Cerebrospinal fluid cytokine, chemokine, and SARS-CoV-2 antibody profiles in children with neuropsychiatric symptoms associated with COVID-19

Binh Ngo [a,b,1], Stacey A. Lapp [c,d,1], Benjamin Siegel [c,e], Vikash Patel [c,d], Laila Hussaini [c,d], Sonali Bora [a,b], Bryan Philbrook [c,e,h], Kristin Weinschenk [a,b], Laura Wright [f], Evan J. Anderson [c,d,g], Christina A. Rostad [c,d,1], Grace Y. Gombolay [e,h,1,*]

[a] Children's Healthcare of Atlanta, Division of Psychiatry, Atlanta, GA, United States
[b] Emory University School of Medicine, Department of Psychiatry, Atlanta, GA, United States
[c] Emory University School of Medicine, Department of Pediatrics, Division of Infectious Diseases, Atlanta, GA, United States
[d] Center for Childhood Infections and Vaccines, Children's Healthcare of Atlanta and Emory University School of Medicine, Atlanta, GA, United States
[e] Children's Healthcare of Atlanta, Division of Pediatric Neurology, Atlanta GA, United States
[f] Children's Healthcare of Atlanta, Department of Neuropsychology, Atlanta GA, United States
[g] Emory University School of Medicine, Department of Medicine, Division of Infectious Diseases, Atlanta, GA, United States
[h] Emory University School of Medicine, Department of Pediatrics, Division of Neurology

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ABSTRACT

Background: Neuropsychiatric symptoms and CSF cytokine, chemokine, and SARS-COV-2 antibody profiles are unknown in pediatric patients with COVID-19 or multisystem inflammatory syndrome (MIS-C), (NP-COVID-19).
Methods: Children at a single pediatric institution quaternary referral center with laboratory-confirmed COVID-19 or MIS-C and neuropsychiatric symptoms were included in this retrospective case series. Clinical symptoms, ancillary testing data, treatments and outcomes are described. Multiplexed electrochemiluminescence assays for cytokines, chemokines and SARS-CoV-2 antibodies were tested in the CSF NP-COVID-19 patients compared to five controls and were analyzed using the Student's t-test.
Results: Three of five NP-COVID-19 patients had psychiatric symptoms, and two patients had encephalopathy and seizures. All patients had full or near resolution of neuropsychiatric symptoms by discharge. One patient received intravenous steroids for treatment for psychiatric symptoms; 3/5 other patients received immunotherapy for MIS-C, including IVIG, high-dose steroids, anakinra, and tocilizumab. Pro-inflammatory chemokines, including MIG, MPC, MIP-1β, and TARC were significantly elevated in NP-COVID-19 patients compared to controls. Two of five patients had elevated CSF neurofilament light chain. CSF SARS-CoV-2 antibody titers to the full-length spike, receptor binding domain and N-terminal domain were significantly elevated. SARS-CoV-2 antibody titers strongly correlated with pro-inflammatory chemokines/cytokines, including IL-1β, IL-2, IL-8, TNF-α, and IFN-γ (P≤0.05 for all).
Conclusions: A spectrum of neuropsychiatric clinical manifestations can occur in children with SARS-CoV-2 infection. CSF pro-inflammatory chemokines and SARS-CoV-2 antibodies may serve as biomarkers of SARS-CoV-2 mediated NP-COVID-19. Additional study is required to understand the pathophysiologic mechanisms of neuroinflammation in children with COVID-19 and MIS-C.

1. Introduction

Severe acute respiratory syndrome coronavirus (SARS-CoV-2) has caused the coronavirus disease 19 (COVID-19) pandemic (World Health Organization). Complications from COVID-19 include respiratory, cardiac, and multi-system organ failure. Children may also develop a

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Kawasaki-like syndrome called Multisystem Inflammatory Syndrome in Children (MIS-C), either coinciding with or following acute SARS-CoV-2 infection (Hobbs et al., 2020). In children, SARS-CoV-2 infection can also manifest with neuropsychiatric symptoms, which include neurological symptoms such as encephalopathy and encephalitis (Paterson et al., 2020) and psychiatric symptoms such as psychosis and mania. The mechanisms of SARS-CoV-2 associated neuropsychiatric clinical manifestations are thought to be multifactorial (Najjar et al., 2020). Psychosocial stressors, including social distancing, enhanced isolation, school closures, fear of the disease, and financial strain due to COVID-19 can exacerbate psychiatric symptoms, such as depression, anxiety, or suicidal ideation (Zalsman et al., 2020). However, neuropsychiatric symptoms may arise from potential biological causes that are incompletely understood, and direct viral invasion, immune cell infiltration, and cytokine storms have been implicated (Iqbal et al., 2020). There is evidence that the systemic inflammatory state could induce neuroinflammatory changes, which result in neuropsychiatric symptoms(Lin et al., 2021).

