Journal of Microbiology, Immunology and Infection 56 (2023) 1169—1177

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.e-jmii.com

Original Article

Risk factors of Omicron variant associated acute encephalitis/encephalopathy in children

Chong-Wei Huang [a,b], Jainn-Jim Lin [a,d,e], Chen-Yen Kuo [a,b], Kuang-Lin Lin [a,e], Yhu-Chering Huang [a,b], Cheng-Hsun Chiu [a,b,f], Yi-Ching Chen [a,b], Chih-Ho Chen [c,f], Yu-Chia Hsieh [a,b,*]

[a] *Department of Pediatrics, Chang Gung Children's Hospital, Chang Gung Memorial Hospital, Chang Gung University, College of Medicine, Taoyuan, Taiwan*
[b] *Division of Pediatric Infectious Diseases, Department of Pediatrics, Chang Gung Memorial Hospital, Chang Gung University College of Medicine, Taoyuan, Taiwan*
[c] *Division of Pediatric Infectious Diseases, Department of Pediatrics, Kaohsiung Chang Gung Memorial Hospital, Kaohsiung, Taiwan*
[d] *Division of Pediatric Critical Care, Department of Pediatrics, Chang Gung Memorial Hospital, Taoyuan, Taiwan*
[e] *Division of Pediatric Neurology, Department of Pediatrics, Chang Gung Children's Hospital and Chang Gung Memorial Hospital, Taoyuan, Taiwan*
[f] *Molecular Infectious Disease Research Center, Chang Gung Memorial Hospital, Taoyuan, Taiwan*

Received 10 March 2023; received in revised form 29 August 2023; accepted 30 August 2023
Available online 4 September 2023

KEYWORDS
COVID-19;
Encephalitis;
Encephalopathy;
Omicron

Abstract *Background:* Outbreak of Omicron BA.2 in Taiwan led to an increased number of acute encephalitis/encephalopathy cases in children and several fatal cases drew public attention. In pre-Omicron period, pediatric cases of COVID-19-associated acute encephalitis have been reported and during Omicron epidemic, febrile convulsions, encephalitis were mentioned more frequently. The outcome of patients with neurological complications was worse. However, few studies investigated the risk factors, pathophysiology and prognosis of COVID-19-associated encephalitis/encephalopathy. Here, we describe the presentation of pediatric cases of COVID-19-associated acute encephalitis/encephalopathy and explore the associated risk factors.
Methods: Pediatric patients with confirmed SARS-CoV-2 infections were prospectively enrolled at admission at Chang Gung Memorial Hospital between April and August 2022. Patients were categorized into groups of acute encephalitis/encephalopathy, febrile convulsions or mild disease. Demographic descriptions, clinical manifestations and laboratory data were collected.

* Corresponding author. Department of Pediatrics, Linkou Chang Gung Memorial Hospital, No. 5, Fuxing Street, Guishan District, Taoyuan City 333, Taiwan. Fax: +886 3 3286210.
E-mail address: ychsieh@cgmh.org.tw (Y.-C. Hsieh).

https://doi.org/10.1016/j.jmii.2023.08.010

Results: Of 288 acute COVID-19 patients, there were 38 (13.2%) acute encephalitis/encephalopathy, 40 (13.9%) febrile convulsions, and 210 (72.9%) mild disease. Among acute encephalitis/encephalopathy group, the mean age was 68.3 ± 45.0 months. The common neurological symptoms were lethargy (65.8%), seizures (52.6%), and impaired consciousness (34.2%). Over 3 years old (adjusted odds ratio [aOR]: 7.57, $p < 0.001$), absolute neutrophil count ≥3150/μL (aOR: 5.46, $p = 0.008$), and procalcitonin ≥0.5 ng/mL (aOR: 4.32, $p = 0.021$) were independent factors for acute encephalitis/encephalopathy.
Conclusions: Most cases of COVID-19-associated acute encephalitis/encephalopathy showed no evidence of direct viral invasion but associations with older age, increased peripheral neutrophil, and serum procalcitonin. These findings may imply the neutrophil-mediated systemic inflammatory response plays an important role on central nerve system, leading to cerebral dysfunction.

Introduction

The emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has had a significant global impact in the last three years. The Omicron variant first reported in South Africa in November 2021 has rapidly replaced the Delta variant globally, and BA.2 sub-variant became the dominant strain in the US between March and June, 2022.[1] In this wave, nearly one-fifth of the cases occurred in children, among whom, 1.5% required hospitalization, 20% admitted due to seizure.[2,3] In hospitalized children, 7–24.8% had neurological involvements, and 0.4–9.1% patients had encephalitis/encephalopathy, which were more common in Omicron-predominant period.[4–6] The outcome of the patients with neurologic complications was worse with longer length of hospital stay, more intensive care unit (ICU) admissions, and higher mortality rate.[4] The spectrum of neurological involvement ranges widely. In the pre-Omicron period, studies focused on life-threatening conditions such as severe encephalopathy, stroke, acute fulminant cerebral edema, Guillain-Barre syndrome and acute disseminated encephalomyelitis (ADEM) in adults or children with chronic comorbidities.[7,8] In the Omicron period, increasing numbers of children admitted owing to febrile seizures, convulsions or other severe neurological complications including severe encephalitis/encephalopathy, aseptic meningitis, and acute necrotizing encephalitis in childhood (ANEC) have been described.[9]

In Taiwan, COVID-19 vaccine coverage rate for children ≤11 years old was low during the first half of 2022. However, due to previous strict non-pharmaceutical interventions policies, the COVID-19 pandemic did not significantly affect Taiwan until the outbreak of the Omicron BA.2 variant in April 2022. Burden of hospitalization has received exclusive attention. Due to a fulminant disease course with poor outcomes in several cases during the first Omicron pandemic, COVID-19-associated encephalitis in pediatric patients was highly publicized in Taiwanese society.[10,11] Therefore, COVID-19-associated acute encephalitis/encephalopathy became our interests.

