSF, contrary to what has been observed among other patient cohorts with neurologic manifestations of COVID-19 (Alexopoulos et al., 2020; Song et al., 2020). This can be in part explained by different extent of the disease encompassing the CNS and the use of assays with different sensitivities and specificities. While it remains possible that this negative result was a sampling error given the lag time between neurologic symptom onset and lumbar puncture from the acute phase of respiratory infection, the current literature has also not provided convincing evidence of CSF viral detection in patients with neurologic manifestations of COVID-19 (Destras et al., 2020; Edén et al., 2020; Helms et al., 2020; Neumann et al., 2020). Case reports have described positive SARS-CoV-2 PCR in the CSF of patients with various forms of encephalitis (Huang et al., 2020; Kamal et al., 2020; Moriguchi et al., 2020); these results have not been reproduced in large-scale studies. Variable viral detection in the brain parenchyma using qRT-PCR for viral RNA and immunohistochemical assays for spike and nucleocapsid proteins has been reported in select autopsy studies (Matschke et al., 2020; Puelles et al., 2020;

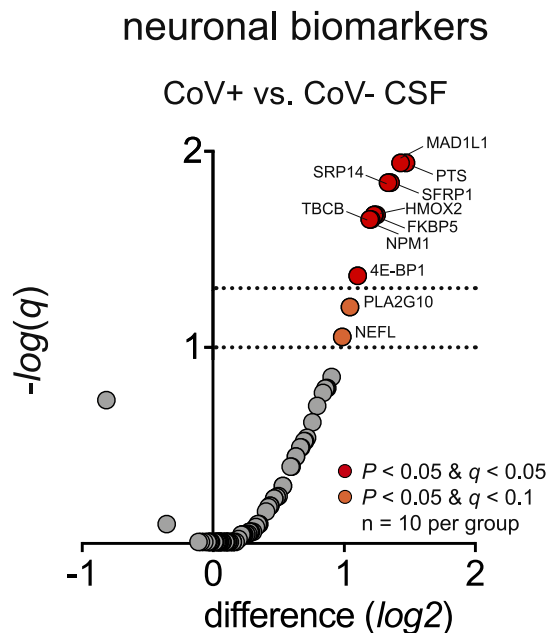


Figure 4. Accumulation of Neuronal Biomarkers in CSF of Patients with Neurologic Manifestations of COVID-19

Targeted proteomic analysis of neurologic damage in CSF of cancer patients by proximity extension assay (O-link). Enrichment plot of 11 individual proteins in the CSF of cancer patients with COVID-19 (CoV+, n = 10) compared with their cancer-matched controls without COVID-19 (CoV-, n = 10; multiple t tests).

Solomon et al., 2020), but not in others (Barton et al., 2020; Schaller et al., 2020), and did not predict severity of intracranial immune infiltration (Matschke et al., 2020). When SARS-CoV-2 was detectable by either modality, clear involvement of the brainstem and cranial nerves was apparent (Matschke et al., 2020; Solomon et al., 2020).

Furthermore, we have found that CSF levels of MMP-10 correlates with the degree of neurologic dysfunction exhibited by our cancer patient cohort. This is consistent with other reports correlating MMP-10 levels with neuronal damage (Thibert et al., 2012). Increased levels of 11 cellular secreted, membranous, cytoplasmic, and nuclear proteins in the CSF, including markers of senescence and neuronal damage, further support the presence of ongoing, COVID-19-induced neurodegeneration. The specific mechanism through which COVID-19 induces this array of biomarkers warrants further investigation, as does the extent of neuronal damage.

Our findings have potentially important diagnostic and treatment implications. A prolonged encephalopathy of unknown etiology accompanies critical infections with COVID-19, and studies to date have not identified any obvious meningitic, ischemic, or ictal cause. This case series characterizes the increase in pro-inflammatory CSF cytokines in cancer patients in the weeks after respiratory illness. Similar to other encephalitides, these cytokines may result in neuronal damage in the absence of obvious radiographic abnormalities.

Dexamethasone, a potent steroid and immunosuppressant, is the only intervention that has been shown in large-scale studies

to reduce the incidence of death in hospitalized patients with severe COVID-19 infections (Group et al., 2020). The impact of dexamethasone on COVID-19-related neurologic toxicity, specifically, has not been defined. Our data demonstrate that the neurologic toxicity associated with COVID-19 is biochemically similar to CAR T cell neurotoxicity. Clinical rubrics for the grading and management of CAR T cell neurotoxicity support early diagnosis and use of anti-inflammatory agents, including dexamethasone (Brouwer et al., 2015; Gust et al., 2018; Norelli et al., 2018).