Although cases have been described in adults with neuropsychiatric symptoms(Iqbal et al., 2020; Benameur et al., 2020; Rogers et al., 2020) along with cerebrospinal fluid (CSF) cytokines (Bodro et al., 2020; Benameur et al., 2020), there is a paucity of literature of the neuropsychiatric manifestations of COVID-19 in children. Here we describe the clinical course and management of five children with neuropsychiatric symptoms in the setting of acute COVID-19 or during acute MIS-C following known or suspected COVID-19, including three patients with new onset psychosis, one patient with febrile status epilepticus, and one patient with a first-time seizure and encephalopathy. To understand the biological basis of these neuropsychiatric symptoms, we analyzed SARS-CoV-2 antibodies, cytokines, and chemokines in CSF from these children.

2. Methods

2.1. Patient enrollment

This study was approved by the institutional review boards (IRBs) of Emory University (IRB00000723, IRB00113456) and Children's Healthcare of Atlanta (IRB00000885). Children 0 to 21 years of age who were hospitalized at Children's Healthcare of Atlanta (CHOA) from January 1, 2020 to December 31, 2020 with suspected or confirmed symptomatic COVID-19 or MIS-C, as defined by the Centers for Disease Control and Prevention, were prospectively enrolled into a specimen collection protocol following informed parental consent and patient assent, as age appropriate. Residual CSF leftover from clinician-ordered testing was obtained from the clinical laboratory and stored at -80°C until subsequent analysis. CSF was available from 5 COVID-19 patients with neuropsychiatric clinical manifestations, including three with psychosis, one with febrile status epilepticus, and one with new-onset seizures. Four of these patients met the CDC case definition for MIS-C (CDC Health Alert Network, May 14, 2020), whereas one had acute COVID-19. Five additional control CSF specimens were obtained from children who were undergoing a lumbar puncture for evaluation for idiopathic intracranial hypertension following informed consent and assent as age appropriate. No controls had psychiatric symptoms or known history of COVID-19. CSF samples were stored at -80°C until subsequent analysis.

Retrospective chart review was performed on all five pediatric patients who presented with neuropsychiatric symptoms in the setting of either COVID-19 or MIS-C. Chart review was focused on systemic symptoms, neuropsychiatric symptoms, psychiatric history, psychosocial risk factors, blood and cerebrospinal fluid (CSF) test results, imaging, treatment, and outcomes.

2.2. CSF analyses

To measure CSF antibodies to SARS-CoV-2, a V-PLEX COVID-19 Coronavirus Panel 2 (IgG) Kit was obtained from MesoScale Discovery (MSD). IgG antibodies to SARS-CoV-2 S1 N-terminal domain (NTD), SARS-CoV-2 S1 receptor binding domain (RBD), SARS-CoV-2 full-length spike (S), and SARS-CoV-2 nucleocapsid (N) were measured in the CSF according to the manufacturer's protocol at CSF dilutions 1:10 and 1:100. The electrochemiluminescence (ECL) signal was detected by a SECTOR Imager 6000, and data was analyzed using the Discovery Workbench software (MSD). Antibody concentrations were calculated by interpolating the ECL signals to a calibration curve using a 4-parameter logistic fitting model. Final antibody concentrations are expressed in arbitrary units (AU)/mL based on known concentrations of three reference standards according to MSD certificate of analysis.

Cytokine testing was similarly performed using the MSD V-PLEX Proinflammatory Panel 1 (interferon-gamma (IFN-γ), interleukin-1 beta (IL-1β), IL-2, IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13, TNF-α), Cytokine Panel 1 (GM-CSF, IL-1α, IL-5, IL-7, IL-12/23 p40, IL-15, IL-16, IL-17A, TNF-β, VEGF) and Chemokine Panel 1 kit (Eotaxin, MIP-1β, eotaxin-3, TARC, IP-10, MIP-1α, MCP-1, MDC, MCP-4). Neurofilament light chain (NFL), BAFF, APRIL/TNFSF13, CXCL-13/BCA-1, and CXCL9/MIG were assayed using a custom U-PLEX kit from MSD. All MSD assays were performed according to manufacturer instructions and plates were read using a SECTOR Imager 6000 with the MSD Discovery workbench software (MSD). Whereas CSF cytokine and chemokine analyses were performed for all ten patients (including five NP-COVID-19 and five controls), one control patient had insufficient CSF volume available to complete antibody analyses.

2.3. Statistical analyses

Statistical analyses were performed using GraphPad Prism version 9.0. CSF cytokine levels and antibody titers from COVID-19 patients with neuropsychiatric symptoms and controls were compared using Student's t-tests. A heatmap was generated of all the cytokines after normalization of the data to 100% of the highest cytokine value. Pearson's correlation coefficient between SARS-CoV-2 antigen-specific antibody titers and cytokines in the CSF were determined, and linear regression analyses were performed with the coefficients of correlation (R^2) shown. *P* values ≤ 0.05 were considered statistically significant.