In the acute phase of COVID-19, seizures, altered consciousness, lethargy, disorientation, hallucinations, and behavioral changes are symptoms of acute encephalitis/encephalopathy. The disease course can be benign, with spontaneous resolution, and it can be morbid or fatal due to rapid progression.[12] It involves several mechanisms, including direct viral invasion, immune-mediated hyperinflammatory response, hypoxia, and metabolic derangements.[12] Direct virus invasion demonstrated possible SARS-CoV-2 neurotropic properties, either hematogenous by infecting central nerve system (CNS) endothelial cells and gaining access across the brain–blood barrier (BBB) or olfactory axonal retrograde dissemination.[13] The second major mechanism is the activation of the innate and adaptive immune systems and an upgraded inflammatory cytokine storm that crosses the disrupted BBB.[14]

Most of the current studies on COVID-19 encephalitis/encephalopathy in children have consisted of case reports or case series, showing a higher incidence rate among patients with chronic illnesses, while rarely discussing the risk factors. We observed that a subset of previously healthy children with acute Omicron infection also developed encephalitis. This study aimed to elucidate the clinical manifestations of COVID-19-associated acute encephalitis/encephalopathy in previously healthy children as well as to identify the risk factors of acute encephalitis/encephalopathy and those associated with impaired consciousness.

Methods

Study population and design

All patients were prospectively enrolled in the admission date at two medical centers, Chang Gung Memorial Hospital – Lin Kou branch and Chang Gung Memorial Hospital – Kaohsiung branch between April 1, 2022, and August 15, 2022, and between May 5, 2022, and July 17, 2022, respectively.

Eligible participants were inpatients ≤18 years with confirmed SARS-CoV-2 infection within 7 days of the acute infection phase. We excluded patients with pre-existing chronic medical conditions (Supplementary Document). Additionally, we excluded patients with incidental positive SARS-CoV-2 test but admitted due to other infections,

patients diagnosed with SARS-CoV-2 over seven days before admission. For the purpose of finding associated factors with acute encephalitis/encephalopathy, we categorized non-encephalitis/encephalopathy patients into two comparison groups, mild disease and febrile convulsion group according to clinical manifestation. Croup patients have distinct clinical features. Moderate to severe cases of croup need active treatment and may even require ICU care; therefore, to remove the confounding factors, they are excluded from the mild disease group. The diagnosis and severity of croup is clinically made (Supplementary Document).

The diagnosis of COVID-19 was based on either positive real-time polymerase chain reaction (PCR) or rapid antigen test results for SARS-CoV-2 in nasopharyngeal or throat swabs. The blood samples were collected at the emergency department or on the first day of admission after obtaining informed consent.

Clinical and laboratory evaluation

We collected demographic, biochemical and radiological data from electronic medical and nursing records of the patients, including age, sex, typical symptoms (fever, cough, rhinorrhea, diarrhea, and abdominal pain), and nervous system symptoms. Patient histories, clinical courses, and diagnoses were confirmed by physicians (pediatric infectiologists, pediatric neurologists, or general pediatric fellows). Laboratory data, including full blood count, differential counts, biochemical data, and serum inflammatory markers were recorded.

Clinical definitions

Encephalitis/encephalopathy

In clinical practice, the definition of acute encephalitis/encephalopathy is based on the International Encephalitis Consortium definition, Brighton criteria.[15–17] The criterion for acute encephalitis is defined as encephalopathy (altered level of consciousness including personality or behavior changes, lethargy, irritation) lasting >24 h with at least two of the following item: [1] fever ≥38 °C, [2] seizure, [3] new onset focal neurological signs, [4] cerebrospinal fluid (CSF) pleocytosis (≥5 WBC/μL in children >2 months old, ≥9 WBC/μL in infant 29–56 days old, ≥19 WBC/μL in infant ≤28 days old), or increased CSF protein content >40 mg/dL; [5] electroencephalography (EEG) compatible with encephalitis e.g.,: diffuse or focal slow activity or epileptiform; [6] abnormal neuroimaging results consistent with encephalitis (Supplementary Document).[15–17] Herein, we analyzed these diagnostic criteria for acute encephalitis/encephalopathy and excluded post-infectious encephalitis due to different mechanism from acute viral encephalitis. That the encephalitis occurs when fever lasts over one week or fever relapses after subsides is considered as post-infectious encephalitis. Impaired consciousness was defined by Glasgow Coma Scale ≤12 and febrile convulsions are defined as previously described (Supplementary Document).

Outcome

The primary outcome is defined as 30-mortality or neurological sequelae on 1-month outpatient department (OPD) follow-up (Supplementary Document).

Statistical analysis

Descriptive continuous data are described as median with interquartile ranges or means with standard deviations, while categorical data are presented as percentages. The chi-square (X^2) test or Fisher's exact test was used to compare categorical data. Independent t-test or one-way ANOVA was used to compare continuous variables among the mild disease, febrile seizure, and acute encephalitis/encephalopathy groups. The Youden's J test was used to determine the optimal cut point values in statistically significant variables. Binary logistic regression was used to model the characteristic factors of acute encephalitis/encephalopathy and impaired consciousness. IBM SPSS for windows version 25.0 (SPSS Corp., Armonk, NY, USA) was used for statistical analysis. $P < 0.05$ was considered to be statistically significant.

Ethical approval

The Institutional Review Board of the Chang Gung Memorial Hospital approved this study (approval number: 202200145A3).

Results

During the study period, 418 children with laboratory-confirmed COVID-19 were admitted to our hospital. 357 (85%) patients were previously healthy after excluding 61 (15%) patients with chronic medical condition. Subsequently, we excluded patients with incidental COVID-19 (n = 26) and positive COVID-19 PCR results (n = 3) over 1 week before admission. The clinical data of 328 patients representing for acute COVID-19 illness in previously healthy children was shown in Supplementary Table 1. For removing other confounding factors, we excluded the moderate and severe croup patients (n = 40). Finally, 288 patients were divided into three groups: mild disease 210 (72.9%) patients, febrile convulsions 40 (13.9%) patients, and acute encephalitis/encephalopathy 38 (13.2%) patients (Fig. 1).

In the mild disease group, the mean age was 36.3 ± 43.4 months, 119 (56.7%) patients were boys and the mean fever duration was 1.9 ± 1.2 days. The top three diagnoses in the mild disease group were as follows: upper respiratory tract infection (31.9%), young infant fever (13.8%), and mild croup (10.5%) (Supplementary Table 2).