Our observational study has several limitations. While our results suggest a trend of cytokine elevation within the CSF, the low number of patients somewhat limits the interpretation of our conclusions. Until these findings can be reproduced in a larger dataset, our conclusions regarding elevated neuroinflammatory markers in patients with neurologic manifestations of COVID-19 should be viewed as hypothesis generating and worthy of further investigation. Moreover, we cannot confirm causality of cytokine accumulation with neurologic dysfunction in this study, because to do so would require invasive CSF collection from COVID-19-positive cancer patients without neurologic manifestations and therefore would not be ethically feasible. Furthermore, mechanical ventilation from alternative pulmonary insults represents a clinical scenario in which CSF testing is often not indicated, therefore this comparison would be challenging to complete even in a center not restricted to cancer patients.

In conclusion, we find that neurologic syndromes associated with moderate and severe COVID-19 infections are accompanied by a wide range of intracranial inflammatory cytokines, mediators that may persist for weeks to months after convalescence of the respiratory syndrome. The global accumulation of both systemically induced and CSF-specific inflammatory mediators in CSF and inability to detect COVID-19 in the CSF suggests that the identification of a single, universal COVID-19 CSF biomarker is unlikely to be successful. However, the correlation of MMP-10 with neurologic dysfunction marks a potential prognostic biomarker for future study. Moreover, our findings further the investigation of early neurologic assessments, such those used for monitoring ICANS (Lee et al., 2019) and support investigation of anti-inflammatory therapies in patients with severe or prolonged COVID-19 disease.

STAR★METHODS

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at <https://doi.org/10.1016/j.ccell.2021.01.007>.

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AUTHOR CONTRIBUTIONS

A.B., B.S., J.A.W., and J.R. conceived the study. J.A.W. performed neurologic examinations, clinical annotations, and collected blood and CSF. J.R. performed the experiments and analyzed and curated the data. N.A.H. and V.D. referred patients to neurology consult service. N.E.B. and T.A.M. developed and performed the CSF COVID-19 RNA PCR method. B.A.V. reviewed neuroimaging data. M.R. reviewed histological staining. C.A.I.-D. provided autopsy samples. E.K.A. reviewed EEG recordings. B.S. provided CSF from retrospective CAR T and AIE cohorts. J.R., J.A.W., and A.B. wrote the manuscript. A.B. supervised the study. All authors reviewed and approved the manuscript.

DECLARATION OF INTERESTS

A.B. is an inventor on United States Provisional Patent Application no. 62/258,044 "Modulating Permeability of The Blood Cerebrospinal Fluid Barrier." A.B. is an unpaid member of the Scientific Advisory Board of EVREN Technologies. B.S. is a paid consultant for Kite Gilead, Juno/Celgene, Janssen, and In8bio. Other authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
anti-ACE-2	R&D	Cat# AF933; RRID:AB355722
Bacterial and Virus Strains		
NA		
Biological samples		
Retrospective collection of human cerebrospinal fluid	This paper	N/A
Plasma and cerebrospinal fluid from COVID-19 patients	This paper	N/A
Human cerebral autopsy tissue	This paper	N/A
Chemicals, Peptides, and Recombinant Proteins		
NA		
Critical commercial assays		
SARS-CoV-2 RNA PCR	Robilotti et al. (2020)	N/A
SARS-COV-2 IgG FDA EUA	Abbott	Cat# 6R86-20
Anti-N IgG ELISA Kit	RayBiotech	Cat# IEQ-CoVN-IgG1
Anti-S1RBD IgG ELISA Kit	RayBiotech	Cat# IEQ-CoVS1RBD-IgG1
Anti-N IgM ELISA Kit	RayBiotech	Cat# IE-CoVN-IgM-1
Anti-N IgA ELISA Kit	RayBiotech	Cat# IE-CoVN-IgA-1
N protein ELISA Kit	RayBiotech	Cat# ELV-COVID19N-1
S2 protein ELISA Kit	RayBiotech	Cat# ELV-COVID19S2-1
Olink Target 96 Inflammation	Olink	https://www.olink.com/products/inflammation/
Olink Target 96 Neuro Exploratory	Olink	https://www.olink.com/products/neuro-exploratory-panel/
Legendplex Human Proinflammatory Chemokines	Biologend	Cat#740003
Legendplex Human Anti-Virus Response	Biologend	Cat#740390
Deposited data		
Targeted proteomics analysis of cerebrospinal fluid from four patient cohorts.	Mendeley Data	https://doi.org/10.17632/s7m535k6nt.1
Experimental Models: Cell Lines		
NA		
Experimental Models: Organisms/Strains		
NA		
Oligonucleotides		
NA		
Recombinant DNA		
NA		
Software and algorithms		
Reactome	Jassal et al., 2020	https://reactome.org
Qiagen Ingenuity Pathway Analysis	Qiagen	https://digitalinsights.qiagen.com/products-overview/discovery-insights-portfolio/analysis-and-visualization/qiagen-ipa/
Prism, v8	Graphpad	https://www.graphpad.com
Other		
NA		