3. Results

Five pediatric NP-COVID-19 patients (Table 1) and five pediatric controls were included in this study. Three of the NP-COVID-19 patients (NP-COVID2, NP-COVID3, NP-COVID4) had new onset psychosis and met the case definition for MIS-C, whereas one patient (NP-COVID1) had acute COVID-19 and febrile seizures with encephalopathy. The clinical description of NP-COVID-19 Patient 4 was previously published (Lin et al., 2020). Patient 5 had a history of sickle cell anemia and presented with a possible focal seizure and encephalopathy. However, stroke evaluation was negative and he met the case definition for MIS-C by exclusion. For the controls, four females and one male were included, with ages between eleven and thirteen who underwent LP evaluation for idiopathic intracranial hypertension but were otherwise healthy.

CSF white blood cell count (WBC) along with protein was normal in all NP-COVID-19 patients. Oligoclonal bands were sent in two NP-COVID-19 patients and were negative. Brain magnetic resonance imaging (MRI) studies were performed for 4/5 (80%) patients, with two normal MRIs, one patient with nonspecific diffusion restriction in the corpus callosum and another patient with punctate T2 hyperintensities. Three patients received immunotherapy for MIS-C, consisting of either intravenous immunoglobulin (IVIG) (n=3), corticosteroids (n=2), anakinra (n=1), or tocilizumab (n=1). The cases are discussed below and a summary of clinical symptoms, clinical course, ancillary testing, and

Table 1

Demographic and clinical information, including symptoms, ancillary testing, treatment and outcomes in neuropsychiatric-COVID-19 (NP-COVID-19) patients.

Patient	NP-COVID1	NP-COVID2	NP-COVID3	NP-COVID4	NP-COVID5
Demographics					
Age (years), Sex	1.4, F	15, M	11, F	14, F	1.4, M
Race	NA	Black	NA	Black	Black
Ethnicity	Hispanic	Non-Hispanic	Hispanic	Non-Hispanic	Non-Hispanic
Clinical Features					
Systemic presentation	Fever	Asymptomatic during initial infection. Later MIS-C: fevers, headache, acute kidney injury, abdominal pain	Fever, malaise, hand swelling and erythema	Myocarditis, culture-negative septic shock	History of sickle cell anemia, Fever
Laboratory Values					
CRP (mg/dL)	4.3	18.6	18.2	10.9	0.7
Ferritin (ng/mL)	NA	264.0	516	857	ND
WBC (K/uL)	4.23	8.30	8.35	9.78	2.2
SARS-CoV-2 PCR	Positive	Positive	Negative	Positive	Negative
SARS-CoV-2 IgG	ND	Positive	Positive	ND	Positive
Neuropsychiatric					
Symptoms	Febrile status epilepticus; altered mental status for 3 days	Disorganization, hyper-sexuality, hyper-religiosity, pressured speech, confusion	Visual hallucinations, agitation	Auditory and visual hallucinations, confusion, neurogenic bladder	Altered mental status, seizure like episode, concern for stroke
Psychiatric symptom onset	None	One week after steroid treatment for MIS-C	One week after somatic COVID-19 symptoms	Three days after somatic acute COVID-19 symptoms	None
Neurological Tests					
CSF WBC (cells/uL)	1	0	1	4	2
CSF protein (mg/dL)	19	28	22	32	36
Oligoclonal Bands	ND	Negative	ND	Negative	ND
Autoimmune Encephalitis Panel*	ND	Negative	ND	Negative	ND
Neuroimaging	MRI brain: small punctate T2 hyperintense lesions	MRI/MRA brain normal	NA	MRI brain and spine: Diffusion restriction in corpus collosum	MRI/MRA brain normal
EEG	Slowing with rare sharps	Excessive beta due to benzodiazepine	NA	Diffuse background slowing	Diffuse slowing
ICU admission	Yes	No**	Yes	Yes	Yes
Hospital LOS (days)	5	8	26	12	7
Treatment/Outcome					
Immunotherapy	None	Steroids, IVIG (for MIS-C); later steroids for psychosis	IVIG, high-dose steroids, anakinra, tocilizumab (for MIS-C)	IVIG (for MIS-C)	None
Psychotropic medication	None	Quetiapine, chlorpromazine	None	None	None
Outcome	Resolved	Psychosis resolved, residual anxiety	Psychosis resolved	Psychosis resolved	Resolved

F: female, M: male, NA: Not available, ND: Not determined, MIS-C – multisystem inflammatory syndrome in children

CRP: C-reactive protein, mg = milligram, dL = deciliter, ng = nanogram, mL= milliliter, K = thousand, uL = microliter, CSF: cerebrospinal fluid, MRI: magnetic resonance imaging, MRA: magnetic resonance angiography, IVIG: intravenous immunoglobulin, ICU: intensive care unit, LOS: length of stay

* Note in CSF and serum where indicated were negative, except mildly elevated anti-GAD65 antibody

** This patient was hospitalized in the ICU during his previous hospitalization for MIS-C but not for the current hospitalization.

management are summarized in Table 1.