Demographic and clinical characteristics

In the acute encephalitis/encephalopathy group, the mean age was 68.3 months (SD 45.0) (2.5–171 months), which was significantly higher than the mean age of the other two groups (p < 0.001), and 71.1% of the children were ≥3

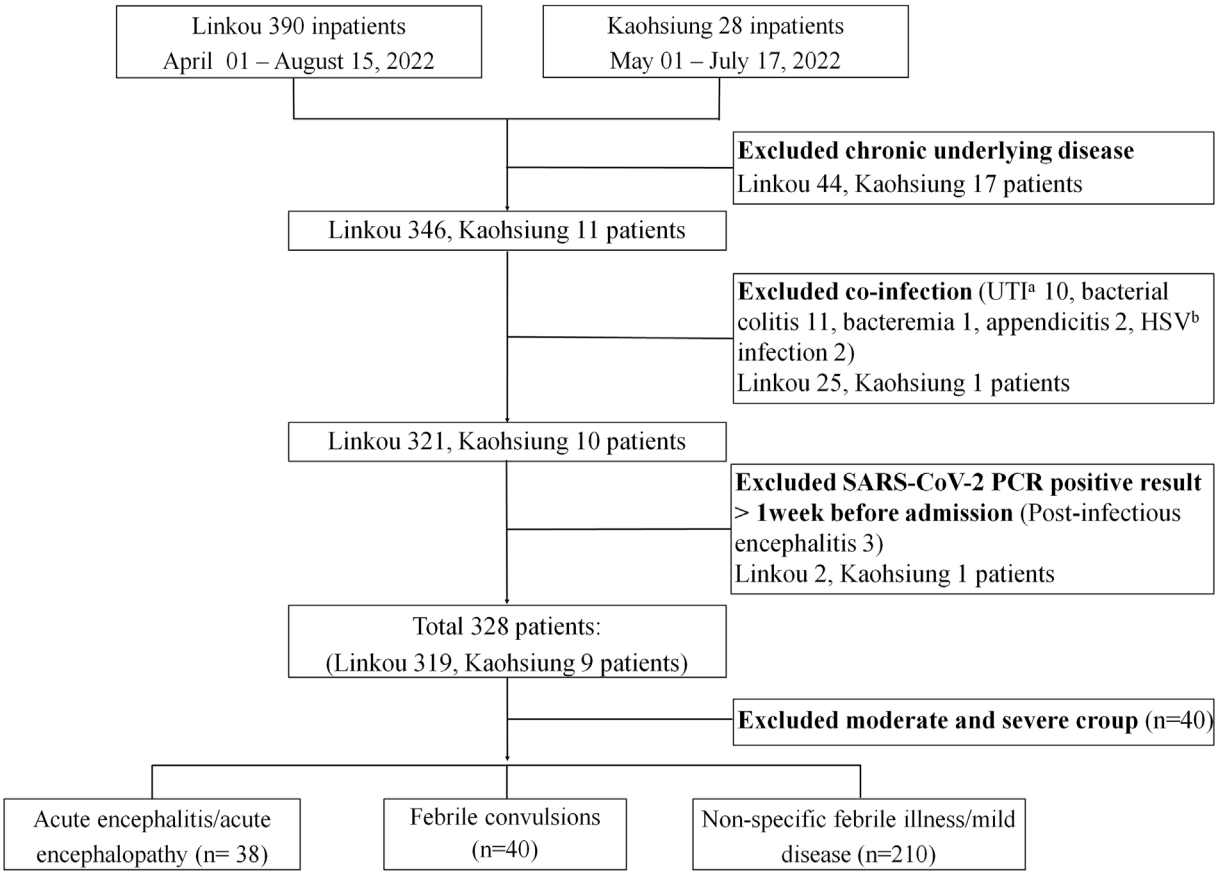

Figure 1. Flow chart describing case selection. [a]Urinary tract infection. [b]Herpes simplex virus.

years; 23 (60.5%) patients were boys (Table 1). Furthermore, the acute encephalitis/encephalopathy group was significantly characterized by longer length of hospital stay (mean [SD] days, 5.8 [3.0] vs. 4.4 [2.8]; p = 0.003), high fever with peak temperature of 39.2 °C (mean [SD] [0.76] vs. 39.13 [0.76]; p = 0.013) than in mild disease group; fever duration and lower throat/nasopharyngeal cycle threshold value was not significantly different between the three groups (Table 1).

Laboratory parameter

The elevated blood neutrophil, lymphopenia and higher neutrophil-to-lymphocyte ratio were significantly seen in acute encephalitis/encephalopathy group than in the mild disease group; a lower platelet count was found in the acute encephalitis/encephalopathy group; however, only 1 (2.7%) patient had thrombocytopenia (Table 1). Patients with acute encephalitis/encephalopathy were prone to elevated serum procalcitonin (PCT) levels than patients with mild disease or with febrile convulsions (percentage test of PCT ≥0.5 ng/mL: 43.5% vs. 11.4% vs. 13.3%, p = 0.001) (Table 1).

Acute encephalitis/encephalopathy

The number of children with COVID-19—associated acute encephalitis/encephalopathy in children increased simultaneously with the peak number of COVID-19 infection-hospitalized patients (Fig. 2). In the 38 cases, 37 (97.4%) patients had fever and the top four neurological symptoms were ranked as follows: lethargy (65.8%), seizure (52.6%), impaired consciousness (GCS ≤12) (34.2%), headache/dizziness (31.6%) (Supplementary Table 3). Of the 38 patients, 12 (31.6%) required ICU care, 2 (5.2%) patients were under mechanical ventilator support and 1 (2.6%) patient died due to ANEC with acute fulminant cerebral edema. Patients with acute encephalitis/encephalopathy with impaired consciousness (GCS ≤12) as symptom presentation were all admitted in ICU and had significantly higher percentage of seizure attack than patients without impaired consciousness (76.9% vs. 40%, p = 0.043) (Table 2). With or without impaired consciousness, over 16% of patients had normal serum CRP levels and there was no significant difference in viral load was found in the throat or nasopharynx (Table 2).

Table 2 and Supplementary Table 4 show subsequent surveys on acute encephalitis/encephalopathy. In the CSF analysis, 14 of the 38 patients underwent spinal tapping; only one patient was diagnosed with rhombencephalitis and had mild pleocytosis in CSF and one patient with fulminant cerebral edema had elevated CSF protein level. All CSF samples for SARS-CoV-2 PCR were negative. Seven of the 28 patients showed positive results on neuroimaging, using either computed tomography (CT) or magnetic resonance imaging (MRI). The MRI neuroimaging features included occipital corona radiata hyperintense spots,

Table 1 Characteristics of included pediatric patients with acute SARS-CoV-2 infection patients according to mild disease, febrile convulsions and acute encephalitis/encephalopathy.

