Coronavirus Infections in the Central Nervous System and Respiratory Tract Show Distinct Features in Hospitalized Children

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Abstract

Background/Aims: Coronavirus (CoV) infections induce respiratory tract illnesses and central nervous system (CNS) diseases. We aimed to explore the cytokine expression profiles in hospitalized children with CoV-CNS and CoV-respiratory tract infections. ***Methods:*** A total of 183 and 236 hospitalized children with acute encephalitis-like syndrome and respiratory tract infection, respectively, were screened for anti-CoV IgM antibodies. The expression profiles of multiple cytokines were determined in CoV-positive patients. ***Results:*** Anti-CoV IgM antibodies were detected in 22/183 (12.02%) and 26/236 (11.02%) patients with acute encephalitis-like syndrome and respiratory tract infection, respectively. Cytokine analysis revealed that the level of serum granulocyte colony-stimulating factor (G-CSF) was significantly higher in both CoV-CNS and CoV-respiratory tract infection compared with healthy controls. Additionally, the serum level of granulocyte macrophage colony-stimulating factor (GM-CSF) was significantly higher in CoV-CNS infection than in CoV-respiratory tract infection. In patients with CoV-CNS infection, the levels of IL-6, IL-8, MCP-1, and GM-CSF were significantly higher in their cerebrospinal fluid samples than in matched serum samples. ***Conclusion:*** To the best of our knowledge, this is the first report showing a high incidence of CoV infection in hospitalized children, especially with CNS illness. The characteristic cytokine expression profiles in CoV infection indicate the importance of host immune response in disease progression.

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Introduction

Coronavirus (CoV) is an enveloped virus with a large positive-sense, single-stranded RNA genome [1–3] belonging to the Coronaviridae family [4]. Human pathogenic CoVs include HCoV-229E, HCoV-OC43, HCoV-HKU1, HCoV-NL63, severe acute respiratory syndrome CoV (SARS-CoV), and Middle East respiratory syndrome

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CoV (MERS-CoV) [5–7]. Human pathogenic CoVs are associated with a wide range of respiratory illnesses, including common colds, pneumonia, and bronchiolitis [7]. Additionally, several studies have described that CoVs are associated with CNS diseases such as acute disseminated encephalomyelitis and multiple sclerosis [8–10]. Respiratory tract infection contributes to high morbidity and mortality with a worldwide disease burden estimated at 112,900,000 disability-adjusted life years and 3.5 million deaths [11]. Furthermore, the mortality of viral encephalitis ranges from 4.6 to 29% and nearly 50% of survivors are at a high risk of developing neurological disorders [12].

The effect of CoV infection is influenced by various factors, including environmental factors, genetic factors, and immune-mediated process [10]. Cytokines are widely recognized as important mediators of inflammatory response [13]. For instance, IL-6 is a proinflammatory cytokine that induces the terminal differentiation of proliferating B cells to plasma cells, stimulates antibody secretion, and enhances T-lymphocyte responses in secondary lymphoid organs [14]. Further, IL-8 is a C-X-C chemokine that functions as a potent chemotactic agent for polymorphonuclear cells and lymphocytes [15] and is associated with blood-brain barrier breakdown [16]. MCP-1 is a C-C chemokine that can initiate the transmigration of monocytes across the blood-brain barrier [16]. Several studies have shown that IL-6, IL-8, and MCP-1 contribute to severe respiratory disease progression in SARS infections [17–19]. Granulocyte colony-stimulating factor (G-CSF) expression is often induced during CoV infections, resulting in systemic (i.e., in the plasma) and local increases in inflammatory fluids either in mice or humans, such as patients with rheumatoid arthritis and severe respiratory syncytial virus infection [20–22]. Recent studies have suggested that granulocyte macrophage colony-stimulating factor (GM-CSF) also has proinflammatory functions and plays critical roles in the development of autoimmune and inflammatory diseases such as autoimmune encephalomyelitis [23, 24].

In this study, we conducted a comprehensive analysis to investigate clinical features and cytokine profiles in hospitalized children diagnosed with either central nervous system (CNS) or respiratory tract infection of CoV.