RESOURCE AVAILABILITY

Lead Contact

Further information and requests should be directed to and will be fulfilled by the Lead Contact, Adrienne Boire (boirea@mskcc.org).

Material availability

Human tissues used in this manuscript are unique biological resources, not available for further distribution.

Data and Code Availability

This study did not generate any new code. All data shown in the manuscript are available upon reasonable request from the from Lead Contact. Targeted proteomics analysis of cerebrospinal fluid from four patient cohorts is available on Mendeley Data (<https://doi.org/10.17632/s7m535k6nt.1>)

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Patients

This study was conducted according to the principles expressed in the Declaration of Helsinki. Ethical approval for COVID-19-related research was obtained from MSKCC Institutional Review Board (IRB) under protocol #20-006. Collection and use of clinical samples were approved by MSKCC IRB #06-107, #12-245, #13-039, and #18-505. Only clinical samples collected in excess of those needed for diagnostic and therapeutic procedures were used for research. All participants provided written informed consent for sample and clinical data collection and subsequent analyses.

Eighteen cancer patients with recent or history of recent respiratory illness or COVID-19 with severe neurologic symptoms were admitted to the MSK Neurology Department between May and August 2020. All patients were tested positive for the presence of COVID-19 viral RNA in nasopharyngeal swab or anti-COVID-19 in serum or plasma as described below, using CDC and FDA approved methods. The neurological symptoms are described in [Table 1](#) and the detailed demographic characteristics are listed in [Table S1](#).

METHOD DETAILS

Clinical Sample Collection

Cerebrospinal fluid in excess of that needed for diagnostic purposes was collected via bedside or fluoroscopically-guided lumbar puncture. At the time of spinal fluid collection, venous blood was collected into a tube containing sodium citrate, or sodium or lithium heparin. CSF samples were kept on ice and processed within two hours from collection. Anti-coagulated blood and CSF were transferred into 15 mL conical tubes and spun at 800g for 10 minutes. Plasma and cell-free CSF was aliquoted and stored at -80°C. Processing and experimentation with non-inactivated body fluids were performed under Bsl2+ containment.

SARS-CoV-2 RNA and Protein Detection

Total nucleic acids were extracted from CSF (200 μ L) on the MagNA Pure compact (Roche Molecular Diagnostics) and eluted in a final volume of 100 μ L. Real-time reverse transcriptase polymerase chain reaction (PCR) was performed on an ABI 7500 Fast (Applied Biosystems) using 5 μ L of extracted nucleic acids and primers and probes targeting two regions of the nucleocapsid gene (N1 and N2) and the human RNase P gene as an internal control as previously described for respiratory samples ([Robilotti et al., 2020](#)). Results were valid only if the RNase P gene was detected in the sample. Samples were considered positive if both analytic targets N1 and N2 were detected and negative if both targets were not detected. N and S (S2 subunit) SARS-CoV-2 structural proteins were detected using commercially available ELISA kits (ELV-COVID19N-1 and ELV-COVID19S2-1, RayBiotech), as recommended by the manufacturer. All ELISA samples were run in technical replicates.

Magnetic Resonance Imaging

Imaging studies were conducted either on 1.5 or 3 Tesla MRI. Images were obtained as part of the patient's routine standard of care imaging, which did not allow standardization of sequences. The most frequently sequences performed were diffusion-weighted imaging (DWI), susceptibility-weighted angiography (SWAN), 2D fluid-attenuated inversion recovery (FLAIR), T2-weighted fast spin-echo and T1-weighted fast spin-echo MRI before and after administration of gadolinium-based contrast agent. MRI parameters of the most commonly used sequences in this study are tabulated in [Table S7](#).

Electroencephalography

Long-term and routine electroencephalography monitoring with video was performed using a Natus 32 channel computerized electroencephalography (EEG) system with digital analysis of video-EEG for spike detection and analysis, computerized automated seizure detection algorithms, and patient "event marker." Electrode set up of 8 channels or greater was performed by the EEG technologist. Standard 10-20 system montages were employed for EEG review.