	Mild disease (n = 210)	Febrile convulsions (n = 40)	Acute Encephalitis/ Encephalopathy (n = 38)	P value
Age, mean ± SD, months	36.26 ± 43.41	27.39 ± 13.85	68.32 ± 45.03	<0.001
Age ≥36 months old, n (%)	70 (33.3)	7 (17.5)	27 (71.1)	<0.001
Sex, no (%)				
Female	91 (43.3)	17 (42.5)	15 (39.5)	0.906
Male	119 (56.7)	23 (57.5)	23 (60.5)	
LOS, mean ± SD, days	4.38 ± 2.83	3.90 ± 1.77	5.76 ± 3.03	0.003
NP Ct value, mean ± SD				
Ct value of documented at admission	18.85 ± 6.06 (n = 170)	18.26 ± 6.37 (n = 31)	16.87 ± 4.01 (n = 32)	0.213
Ct value of symptoms onset in 24 h	18.20 ± 5.72 (n = 145)	18.26 ± 6.37 (n = 31)	15.71 ± 3.04 (n = 23)	0.133
Ct value of symptoms onset in 48 h	18.51 ± 5.76 (n = 156)	18.26 ± 6.37 (n = 31)	15.71 ± 2.91 (n = 27)	0.057
Fever, n (%)	184 (87.6)	40 (100)	37 (97.4)	0.015
Duration, mean ± SD, days	1.92 ± 1.18	2.05 ± 0.94	1.78 ± 0.92	0.578
Peak, mean ± SD, °C	39.13 ± 0.76	39.31 ± 0.60	39.20 ± 0.76	0.013
Laboratory data, mean ± SD				
WBC, 1000/μL	7.15 ± 3.20	7.11 ± 2.02	6.37 ± 2.32	0.329
Neutrophil, 1000/μL	3.83 ± 2.79 (n = 206)	5.11 ± 1.56 (n = 38)	4.53 ± 2.41 (n = 37)	0.013
ANC ≥3150/μL, n (%)	98 (47.6)	34 (89.5)	28 (75.7)	<0.001
Lymphocyte, 1000/μL	2.47 ± 1.69	1.24 ± 0.68	1.32 ± 0.78	<0.001
Segment, %	51.37 ± 21.68	71.99 ± 9.49	68.16 ± 2.74	<0.001
Lymphocyte, %	35.99 ± 19.46	17.55 ± 7.94	23.46 ± 15.49	<0.001
N/L ratio	2.81 ± 3.77	5.74 ± 4.41	6.29 ± 7.28	<0.001
Hemoglobin, g/dL	12.06 ± 1.77	12.14 ± 0.72	12.60 ± 1.09	0.161
Platelet, 1000/μL	289.50 ± 108.58	246.71 ± 61.31	233.62 ± 60.60	0.001
D-dimer, ng/mL	1090.9 ± 1086.4 (n = 44)	473.1 ± 255.8 (n = 7)	1146.4 ± 2061.9 (n = 23)	0.529
CRP, mg/L	8.18 ± 21.19 (n = 204)	2.70 ± 2.45 (n = 40)	7.35 ± 14.15 (n = 37)	0.244
Procalcitonin, ng/mL	0.27 ± 0.48 (n = 105)	1.08 ± 2.57 (n = 15)	2.39 ± 4.23 (n = 23)	<0.001
PCT ≥0.5 ng/mL, n (%)	12 (11.4)	2 (13.3)	10 (43.5)	0.001
CK, U/L	923.0 ± 3210.6 (n = 53)	175.9 ± 69.5 (n = 21)	182.3 ± 123.6 (n = 28)	0.278
AST, U/L	51.99 ± 71.16 (n = 190)	37.31 ± 14.13 (n = 36)	42.57 ± 58.22 (n = 35)	0.383
ALT, U/L	43.04 ± 100.57 (n = 119)	20.05 ± 9.29 (n = 21)	20.41 ± 21.48 (n = 22)	0.341
Ferritin, mg/mL	188.08 ± 190.13 (n = 32)	78.03 ± 3.46 (n = 3)	304.84 ± 693.73 (n = 23)	0.554
LDH, U/L	322.69 ± 100.86 (n = 34)	345.33 ± 23.42 (n = 6)	349.48 ± 129.15 (n = 21)	0.648
BNP, pg/mL	23.00 ± 17.58 (n = 14)	67.00 ± 1.41 (n = 2)	79.96 ± 63.10 (n = 14)	0.009

Abbreviations: SD, standard deviation; LOS, length of stay; NP, nasopharyngeal; Ct, cycle threshold; WBC, white blood cell; ANC, absolute neutrophil count; N/L, neutrophil-to-lymphocyte ratio; CRP, C-reactive protein; PCT, procalcitonin; CK, creatine kinase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; LDH, lactate dehydrogenase; BNP, B-type Natriuretic Peptide.

lacuna infarcts, and hypodense lesion over occipital lobe. On EEG, 11 of the 17 patients had positive findings compatible with encephalitis.

Characteristic factors for acute encephalitis/ encephalopathy

Table 3 shows the logistic regression multivariate analysis of the distinguishing factors associated with acute encephalitis/encephalopathy in previously healthy children excluding croup patients. Over 3 years old (aOR: 7.57, 95% CI: 2.46–23.31, $p < 0.001$), neutrophil ≥3150/μL (aOR: 5.46, 95% CI: 1.56–19.08, $p = 0.008$), and procalcitonin ≥0.5 ng/mL (aOR: 4.32, 95% CI: 1.25–14.97, $p = 0.021$) were independently associated with acute encephalitis/encephalopathy in children hospitalized with COVID-19.

Treatment and outcome

The Supplementary Table 4 summarized the treatment strategy and outcome of the encephalitis/encephalopathy patients. The ICU-patients received significantly higher percentages of remdesivir, intravenous immunoglobulin and pulse therapy than non-ICU patients. The mortality was 2.6% (1/38) and the 1-month outpatient department follow-up revealed 31.6% (12/38) patients with neurological sequelae. ICU patients had significantly higher percentage of sequelae than non-ICU patients (75% vs. 15.4%, $p = 0.01$).