CSF testing for cytokine and chemokine profiles was performed and compared to a panel of CSF from five pediatric controls. A heatmap of all cytokines and chemokines was generated by normalizing levels to 100% of the highest level of each cytokine (Fig. 1), demonstrating unique cytokine/chemokine profiles in each patient. Pro-inflammatory chemokines MIG, MIP-1β, MDC, and TARC were significantly higher in the CSF of NP-COVID-19 patients compared to controls. Pro-inflammatory chemokine Eotaxin, and pro-inflammatory cytokines including IL-13, and IL-15 also trended toward significance (Fig. 2). While no overall difference in neurofilament light (NFL) chain was detected between NP-COVID-19 and controls, 2/5 (40%) NP-COVID-19 patients had highly elevated levels of NFL (Fig. 2).

We then evaluated the CSF of NP-COVID-19 patients and 4/5 controls for SARS-CoV-2 IgG antibodies using an MSD V-PLEX multiplex panel, which included the full-length spike protein, the spike S1 receptor binding domain (RBD), S1 N-terminal domain (NTD), and nucleocapsid protein. NP-COVID-19 patients had significantly higher CSF IgG antibody titers to SARS-CoV-2 full-length spike, RBD, and NTD when compared to pediatric controls, as no controls had SARS-CoV-2 antibodies, with trends toward significance for nucleocapsid antibody titers (Fig. 3). Antibodies to all four SARS-CoV-2 antigens correlated with pro-inflammatory cytokines and chemokines in the CSF by Pearson's

correlation (Fig. 4). Linear regression analyses demonstrated that antibodies to the N protein correlated most strongly with CSF pro-inflammatory cytokines, including GM-CSF, IL-2, IL-8, IL-13, IP-10, MCP-1, MIP-1 β, and TNF-α (Fig. 5).

3.1. Case summaries

3.1.1. Neuropsychiatric (NP)-COVID-19 patients

3.1.1.1. NP-COVID1. A 17-month-old twin born at 37 weeks with history of hypotonia and global developmental delay presented with febrile status epilepticus. She had a generalized tonic-clonic seizure with a 102°F fever on the day of admission. The seizure abated after multiple doses of benzodiazepines and intravenous fosphenytoin. She tested positive for SARS-CoV-2 by nasopharyngeal-PCR (NP-PCR) and for rhinovirus/enterovirus on a multiplexed respiratory viral panel (Bio-Fire). CSF studies were normal including a negative meningitis-encephalitis panel (BioFire) that included negative enteroviral PCR. Prolonged EEG demonstrated focal slowing with rare sharp waves in the left temporal region with some background slowing but no seizures. MRI demonstrated a few small punctate T2-weighted nonspecific hyperintense lesions but was otherwise normal. She returned to her baseline after three days and was discharged home, with no further seizures. Of
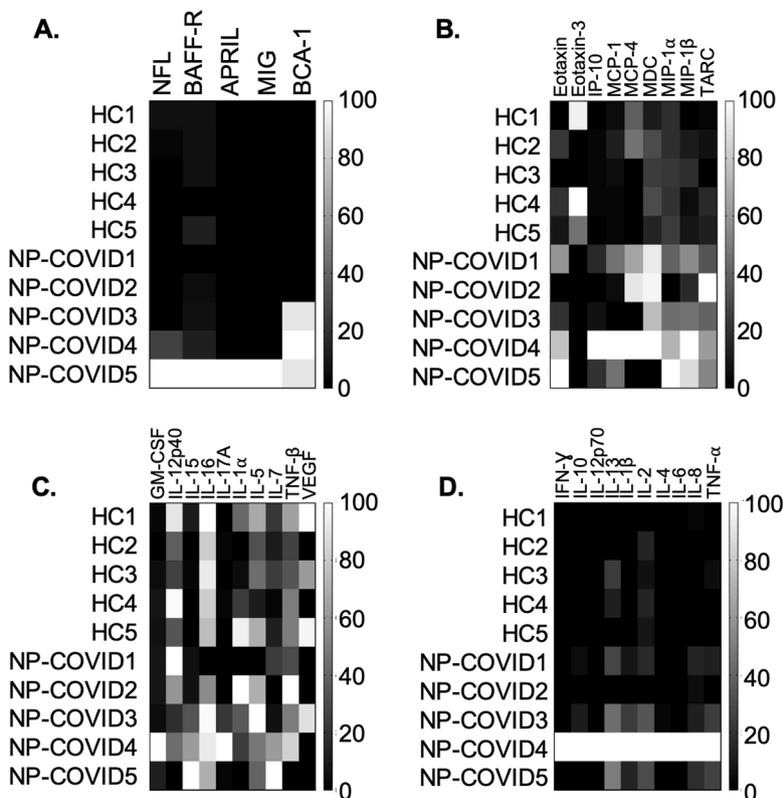
Fig. 1. Cerebrospinal fluid cytokines and chemokines in children with COVID-19 associated neuropsychiatric symptoms and healthy controls. Heatmaps of (A) neuroinflammatory cytokines, (B) chemokines, (C) cytokines, and (D) pro-inflammatory cytokines are shown, with data normalized to the highest concentration detected for each analyte.
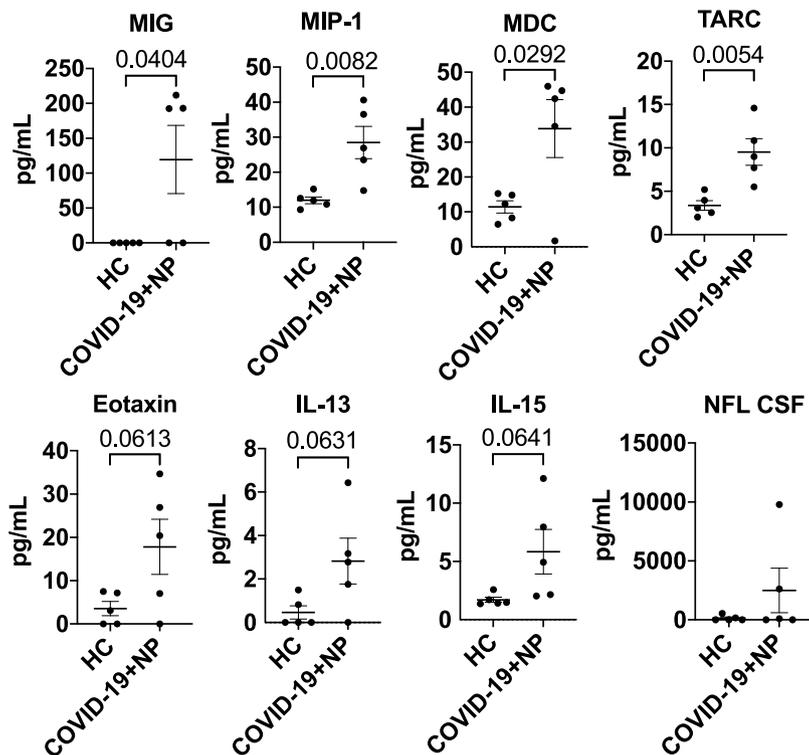
Fig. 2. Comparison of cerebrospinal fluid cytokines and chemokines between children with COVID-19 associated neuropsychiatric symptoms (COVID-19+NP) vs. controls (HC). Statistical comparisons were made using Student's t-test. Comparisons with P-values $\leq$ 0.1 are shown. MIG (Monokine induced by gamma interferon), MIP-1β (macrophage inflammatory protein-1 beta), MDC (Macrophage-derived chemokine), TARC (thymus and activation regulated chemokine), IL-13 = interleukin-13, IL-15 = interleukin-15, and NFL = neurofilament light chain.