Clinical Laboratory Analyses

Levels of CSF protein and glucose and serum D-dimer, ferritin, CRP and IL-6 were determined per standard laboratory methods using FDA-approved, *in vitro* diagnostics (IVD) kits.

Detection of Anti-SARS-CoV-2 Immunoglobulins

Clinical IgG test against SARS-CoV-2 was performed using FDA EUA kit from Abbott (6R86-20). Experimental IgG tests against SARS-CoV-2 N and S1RBD proteins were detected in plasma and CSF using quantitative ELISA kits (IEQ-CoVN-IgG1 and IEQ-CoVS1RBD-IgG1, RayBiotech). Samples were analyzed as recommended by manufacturer, except that the plasma was diluted 1,500x and CSF 750x in 1x sample buffer. IgM and IgA against SARS-CoV-2 N protein were detected in plasma and CSF using semi-quantitative ELISA kits (IE-CoVN-IgM-1 and IE-CoVN-IgA-1, RayBiotech), as recommended by the manufacturer. All samples were run in technical replicates. These kits were for research use only and did not have FDA approval at the time of initial submission.

ACE2 Immunohistochemistry

Human autopsy tissue was collected under MSKCC IRB #18-065 and #18-292 from patients that provided written informed consent. Tissue was de-paraffinized, antigens were retrieved, and the procedure was performed essentially as described in (Chi et al., 2020). Primary anti-ACE-2 antibody (AF933, R&D) was used as recommended by the manufacturer, followed by the incubation with HRP-conjugated anti-goat secondary antibody (Immpress HRP Anti-Goat IgG, MP-7405, Vector Laboratories) and subsequently DAB EqV (SK-4103, Vector Laboratories). Nuclei were counterstained with hematoxylin (S3309, Dako). Stained, dehydrated slides were mounted in Vectamount (H-5000, Vector Laboratories), dried and scanned with Mirax (Zeiss).

CSF Proteomics and Data Analysis

CSF collected from patients with neurologic complications of COVID-19 via lumbar puncture was processed within two hours post collection, as described above, aliquoted and stored at -80°C . Retrospectively collected samples from primary and metastatic tumor-matched patients without COVID-19 who underwent lumbar puncture to rule out leptomeningeal spread of disease, patients with severe CAR T neurotoxicity (grade 3-4) and patients with autoimmune encephalitis were obtained from MSK Brain Tumor Center CSF Bank. Control patient cohort was selected from a random pool of potential matches. Samples were slowly thawed on ice and inactivated for COVID-19 as follows: 45 μL of CSF was mixed with 5 μL of 10% Triton X-100 (Sigma, T8787) in saline and incubated at room temperature for two hours. Samples were then dispensed in randomized fashion into 96-well PCR plate and stored at -80°C until further analysis. Relative levels of 92 inflammatory proteins were detected using proximity extension assay (Olink Target 96 Inflammation and Olink Target 96 Neuro Exploratory, Olink). Protein abundance values are shown in NPX units (\log_2 scale). Analytical measuring range for each protein is available online (www.olink.com) or from the corresponding author upon request. Cytometric bead arrays were performed with Legendplex Human Anti-Viral Response (Biolegend, 740003) and Legendplex Human Proinflammatory Chemokines (Biolegend, 740390), as recommended by the manufacturer. Source data used to generate plots in Figures 2 and 3 were submitted to Mendeley (<https://doi.org/10.17632/s7m535k6nt.1>).

Calculations of Composite Signature and Computational Analyses

Inflammatory signature was constructed as follows: z-score for each of the twelve analytes in this dataset was computed for all patients, the sum of all z-scores for a patient then represented an inflammatory score plotted in Figure 1. Pathway analysis was performed with Reactome (www.reactome.org) and IPA (Qiagen).

Statistics

Sample size was not pre-determined and no patients, data points or samples were excluded. Differences in inflammatory protein abundance between COVID-19 positive subjects and control cohort were determined using multiple t tests (unpaired, two-tailed) with Benjamini and Hochberg correction for FDR. Proteins with P and q values lower than 0.05 were considered significant. Differences in inflammatory score between patient cohorts were determined with Mann-Whitney U test (unpaired, two-tailed). For multi-group comparisons, one-way ANOVA P values are reported (two-tailed). Paired plasma-CSF analyses were performed with Wilcoxon matched-pairs signed rank test. Inflammatory score was computed as described above. Number of replicates is stated in corresponding figure legends. For correlations, both Person's and Spearman's R is reported. Data used to generate figures in this study were submitted as "Source Data" tables. Statistical analyses were conducted in Prism (v8, GraphPad).