Materials and Methods

Patients and Samples

The inclusion criteria in this study for the diagnosis of clinically suspected acute encephalitic patients were modified according to the previous study as follows [25]: (1) age <16 years and (2) hospitalized with at least 2 of the following encephalitis-like symptoms or signs with a duration of illness of <7 days: fever ≥38°C (axillary), headache, neck stiffness, convulsion, altered levels of consciousness >24 h, and focal neurological signs. A total of 183 hospitalized children with clinically suspected acute encephalitis and 236 children with acute respiratory tract infection were enrolled from May 2014 to April 2015 at the Children's Hospital of Chenzhou (Hunan Province, China). On the day of admission, blood samples were collected from patients with respiratory tract infection and paired samples of blood and cerebrospinal fluid were collected from patients with clinically suspected acute encephalitis. Then, to exclude bacteria, fungus, or *Mycobacterium tuberculosis* infection from subsequent data analysis, 1 ml of cerebrospinal fluid specimens were subjected to microbiological investigations. Control blood samples were collected from age-matched children (*n* = 26) without infection who underwent surgery. The patients' clinical data were collected for further analysis. The study's protocol was approved by the Ethics Committee of the Hospital for Human Studies and written consent forms were obtained from the parents of the participants. The course of illness was defined in this study as the period from the onset of symptoms to the disappearance of symptoms.

CoV Detection

All CoVs infections were identified by detection of anti-CoV IgM (Boyan, Shanghai) by ELISA according to the manufacturer's instructions. In brief, 10 μL serum/cerebrospinal fluid samples mixed with 40 μL sample diluents were incubated with pan-CoV antigen-coated 96-well plates, and anti-human IgM antibodies labeled with peroxidase were then analyzed by an automatic microplate reader.

Cytokine Measurement

Expression levels of multiple cytokines (IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12p70, IL-13, IL-17A, G-CSF, GM-CSF, IFN-γ, MCP-1, MIP-1β, and TNF-α) were measured in the serum samples of patients with respiratory tract infection, serum samples of healthy controls, and matched paired serum and cerebrospinal fluid samples of patients with viral encephalitis-like syndrome by the Bio-Plex Assay (Bio-Rad, USA) following the manufacturer's instructions.

Statistical Analyses

Differences between continuous variables were evaluated by the Student *t* test or Mann-Whitney U test, while differences between categorical variables were evaluated by the χ^2 test. Statistical analyses were carried out by the SPSS 18.0 software. The results were considered significant for 2-sided *p* values of ≤0.05.

Results

Clinical Characteristics of Hospitalized Children with CoV Infection in the CNS and Respiratory Tract

Among 183 hospitalized children with clinically suspected acute encephalitis, 22 (12.02%) were identified with CoV infection. Vomiting (36.4%), headache (45.5%), and fever (81.8%) were the most common symptoms of these patients (Table 1). Among CoV-en-

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Table 1. Clinical characteristics of hospitalized children with central nervous system coronavirus infection

Patient characteristics	Values (*n* = 22)
Demographics	
Male	18 (81.8)
Age, months	36.00 (0.83–72.00)
Rural	17 (77.3)
Course of illness[a], day	14.50 (10.00–22.25)
Symptom	
Peak body temperature, °C	38.50 (37.60-39.10)
History of fever	18 (81.8)
Headache	10 (45.5)
Vomiting	8 (36.4)
Diarrhoea	1 (4.5)
Seizure	5 (22.7)
Altered mental status	0 (0)
Sign	
Neck stiffness	7 (31.8)
Kering sign	2 (9.1)
Brudzinski sign	1 (4.5)
Babinski sign	2 (9.1)
Image (normal/abnormal)	
MRI or CT (*n* = 16)	8/8
EEG (*n* = 3)	3/0
Laboratory findings	
Pleocytosis	10 (45.5)
Normal CSF glucose	18 (81.9)
Elevated CSF protein	8 (36.4)
Outcome at discharge	
Full recovery	22 (100)
Mild neurological sequelae	0 (0)

Values are presented as *n* (%) or medians (range). [a] Course of illness is from the onset of symptoms to the disappearance of symptoms.