note, a possible genetic predisposition was suspected as her identical twin sister also had a febrile seizure during the same week. However, COVID-19 testing was not available for her twin.

3.1.1.2. NP-COVID2. A 15-year-old previously healthy male was hospitalized with MIS-C approximately one month following diagnosis of asymptomatic SARS-CoV-2 infection by a positive SARS-CoV-2 NP-PCR.
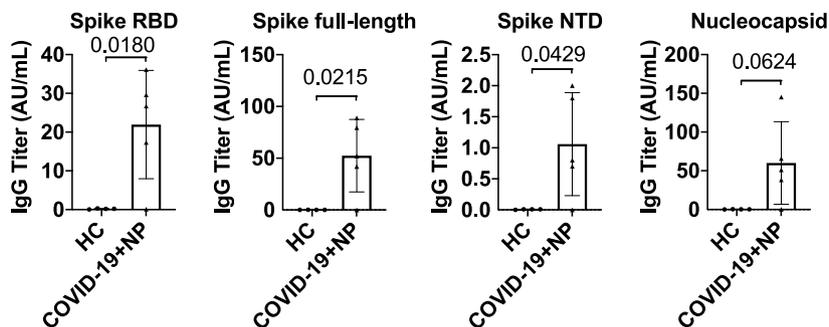
Fig. 3. SARS-CoV-2 antibodies in the cerebrospinal fluid of children with COVID-19 associated neuropsychiatric symptoms vs. controls. Statistical comparisons were made using Student's t-test. P-values are shown. RBD, receptor binding domain. NTD, N-terminal domain. HC, healthy control. COVID-19+NP, child with COVID-19 associated neuropsychiatric symptoms. AU/mL, arbitrary units/mL.
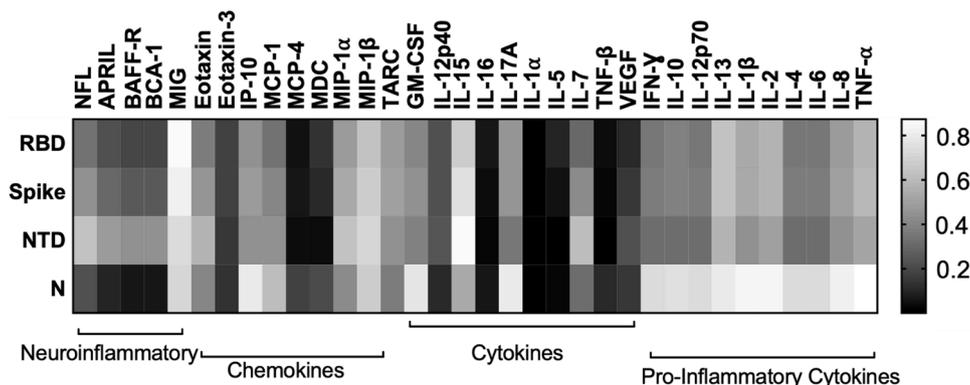