Discussions

The COVID-19 outbreak in Taiwan was unique and different from other countries due to the lack of prior SARS-CoV-2

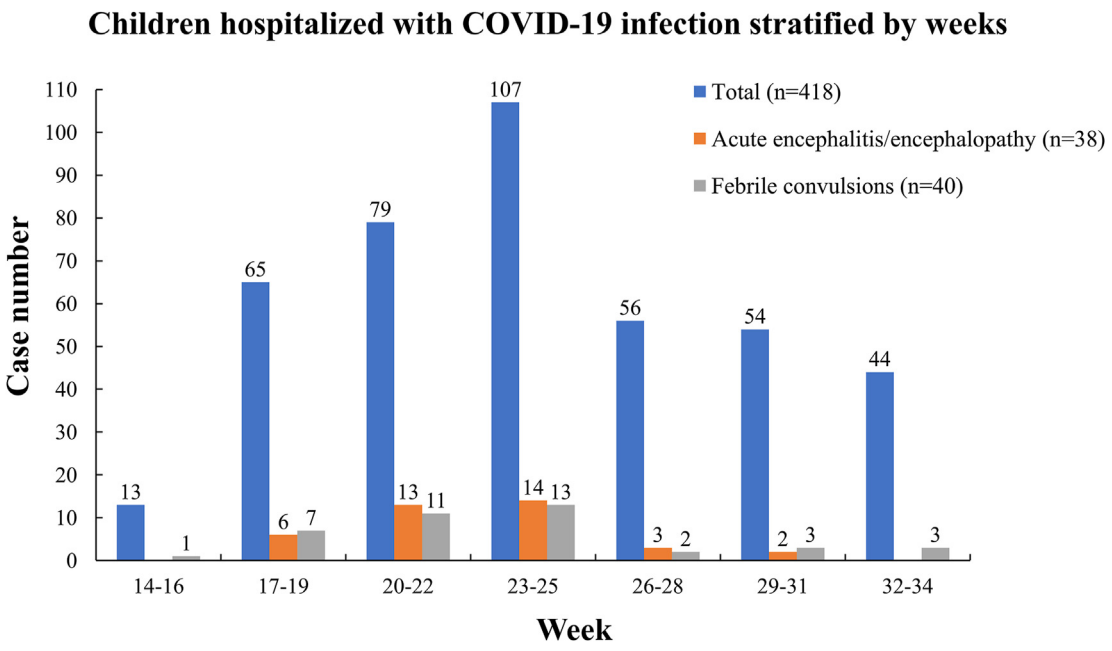

Figure 2. Children hospitalized with COVID-19 infection stratified by weeks during study period.

infection in children. The majority of pediatric patients had not been previously infected or vaccinated, and consequently, they contracted the primary Omicron variant infection. The natural infection of the Omicron variant in previously healthy children in our study is characterized by a significant level of neurological involvements, including encephalitis/encephalopathy and febrile convulsions. The increasing numbers of pediatric patients with neurological complications during the Omicron epidemic may be due to different mechanism of cell entry.[18] The Omicron variant has neurotropic property and uses cathepsin-mediated pathways to invade cells.[19] Consequently, the spectrum of pediatric diseases in children predominantly encompasses upper respiratory tract infections, croup, encephalitis, and seizures, whereas occurrences of pneumonia are less frequent.[20]

The pathophysiology of SARS-CoV-2-associated acute encephalitis/encephalopathy is mainly attributed to indirect mechanisms including hypoxemia, immune-mediated hypercytokinemia or microvascular thrombosis rather than direct infection of neurons.[12,21] COVID-19 associated encephalitis develops within an average of 14.5 days after the onset of respiratory tract symptoms in adult but usually occurs rapidly during acute infection phase in children.[22,23] In adult, cerebral vascular endothelial cells injury or lung parenchymal disruption, and the subsequent thromboembolism events or hypoxic/metabolic changes are primarily responsible for brain dysfunction.[22] Clinical physicians have sufficient time to monitor disease progression.

Omicron infection rarely causes pneumonia or lower respiratory tract disease in children, and the hypoxic event could not be the cause of brain dysfunction. The rapid disease course of COVID-19 encephalitis/encephalopathy is suspected due to immune-mediated hyperinflammation. The CNS innate immune signaling pathway is provoked by SARS-CoV-2 spike protein (S1) binding to ACE-2 expressing cells in CNS and the circulation of cytokines (IL-10, IL-18) and chemokines (CCL2, CCL3, CXCL10) recruits neutrophils, macrophages, monocytes, and T cells, which subsequently secrete pro-inflammatory cytokines (IL-6, IL-17, TNF-α, IL-1β).[24,25] These hyper-inflammatory conditions can cause endothelium injury, increase the permeability of BBB, induce lymphocytic apoptosis, and activate CNS microglia cells. The cytokines across the broken BBB account for cerebral dysfunction and encephalopathy.[26,27]

Concerning the possibility of rapidly deteriorating and missing the golden time for treatment, when children present with fever, altered consciousness, seizures, behavioral changes or post-ictal drowsiness in the emergency department, a precise judgment to differentiate encephalitis from febrile convulsions or mild disease is important for clinicians.

Several studies have demonstrated that a higher number of neutrophils, relative lymphopenia, and increased neutrophil-to-lymphocyte ratios are associated with severe COVID-19 disease.[28–30] The elevated neutrophil count was seen in peripheral blood circulation and in the nasopharyngeal epithelium, pulmonary capillaries and alveolar spaces.[31] In addition to the increase in number, neutrophil activation signature genes in blood transcriptomes were upregulated upon severe infection and dysregulation of the immune response, including impaired T cell regulation and over-activation of innate immunity.[28] These conditions can heighten the release of neutrophil extracellular traps associated with unfavorable coagulopathy and immunothrombosis and play a role in developing neurological dysfunction or lung fibrosis.[28,32] Additionally, the early neutrophil and macrophage migration into brain causing BBB dysfunction and neuroinflammatory pathogenesis also accounted for other viral encephalitis.[26,33,34]

Procalcitonin is another well-known prognostic biomarker for COVID-19 severity prediction; high serum procalcitonin is related to up-regulation of interleukin-6 and tumor necrosis factor alpha; the hypercytokinemia is

Table 2 Characteristics of patients with acute encephalitis/encephalopathy and comparison of GCS >12 and GCS ≤12.