Table 2. Clinical characteristics of hospitalized children with respiratory tract coronavirus infection

Patient characteristics	Values (*n* = 26)
Demographics	
Male	20 (76.9)
Age, month (range)	12.00 (7.75–60.00)
Rural	15 (57.7)
Course of illness[a], day	7.00 (5.00–8.00)
Symptom	
Peak body temperature, °C	37.30 (36.88–39.40)
History of fever	13 (50.0)
Cough	23 (88.5)
Wheeze	13 (50.0)
Short of breath	1 (3.8)
Produce sputum	8 (30.8)
Vomiting	0 (0)
Diarrhea	1 (3.8)
Radiography	
Interstitial infiltrates	7 (26.9)
Alveolar infiltrates	12 (46.2)
Normal	7 (26.9)
Treatment	
Inhalation of pulmicort respules	22 (84.6)
Oxygen therapy	1 (3.8)
Mechanical ventilation	1 (3.8)
Fiberoptic bronchoscopy alveolar lavage	7 (26.9)
Outcome at discharge	
Full recovery	22 (84.6)
Deterioration	4 (15.4)

Values are presented as *n* (%) or medians (range). [a] Course of illness is from the onset of symptom to the disappearance of symptom.

cephalitis patients, there were 18 males and 4 females with an average age of 36 months. Most of these patients (77.3%) lived in rural areas. Sixteen patients underwent MRI or CT, of which 8 (50%) showed abnormal imaging accompanied with corresponding neurological defects. Among these 8 patients, 2 (25%), including 1 with CT and 1 with MRI, showed abnormity located in the temporal lobe accompanied with seizures; 2 patients (25%) with MRI showed abnormity located in the periventricular region accompanied with headaches; and 4 patients (50%), including 1 patient with CT and 3 patients with MRI, showed abnormity located in the basal ganglia and thalamus accompanied with fever and/or vomiting. In this study, cerebrospinal fluid was analyzed for all patients with CoV-associated encephalitis. Ten patients (45.5%) presented with cerebrospinal fluid pleocytosis,

18 (81.9%) showed normal cerebrospinal fluid glucose, and 8 (36.4%) had elevated cerebrospinal fluid protein levels. Three of the 22 patients with CoV-associated encephalitis underwent EEG, and all the results were normal (Table 1).

Among the 236 hospitalized children with acute respiratory tract symptoms, 26 (11.02%) were identified as having CoV infection. The main symptoms were coughing (88.5%), wheezing (50%), and fever (50%) (Table 2). There were 20 males and 6 females with an average age of 12 months. Also, most of these patients (57.7%) lived in rural areas, and 22 patients (84.6%) received treatment with Pulmicort Respules (inhalation). All the patients with respiratory tract infection had undergone a chest X-ray: 7 (26.9%) showed interstitial infiltrates, which included 3 sole interstitial infiltrates and 4 bilateral intersti-