Fig. 4. Heat map showing correlation between SARS-CoV-2 antibodies in the cerebrospinal fluid vs. cytokine levels. Pearson's correlation coefficients were calculated, and the coefficients of determination (R^2) are shown. RBD, receptor binding domain. NTD, N-terminal domain. N, nucleocapsid.
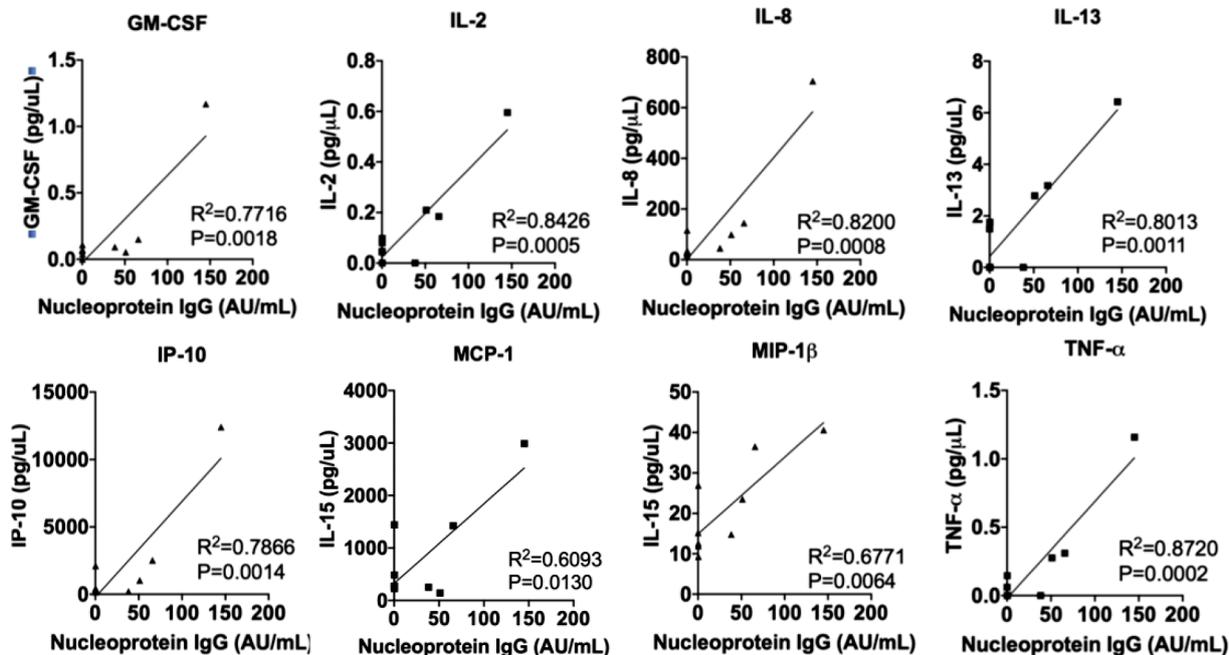

Fig. 5. Linear regression analyses showing the correlation between SARS-CoV-2 nucleocapsid protein IgG antibody titers in the CSF vs. cytokine concentrations. Chemokines and cytokines with statistically significant correlations are shown, with their coefficients of determination (R^2) and P-values. $P \leq 0.05$ was considered statistically significant.

He had no reported past psychiatric history or psychosocial risk factors. His presenting MIS-C symptoms included headache, nausea, abdominal pain, fever, acute renal failure and second-degree heart block. His serum was positive for SARS-CoV-2 nucleocapsid protein IgG (Abbott) with elevated CRP and erythrocyte sedimentation rate (ESR). During his hospitalization for MIS-C, he remained on room air with normal cardiac function. He was treated with intravenous methylprednisolone and IVIG with resolution of his symptoms and normalization of blood inflammatory markers. The patient was discharged home.

Approximately five days after completing steroids, he developed acute onset of progressive confusion, obsession with numbers, hyper-religiosity, hyper-sexuality, visual hallucinations, fast speech, and disorganized thoughts. He was readmitted for acute psychosis. He had normal inflammatory markers and normal CSF cell counts and protein. Magnetic resonance imaging (MRI) and magnetic resonance angiography (MRA) of the brain and EEG were also normal. He was treated with pulse-dose methylprednisolone for five days. Agitation and aggression were managed first by quetiapine, and then more effectively by chlorpromazine. He returned to near baseline on the fifth day of steroids, and he was discharged with both steroid and chlorpromazine tapers. At two-month follow-up, he exhibited no psychotic symptoms and was no longer requiring steroids or chlorpromazine.

During the follow-up appointment, he completed a psychological diagnostic interview and a series of PROMIS (Patient-Reported Outcomes Measurement Information System) short forms assessing anxiety, depressive symptoms, and psychological stress. Based on the diagnostic interview, he met criteria for generalized anxiety disorder; however, his scores on the anxiety and depression short forms were not clinically elevated. The patient also endorsed having a history of milder anxiety prior to his hospitalization for MIS-C. Although his anxiety and depression short forms were within the normal range, his psychological stress experiences short form was clinically elevated.