	Acute Encephalitis/Encephalopathy		
	GCS >12, n (%)	GCS ≤12, n (%)	P-value
Cases numbers	25	13[a]	
Age, mean ± SD, months	70.0 ± 41.6	65.0 ± 52.7	0.553
Sex			
Male	14 (56)	9 (69.2)	0.751
Female	11 (44)	4 (30.8)	
LOS, mean ± SD, days	4.6 ± 1.2	8.1 ± 4.1	0.009
ICU admission, no (%)	0 (0)	12 (92.3)	<0.001
Mechanical ventilator, no (%)	0 (0)	2 (15.4)	0.111
NP Ct, mean ± SD			
Ct value of documented at admission	15.67 ± 2.33 (n = 19)	18.61 ± 5.27 (n = 13)	0.078
Ct value of symptoms onset in 24 h	15.47 ± 2.38 (n = 14)	16.09 ± 4.00 (n = 9)	0.681
Ct value of symptoms onset in 48 h	15.52 ± 2.30 (n = 18)	16.09 ± 4.00 (n = 9)	0.938
Onset time, mean ± SD, days	1.52 ± 0.92	2.08 ± 1.71	0.289
Fever, n (%)	25 (100)	12 (92.3)	0.342
Duration, mean ± SD, days	1.8 ± 0.9	1.8 ± 1.1	0.879
Peak, mean ± SD, °C	39.4 ± 0.6	39.9 ± 1.0	0.147
Seizure, n (%)	10 (40)	10 (76.9)	0.043
CSF study number, n (%)	6 (24)	8 (61.5)	0.028
Elevated protein in CSF, n (%)	0 (0)	1 (12.5)	
Pleocytosis for age in CSF, n (%)	0 (0)	1 (12.5)	
Positive SARS-CoV-2 PCR in CSF, n (%)	0 (0)	0 (0)	
Laboratory findings, Mean ± SD			
WBC, 1000/μL	6.04 ± 2.10 (n = 24)	6.97 ± 2.65 (n = 13)	0.251
Neutrophil, 1000/μL	4.18 ± 2.18	5.17 ± 2.75	0.236
Lymphocyte, 1000/μL	1.28 ± 0.80	1.39 ± 0.79	0.702
N/L ratio	5.67 ± 5.83	7.46 ± 9.57	0.481
Hemoglobin, g/dl	12.73 ± 0.92	12.35 ± 1.37	0.315
Platelet, 1000/μL	217.33 ± 54.50	263.69 ± 61.77	0.024
CRP, mg/L	8.99 ± 17.08 (n = 24)	4.31 ± 5.10 (n = 13)	0.344
CRP ≥5 mg/L, n (%)	7 (29.2)	5 (38.5)	0.716
Procalcitonin, ng/mL	1.38 ± 2.27 (n = 11)	3.32 ± 5.40 (n = 12)	0.271
PCT ≥0.5 ng/mL, n (%)	5 (45.5)	5 (41.7)	1.00
CK, U/L	200.94 ± 141.33	157.33 ± 95.22	0.365
Ferritin, mg/mL	215.26 ± 270.07 (n = 11)	386.96 ± 938.53 (n = 12)	0.565
AST, U/L	48.82 ± 72.84	32.00 ± 11.36	0.417
ALT, U/L	23.10 ± 31.11	18.17 ± 8.75	0.604
LDH, U/L	351.56 ± 106.97	347.91 ± 148.32	0.951
IL-6, pg/mL	2.51 ± 1.74 (n = 3)	2222.34 ± 7018.44 (n = 11)	0.022

Abbreviations: GCS, Glasgow Coma Scale; SD, standard deviation; LOS, length of stay; ICU, intensive care unit; NP, nasopharyngeal; Ct, cycle threshold; CSF, cerebral spinal fluid; PCR, polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; WBC, white blood cell; N/L ratio, neutrophil-to-lymphocyte ratio; CRP, C-reactive protein; CK, creatine kinase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; LDH, lactate dehydrogenase; IL-6, interleukin-6. [a] One 6 years old girl was not admitted to ICU with diagnosis of mild encephalitis. The patient presented with fever, frequent generalized tonic-clonic seizures and prolonged post-ictal lethargy (GCS = 12). Consciousness regained within one day after steroid treatment.

associated with severe COVID-19 disease.[35] The acute encephalopathy with biphasic seizures and late reduced diffusion (AESD), one subtype of acute encephalopathy, caused by viral infection were reported to have elevated serum procalcitonin level.[36] However, we did not examine the neutrophil and T-cell functions, and lack of serum cytokines level, which are our confounding factors. Whether neutrophil activation or impairment of adaptive T cell immunity due to lymphopenia contributed to immune-mediated CNS hyperinflammation required more studies to prove.

In clinical practice, if previously healthy children with acute COVID-19 infection display neurological symptoms, the clinicians should be aware of encephalitis/encephalopathy rather than febrile convulsions when the patients were over 3 years old with higher neutrophil and procalcitonin levels although the sensitivity and specificity of these parameters remain to be determined.

In our study, we found no significantly difference in throat or nasopharyngeal viral load among patients with acute encephalitis/encephalopathy, febrile convulsions and mild disease groups. Although, higher viral load in the

Table 3 The univariate and multivariate analysis by logistic regression for risk factors of acute encephalitis/encephalopathy.