Fig. 1. Peripheral blood cell counts of lymphocytes (**a**), eosinophils (**b**), neutrophils (**c**), and monocytes (**d**) among patients with coronavirus infection of the central nervous system (CoV-CNS, $n = 22$), patients with coronavirus infection of the respiratory tract (CoV-respiratory tract, $n = 26$), and healthy controls ($n = 26$). Cell counts are expressed as 10^9 cells/L. Data are plotted as medians with the 10th–90th percentile ranges. The Student t test or Mann-Whitney U test was performed. p values were derived from a two-tailed test; * $p \leq 0.05$.
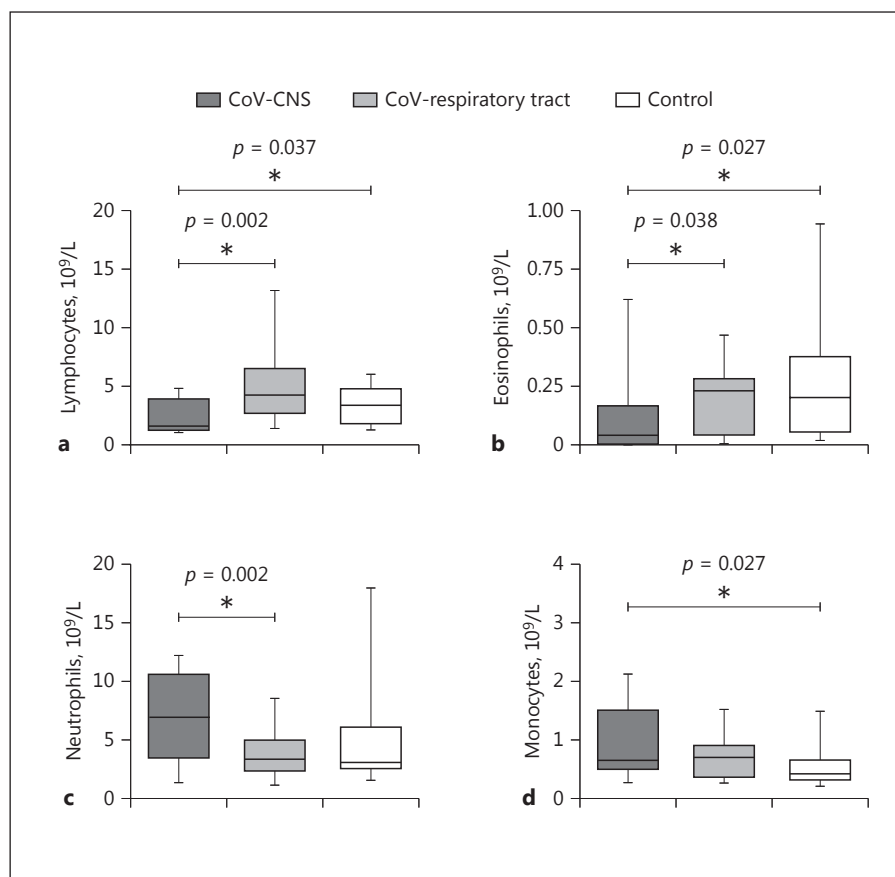
tial infiltrates; 12 (46.2%) had alveolar infiltrates; and 7 (26.9%) had a normal chest X-ray finding (Table 2).

CoV Infection Changes Peripheral Blood Cell Count

To further explore the difference between CoV-CNS and CoV-respiratory tract infections, the peripheral blood cell count was analyzed in both infections. Our results show that lymphocyte and eosinophil counts were significantly lower in patients with CoV-CNS infection than in patients with CoV-respiratory tract infection and healthy controls (Fig. 1a, b). In contrast, the neutrophil cell count was significantly lower in patients with CoV-respiratory tract infection than in patients with CoV-CNS infection (Fig. 1c). The monocyte count was significantly higher in patients with CoV-CNS infection than in healthy controls (Fig. 1d).

Cytokine Expression by CoV Infection in the CNS and Respiratory Tract

To further characterize host immune response in CoV-CNS and CoV-respiratory tract infections, a cyto-kine expression profile was determined using serum and/ or matched cerebrospinal fluid samples. This analysis revealed that the serum level of G-CSF was not significantly different between patients with CoV-CNS and CoV-respiratory tract infections, but it was significantly higher in both of these patient groups than in healthy controls (Fig. 2a). The serum level of GM-CSF was significantly higher in patients with CoV-CNS infection than in patients with CoV-respiratory tract infection and healthy controls (Fig. 2b). Other cytokines showed similar expression profiles for patients with CoV infections in the CNS and respiratory tract as well as for healthy controls (data not shown).

Additionally, an analysis of the cytokine expression profile in matched-paired serum/cerebrospinal fluid samples of CoV-CNS patients showed that expression levels of GM-CSF, IL-6, IL-8, and MCP-1 were significantly higher in cerebrospinal fluid than in the serum (Fig. 3a–d). Other cytokines also showed similar expression profiles in matched cerebrospinal fluid and serum samples (data not shown).