3.1.1.3. NP-COVID3. An 11-year-old previously healthy girl developed fever, headache, conjunctival injection, rash, hand swelling, and malaise approximately six weeks following acute COVID-19 diagnosis by SARS-CoV-2 NP-PCR. She had psychosocial risk factors including recent immigration to the United States from West Africa and resultant separation from her parents, but no past psychiatric history. She had elevated CRP, transaminitis, and mild thrombocytopenia. On day six of illness, she was admitted for MIS-C and repeat SARS-CoV-2 PCR was negative but SARS-CoV-2 IgG was positive by qualitative nucleocapsid protein assay (Abbott). She developed acute respiratory insufficiency requiring positive pressure ventilation and vasopressor support for shock. On day eight of illness, she hit her head, with normal head computed tomography (CT). She then developed hallucinations on day nine, and, by day ten, she was agitated and encephalopathic requiring intubation for airway protection. She was extubated on day fourteen and returned to normal mental status by day fifteen. She received IVIG, anakinra, methylprednisolone, and tocilizumab for treatment of MIS-C with blood inflammatory markers normalizing over time. By day 26 she was at her clinical baseline.

3.1.1.4. NP-COVID4. A 14-year-old previously healthy female presented to the emergency department with three days of fever, vomiting, diarrhea, cough, and unsteady gait. She had no reported past psychiatric history or psychosocial risk factors. On presentation she had high fever, elevated inflammatory markers, cardiac dysfunction, and shock requiring milrinone and epinephrine. Initial SARS-CoV-2 NP-PCR was negative, but repeat testing on hospital day three was positive, as was SARS-CoV-2 nucleocapsid protein IgG (ARUP). She was diagnosed with MIS-C associated with acute COVID-19. On day one, she developed nonsensical speech and visual and auditory hallucinations. She also developed urinary retention. Brain MRI showed nonspecific restricted diffusion in the splenium of the corpus collosum (Lin et al., 2020), with

normal full MRI spine. Routine CSF studies were normal. EEG showed diffuse slowing consistent with nonspecific encephalopathy. She received IVIG with subsequent resolution of inflammatory markers and neurologic symptoms. Repeat MRI at 6 weeks later showed resolution of the diffusion restriction. At her two-month follow-up appointment, she was at her mental status baseline and had no further immunomodulatory therapy.

3.1.1.5. NP-COVID5. A 17-month-old boy with history of prematurity (born at 28 weeks), sickle cell anemia, and gross motor and speech delays presented with fever, altered mental status and possible seizures approximately one month following COVID-19 exposure. He had a fever the day prior to admission. On day of admission, he had lethargy and an episode of whole-body stiffening. Head CT was normal but his hemoglobin had decreased from 9.0 to 6.8 grams/deciliter with new splenomegaly so he was transferred to our institution with concern for splenic sequestration. En route, he developed multiple episodes of left gaze deviation and lip smacking concerning for possible seizures. He received a blood transfusion for his anemia. MRI/MRA of the head was normal. EEG showed diffuse slowing and captured one event of generalized stiffening without electrographic correlate. CSF studies were normal including a meningitis-encephalitis RT-PCR panel (BioFire). His SARS-CoV-2 NP-PCR was negative, but SARS-CoV-2 nucleocapsid IgG (Abbott) was positive. Otherwise, extensive workup did not reveal an etiology of his symptoms, and he therefore met the case definition for MIS-C by exclusion. His mental status returned to his baseline five days after admission without any immunotherapy.

4. Discussion

In this case series, we describe neuropsychiatric symptoms in pediatric patients with acute COVID-19 and MIS-C, including acute onset psychosis, encephalopathy, and/or seizures. The three patients with acute onset psychosis and one patient with seizures and encephalopathy met the CDC case definition for MIS-C (CDC Health Alert Network, May 14, 2020), whereas one patient with febrile status epilepticus presented with acute COVID-19.

The mechanisms of neuropsychiatric manifestations in patients with COVID-19 or MIS-C are incompletely understood, but are thought to represent a spectrum of distinct clinical syndromes. These have been loosely classified as those associated with direct viral invasion (e.g. SARS-CoV-2 encephalitis), delirium and encephalopathy, post-infectious immune-mediated phenomena, seizures, cerebrovascular events, and psychosocial and cognitive sequelae (Najjar et al., 2020; Lindan et al., 2020). Neuroinflammation stemming from systemic inflammation could then lead to neuropsychiatric symptoms. However, other causes may also be contributing to neuropsychiatric symptoms, including psychosocial risk factors, or iatrogenic factors such as treatment adverse effects (Parra et al., 2020). While one patient in our cohort did have a psychosocial stressor of recent immigration and temporary separation from her parents, it is difficult to determine how this contributed to her clinical presentation. Some patients in our cohort received immunomodulatory therapy for MIS-C, including NP-COVID2 who developed neuropsychiatric symptoms several days following completion of a course of corticosteroids. While his symptoms improved after receiving additional steroids, it is unclear to what extent steroids exacerbated versus alleviated his neuropsychiatric symptoms.