Risk factors	Univariate analysis		Multivariate analysis	
	Odds ratio (95% CI)	P value	Odds ratio (95% CI)	P value
Age ≥3 years old	5.52 (2.60–11.68)	<0.001	7.57 (2.46–23.31)	<0.001
PCT ≥0.5 ng/mL	5.82 (2.15–15.76)	0.001	4.32 (1.25–14.97)	0.021
Neutrophil ≥3150/μL	2.64 (1.20–5.83)	0.016	5.46 (1.56–19.08)	0.008
Lymphocyte ≤1000/μL	3.19 (1.55–6.59)	0.002	0.86 (0.24–3.05)	0.810
Platelet ≤150,000/μL	0.58 (0.07–4.66)	0.611	–	–

Variables with p value < 0.1 were calculated in the multivariate analysis. Abbreviations: PCT, procalcitonin; CI, confident interval; "–," no data in multivariate analysis.

respiratory tract and delayed virus clearance in adult are associated with greater severity of COVID-19 in several reports, more longitudinal studies including viral load in blood are needed to clarify the relationship in encephalitis.[37]

Both of influenza and COVID-19-associated encephalitis/encephalopathy can occur in previously healthy children and have rapid onset disease course. No virus detection and rare pleocytosis in CSF is their characteristics.[38,39] The risk factors for poor outcomes in influenza-associated encephalitis were high CSF protein, under 2 years of age, thrombocytopenia and severe transaminase elevation, which was not observed in COVID-19-associated acute encephalitis/encephalopathy.[40]

Our study had some limitations including smaller number of cases, no genomic analysis of infected strains, and the absence data of inflammatory cytokines in CSF. Our study has some strengths. First, all the patients were primary care patients with reliable clinical information. Second, because few children between 0 and 11 years had COVID-19 vaccination or previous COVID-19 infection, the natural characteristics of Omicron infection in children could be presented well.

In conclusion, the majority of cases of COVID-19-associated acute encephalitis/encephalopathy showed no evidence of direct viral invasion but was associated with increased age, peripheral neutrophil count, and procalcitonin levels. These findings suggest that neutrophil-mediated systemic hyper-inflammatory response or maladaptive innate immunity might play an important role on the central nerve system, leading to cerebral dysfunction.

Contributors

C.W. Huang, Y.C. Hsieh, Y.C. Huang and C.H. Chiu participated in the study conception and design. C.Y. Kuo, C.H. Chen and C.W. Huang participated in acquisition of data (laboratory or clinical). C.W. Huang, J.J. Lin, K.L. Lin, and Y.C. Chen participated in data analysis and interpretation. C.W. Huang, Y.C. Hsieh, J.J. Lin and K.L. Lin participated in drafting manuscript and critical revision. Y.C. Hsieh, K.L. Lin, Y.C. Huang and C.H. Chiu were the co-chief investigators.

Funding

The study was supported in part by the Research Center for Epidemic Prevention Science Project, National Science and Technology Council, Taiwan; Chang Gung Memorial Hospital (award number: NSTC 111-2321-B-182-001).

Declaration of competing interest

None of the authors have any conflicts of interest to disclose.

Acknowledgments

The authors thank all physicians and pediatricians who participated in this study and thank Ping-Hsuan Huang for the statistical assistance and wish to acknowledge the support of the Maintenance Project of the Center for Big Data Analytics and Statistics (Grant CLRPG3D0049) at Chang Gung Memorial Hospital for study design and monitor, data analysis and interpretation.

References

1. Paton RS, Overton CE, Ward T. The rapid replacement of the SARS-CoV-2 Delta variant by Omicron (B.1.1.529) in England. *Sci Transl Med* 2022;**14**(652):eabo5395.
2. Ruth Link-Gelles P. Effectiveness of 2, 3, and 4 COVID-19 mRNA vaccine doses among immunocompetent adults during periods when SARS-CoV-2 Omicron BA.1 and BA.2/BA.2.12.1 sublineages predominated — VISION Network, 10 States, December 2021–June 2022. *MMWR* 2022;**71**(29).
3. Cloete J, Kruger A, Masha M, du Plessis NM, Mawela D, Tshukudu M, et al. Paediatric hospitalisations due to COVID-19 during the first SARS-CoV-2 omicron (B.1.1.529) variant wave in South Africa: a multicentre observational study. *Lancet Child Adolesc Health* 2022;**6**(5):294–302.
4. Antoon JW, Hall M, Howard LM, Herndon A, Freundlich KL, Grijalva CG, et al. COVID-19 and acute neurologic complications in children. *Pediatrics* 2022;**150**(5).
5. Yamagishi H, Tamura D, Aoyagi J, Suzuki S, Mizobe Y, Wakae K, et al. Impact of the omicron phase on a highly advanced medical facility in Japan. *Front Pediatr* 2023;**11**:1201825.
6. Tso WWY, Kwan MYW, Wang YL, Leung LK, Leung D, Chua GT, et al. Severity of SARS-CoV-2 Omicron BA.2 infection in unvaccinated hospitalized children: comparison to influenza and parainfluenza infections. *Emerg Microb Infect* 2022;**11**(1):1742–50.
7. Larovere KL, Riggs BJ, Poussaint TY, Young CC, Newhams MM, Maamari M, et al. Neurologic involvement in children and adolescents hospitalized in the United States for COVID-19 or multisystem inflammatory syndrome. *JAMA Neurol* 2021;**78**(5):536.