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Fig. 2. Expression levels of serum G-CSF (**a**) and GM-CSF (**b**) among patients with coronavirus infection of the central nervous system (CoV-CNS, $n = 22$), patients with coronavirus infection of the respiratory tract (CoV-respiratory tract, $n = 26$), and healthy controls ($n = 26$). Data are expressed as medians with the 10–90th percentile ranges. The Student t test or Mann-Whitney U test was performed. p values were derived from a two-tailed test; * $p \leq 0.05$.
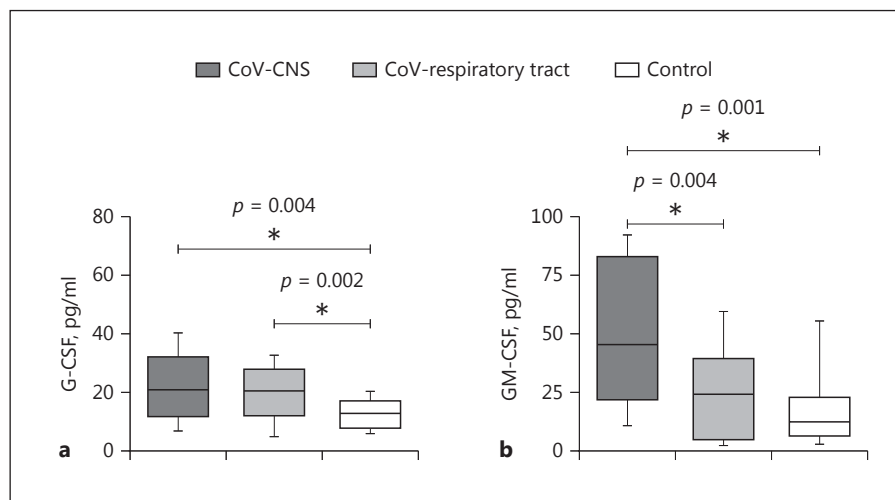

Fig. 3. Expression levels of GM-CSF (**a**), IL-6 (**b**), IL-8 (**c**), and MCP-1 (**d**) levels in matched serum (PLA) and cerebrospinal fluid (CSF) from patients with coronavirus infection (CoV) of the central nervous system ($n = 17$). Data are expressed as medians with the 10–90th percentile ranges. Student's t tests or Mann-Whitney U tests were performed. p values were derived from a two-tailed test; * $p \leq 0.05$, ** $p \leq 0.001$.
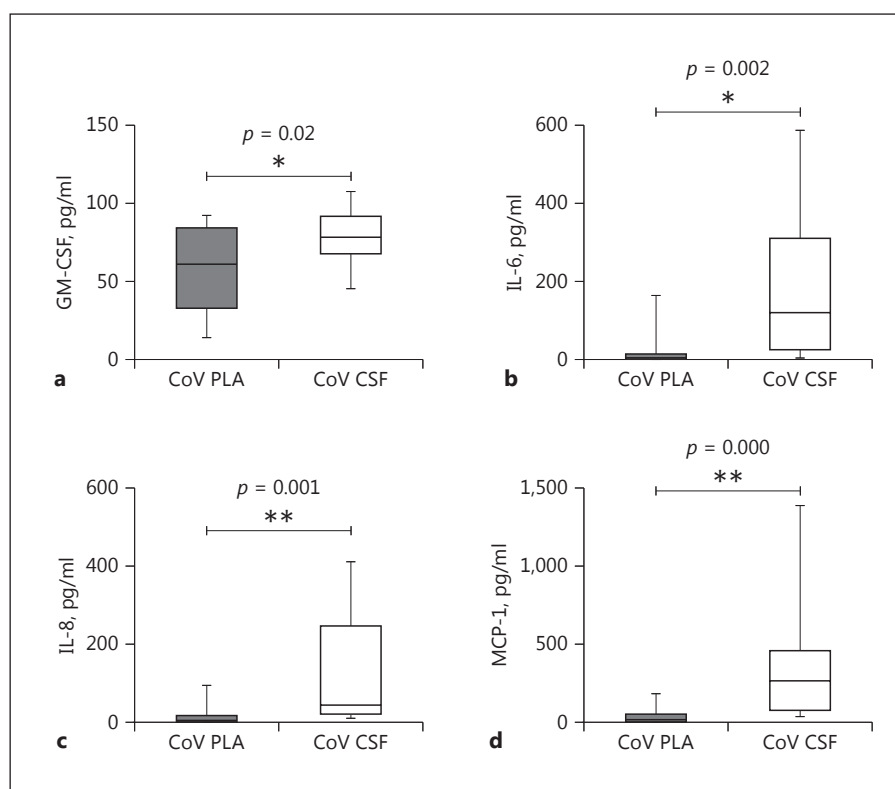
Discussion