Adult patients with COVID-19 and neurological symptoms often have normal neuroimaging and normal routine CSF studies (including WBC, protein, oligoclonal bands, and IgG index) (Mao et al., 2020). In pediatric patients, however, other imaging findings such as acute disseminated encephalomyelitis, myelitis, and neural enhancement have been reported (Lindan et al., 2020). While treatment with steroids (Pilotto et al., 2020), IVIG (Muccioli et al., 2020a), and tocilizumab (Muccioli et al., 2020b) have been reported in management of COVID-19

related encephalopathy, the majority of adult patients do not seem to require immunotherapy, instead responding to low doses of antipsychotics (Parra et al., 2020; Paterson et al., 2020). In our pediatric NP-COVID-19 cohort, two of five did not receive immunotherapy and had resolution of symptoms. The clinical outcomes of COVID-19-associated neuropsychiatric symptoms in our cohort of patients was very good.

Although MIS-C has previously been associated with encephalopathy in children (Abdel-Mannan et al., 2020), our study is the first to describe psychiatric symptoms in association with MIS-C. We also describe the CSF cytokine and chemokine profiles with evidence of neuroinflammation despite normal routine CSF studies. Moreover, we identified a correlation of CSF cytokines and chemokines with SARS-CoV-2 antibody titers. In our cohort, pro-inflammatory chemokines MIG, MPC, TARC, and MIP-1β were significantly elevated in NP-COVID-19 patients as compared to controls. CSF pro-inflammatory chemokine eotaxin and pro-inflammatory cytokines, including IL-13 and IL-15, also trended toward significance. SARS-CoV-2 antibodies to the full-length spike, RBD, and NTD were significantly elevated, with trends towards significance with nucleocapsid protein antibodies. SARS-CoV-2 antibodies to all antigens tested significantly correlated with multiple pro-inflammatory cytokines and chemokines, which may be in part attributable to systemic hyperinflammatory response with resultant compromise of the blood-brain barrier and neuroinflammation. Interestingly, the patient who presented with febrile status epilepticus in the setting of acute COVID-19 had minimal CSF SARS-CoV-2 antibody titers and inflammatory cytokines. We suspect the pathophysiology of her clinical presentation differed from the others, as she likely had lowering of her seizure threshold during an acute infection in the setting of genetic predisposition with her history of developmental delay, hypotonia, and identical twin with febrile seizures.

Neurofilament light chain (NFL) is expressed in neurons and is a marker for neuronal injury. NFL has been used as a biomarker in other diseases including multiple sclerosis (MS) and Alzheimer's disease (Gordon, 2020). NFL has been reported elevated in 2/6 adult COVID19 patients with neurological symptoms. (Eden et al., 2020) We also observed elevated NFL in two of our patients. NFL has been used in determining long-term prognosis, including predicting clinical progression or neurodegeneration in MS; thus, how CSF NFL is associated with long-term prognosis in COVID-19 patients is yet to be examined.

This case series is limited by its small sample size and varied medical work-up between patients. Moreover, the autoimmune encephalitis panel was only sent on two of five patients. Another limitation is that paired serum/plasma samples were not available in all patients, so whether the CSF cytokine/chemokine signatures reflect a crossing of the blood brain barrier versus CSF specific inflammation is unknown. Our ideal would have been to compare the CSF of NP-COVID-19 group with the CSF of COVID-19 positive patients without neuropsychiatric symptomatology. However, it is not standard of care to obtain CSF samples in these patients, thus limiting the availability of these samples for comparison.

In conclusion, the present case series describes multiple clinical phenotypes of acute neuropsychiatric symptoms in pediatric patients with MIS-C and acute COVID-19. We also identified cytokine, chemokine, and SARS-CoV-2 antibody profiles in children with COVID-19 associated neuropsychiatric symptoms, which may serve as biomarkers for SARS-CoV-2 mediated disease and provide insight into underlying pathophysiology of COVID-19 associated neuroinflammation. Future studies employing a prospective, longitudinal design would help identify the psychosocial and biologic underpinnings of the phenotypes illustrated in this series.

Conflicts of interest

Funding

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author statements

Binh Ngo, MD: methodology, investigation, resources, writing – original draft, writing – review and editing

Stacey A. Lapp, MS, MPH: methodology, investigation, formal analysis, writing – review and editing

Benjamin Siegel, MD: methodology, formal analysis, investigation, writing – original draft, writing – review and editing

Vikash Patel, MS: investigation, edited final version

Laila Hussaini, MS: investigation, edited final version

Sonali Bora, MD: conceptualization, methodology, writing – review and editing

Bryan Philbrook, MD: conceptualization, methodology, writing – review and editing

Kristin Weinschenk, MD: conceptualization, methodology, writing – review and editing

Laura Wright, PhD: investigation, writing – review and editing

Evan J. Anderson, MD: methodology, resources, writing – review and editing

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