8. Mao L, Jin H, Wang M, Hu Y, Chen S, He Q, et al. Neurologic manifestations of hospitalized patients with coronavirus disease 2019 in Wuhan, China. *JAMA Neurol* 2020;**77**(6):683.
9. Ludvigsson JF. Convulsions in children with COVID-19 during the Omicron wave. *Acta Paediatr* 2022;**111**(5):1023—6.
10. Lin JJ, Tu YF, Chen SJ, Kuo YT, Mei-Jy J, Hsin-Ju Ko M, et al. Fatal fulminant cerebral edema in six children with SARS-CoV-2 omicron BA.2 infection in Taiwan. *J Pediatric Infect Dis Soc* 2022;**12**(2):99—103.
11. Chen CS, Chang CN, Hu CF, Jian MJ, Chung HY, Chang CK, et al. Critical pediatric neurological illness associated with COVID-19 (Omicron BA.2.3.7 variant) infection in Taiwan: immunological assessment and viral genome analysis in tertiary medical center. *Int J Infect Dis* 2022;**124**:45—8.
12. Azizi SA, Azizi SA. Neurological injuries in COVID-19 patients: direct viral invasion or a bystander injury after infection of epithelial/endothelial cells. *J Neurovirol* 2020;**26**(5):631—41.
13. Keyhanian K, Umeton RP, Mohit B, Davoudi V, Hajighasemi F, Ghasemi M. SARS-CoV-2 and nervous system: from pathogenesis to clinical manifestation. *J Neuroimmunol* 2021;**350**:577436.
14. Chen Z, Li G. Immune response and blood—brain barrier dysfunction during viral neuroinvasion. *Innate Immun* 2021; **27**(2):109—17.
15. Venkatesan A, Tunkel AR, Bloch KC, Lauring AS, Sejvar J, Bitnun A, et al. Case definitions, diagnostic algorithms, and priorities in encephalitis: consensus statement of the international encephalitis consortium. *Clin Infect Dis* 2013;**57**(8): 1114—28.
16. Messacar K, Fischer M, Dominguez SR, Tyler KL, Abzug MJ. Encephalitis in US children. *Infect Dis Clin North Am* 2018; **32**(1):145—62.
17. Sejvar JJ, Kohl KS, Bilynsky R, Blumberg D, Cvetkovich T, Galama J, et al. Encephalitis, myelitis, and acute disseminated encephalomyelitis (ADEM): case definitions and guidelines for collection, analysis, and presentation of immunization safety data. *Vaccine* 2007;**25**(31):5771—92.
18. Du X, Tang H, Gao L, Wu Z, Meng F, Yan R, et al. Omicron adopts a different strategy from Delta and other variants to adapt to host. *Signal Transduct Target Ther* 2022;**7**(1):45.
19. Pia L, Rowland-Jones S. Omicron entry route. *Nat Rev Immunol* 2022;**22**(3):144.
20. Tunç EM, Koid Jia Shin C, Usoro E, Thomas-Smith SE, Trehan I, Migita RT, et al. Croup during the coronavirus disease 2019 Omicron variant surge. *J Pediatr* 2022;**247**:147—9.
21. Solomon T. Neurological infection with SARS-CoV-2 — the story so far. *Nat Rev Neurol* 2021;**17**(2):65—6.
22. Garg RK, Paliwal VK, Gupta A. Encephalopathy in patients with COVID-19: a review. *J Med Virol* 2021;**93**(1):206—22.
23. Siow I, Lee KS, Zhang JJY, Saffari SE, Ng A. Encephalitis as a neurological complication of COVID-19: a systematic review and meta-analysis of incidence, outcomes, and predictors. *Eur J Neurol* 2021;**28**(10):3491—502.
24. Chang YM, Chen CH, Wang JN, Cheng CM, Tu YF, Shen CF. Case report: Distinct neurologic manifestation and cytokine profile of a child with COVID-19-associated acute fulminant encephalitis. *Front Med (Lausanne)* 2023;**10**:1209656.
25. Cheng CY, Tsai CH, Wang HP, Chiu WT, Hung HC, Chi CY, et al. Successful treatment of acute encephalitis and hepatitis in a child with COVID-19 infection. *J Formos Med Assoc* 2023; **122**(2):182—6.
26. Michael BD, Bricio-Moreno L, Sorensen EW, Miyabe Y, Lian J, Solomon T, et al. Astrocyte- and neuron-derived CXCL1 drives neutrophil transmigration and blood-brain barrier permeability in viral encephalitis. *Cell Rep* 2020;**32**(11):108150.
27. Najjar S, Najjar A, Chong DJ, Pramanik BK, Kirsch C, Kuzniecky RI, et al. Central nervous system complications associated with SARS-CoV-2 infection: integrative concepts of pathophysiology and case reports. *J Neuroinflammation* 2020; **17**(1).
28. Qin C, Zhou L, Hu Z, Zhang S, Yang S, Tao Y, et al. Dysregulation of immune response in patients with coronavirus 2019 (COVID-19) in Wuhan, China. *Clin Infect Dis* 2020;**71**(15):762—8.
29. Laforge M, Elbim C, Frère C, Hémadi M, Massaad C, Nuss P, et al. Tissue damage from neutrophil-induced oxidative stress in COVID-19. *Nat Rev Immunol* 2020;**20**(9):515—6.
30. Schimke LF, Marques AHC, Baiocchi GC, de Souza Prado CA, Fonseca DLM, Freire PP, et al. Severe COVID-19 shares a common neutrophil activation signature with other acute inflammatory states. *Cells* 2022;**11**(5).
31. Chua RL, Lukassen S, Trump S, Hennig BP, Wendisch D, Pott F, et al. COVID-19 severity correlates with airway epithelium-immune cell interactions identified by single-cell analysis. *Nat Biotechnol* 2020;**38**(8):970—9.
32. Zuo Y, Yalavarthi S, Shi H, Gockman K, Zuo M, Madison JA, et al. Neutrophil extracellular traps in COVID-19. *JCI Insight* 2020;**5**(11).
33. Grist JJ, Marro B, Lane TE. Neutrophils and viral-induced neurologic disease. *Clin Immunol* 2018;**189**:52—6.
34. Marques CP, Cheeran MCJ, Palmquist JM, Hu S, Urban SL, Lokensgard JR. Prolonged microglial cell activation and lymphocyte infiltration following experimental herpes encephalitis. *J Immunol* 2008;**181**(9):6417—26.
35. Tong-Minh K, van der Does Y, Engelen S, de Jong E, Ramakers C, Gommers D, et al. High procalcitonin levels associated with increased intensive care unit admission and mortality in patients with a COVID-19 infection in the emergency department. *BMC Infect Dis* 2022;**22**(1):165.
36. Fujii Y, Yashiro M, Yamada M, Kikkawa T, Nosaka N, Saito Y, et al. Serum procalcitonin levels in acute encephalopathy with biphasic seizures and late reduced diffusion. *Dis Markers* 2018; **2018**:1—4.
37. Wang Y, Zhang L, Sang L, Ye F, Ruan S, Zhong B, et al. Kinetics of viral load and antibody response in relation to COVID-19 severity. *J Clin Invest* 2020;**130**(10):5235—44.
38. Huang YC, Li WC, Tsao KC, Huang CG, Chiu CH, Lin TY. Influenza-associated central nervous system dysfunction in Taiwanese children: clinical characteristics and outcomes with and without administration of oseltamivir. *Pediatr Infect Dis J* 2009;**28**(7):647—8.
39. Jarius S, Pache F, Körtvelyessy P, Jelčić I, Stettner M, Franciotta D, et al. Cerebrospinal fluid findings in COVID-19: a multicenter study of 150 lumbar punctures in 127 patients. *J Neuroinflammation* 2022;**19**(1).
40. Morishima T, Togashi T, Yokota S, Okuno Y, Miyazaki C, Tashiro M, et al. Encephalitis and encephalopathy associated with an influenza epidemic in Japan. *Clin Infect Dis* 2002; **35**(5):512—7.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jmii.2023.08.010.