CoV infection was observed in 12.02% of hospitalized children with CNS infection and 11.02% of hospitalized children with acute respiratory tract infection. While the incidence of CoV infection in children with acute respiratory tract infection has been previously illustrated [1], to our knowledge this is the first report showing the high incidence of CoV infection in hospitalized children with CNS infection. Since there was no significant difference in the age of patients with CoV-CNS infection, CoV-respiratory tract infection, and healthy controls, the difference in immune blood cell counts between CNS and respiratory tract infection could not be attributed to the difference in patient age, but rather to the difference in the nature of CoV tropism.

We observed a significant increase in the serum G-CSF level in patients with either CoV-CNS or CoV-respiratory tract infections. G-CSF is one of the key regulators of granulocytosis, which plays a central role in stimulating the proliferation of granulocytic precursors, enhances their terminal differentiation, and stimulates their release from the bone marrow into the peripheral blood [20]. In addition, GM-CSF stimulates stem cells to produce granulocytes (neutrophils, eosinophils, and basophils) and monocytes [26]. Our results showed that (1) CoV-CNS infection induces a high level of GM-CSF either in serum or cerebrospinal fluid, and (2) peripheral cell counts of neutrophil cells and monocytes were significantly higher in patients with CoV-CNS infection than in patients with CoV-respiratory tract infection and healthy controls. These findings may suggest that GM-CSF plays an important role in controlling CNS infection through inducing neutrophils and monocyte proliferation and/or accumulation in the infection site [27]. To our knowledge, GM-CSF can promote leukocyte chemotaxis and adhesion, upregulate the antimicrobial functions of neutrophils as a secondary immune response to the virus, and provide protection against viral encephalitis [28–30]. In addition, the increased neutrophils can afford protection against viral infection. Several reports have implied direct roles for them in the targeting of infected cells or virions: neutrophils can adhere to infected cells after complement activation and phagocytose antibody-coated virions [31, 32]. However, this issue needs to be further investigated through in vitro studies examining the production of GM-CSF from blood cells isolated from patients with CoV-CNS.

Although high serum levels of IL-6, IL-8, and MCP-1 in SARS- and MERS-infected patients have been illustrated [17–19], we did not find a significant increase in the serum level of these cytokines among our patient cohort. However, we found that IL-6, IL-8, and MCP-1 were significantly accumulated in the cerebrospinal fluid of patients with CoV-CNS infection. IL-6 has neurotrophic and neuroprotective effects and can increase blood-brain barrier permeability [33]. A high level of IL-6 leads to progressive neurological disorders with neurodegeneration and cognitive decline [34]. The higher level of cerebrospinal fluid IL-8 seen in this study is consistent with the fact that CNS viral infection might induce proliferation of microglia and astrocytes, resulting in the release of IL-8 [35]. An experimental Japanese encephalitis mouse model demonstrated that IL-8 plays an important role in inflammatory responses involving injury to the brain [36]. MCP-1 is a C-C chemokine that can initiate the transmigration of monocytes across the blood-brain barrier and recruit inflammatory cells into the CNS, thereby facilitating the entry of virus-infected cells, as well as amplifying the inflammatory response, which damage the brain [16, 37]. These accumulated cytokines may also contribute to immune damage in the CNS of patients with CoV infection similar to that observed in other viral encephalitis.

Even though there are important outcomes of this study, there are also several limitations: (1) the sample size was small and (2) we collected samples only at admission. Future studies with a larger sample size analyzing blood and cerebrospinal fluid samples collected at multiple time points are needed to monitor the kinetics of cytokine expression profile during the course of illness.

In conclusion, this study suggests that CoV infection of the CNS is common and multiple cytokine expression profiles are involved in the initial host's immune response to the infection, which could induce immune impairment in the brain. Therefore, this study highlights the importance of the neurotropic ability of CoV and its involvement in the CNS, especially in children who need more attention to control this serious viral infection.

Disclosure Statement

All authors have no conflicts of interest regarding the work reported in this paper.

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