Original Contribution Kitasato Med J 2022; 52: 47-53

Characteristics of virologically proven human herpes virus-6 and -7 acute encephalopathy with biphasic seizures and late reduced diffusion in children with status epilepticus

Yutaka Nonoda, Takashi Ito, Taira Toki, Hironao Shirai,
Eri Mineo, Hisashi Ando, Kenji Ishikura

Department of Pediatrics, Kitasato University School of Medicine

Objective: To disclose the clinical features of human herpes virus-6 and -7 (HHV-6,7) polymerase chain reaction (PCR)-positive acute encephalopathy with biphasic seizures and late reduced diffusion (AESD) in an acute care hospital.
Methods: By reviewing their medical records, we retrospectively investigated a total of 340 patients who had been admitted to our hospital because of status epilepticus.
Results: HHV-6,7 PCR was performed for 62 patients, 29 of whom were HHV-6,7 PCR-positive, of whom 7 patients satisfied the diagnostic criteria of AESD. And 5 of those 7 patients with HHV-6,7 PCR-positive AESD had uniformly similar clinical courses. The most common age of onset was 1－2 years old in children who had a family history of seizures. The patients had fever and status epilepticus lasting longer than 30 minutes, and the initial seizure was difficult to stop. The fever lasted 2－5 days, and a rash appeared 3－5 days after the fever. The patient regained consciousness, however, late-onset seizures with repetitive short focal seizures of about 1 minute occurred 4－6 days after the initial seizure. Diffusion-weighted images of the brain MRI after the late-onset seizure showed a BTA (bright tree appearance) in the subcortical white matter of the bilateral or dominant frontal lobe.
Conclusion: These cases had similar epidemiological characteristics to those in previous reports and nationwide surveys.

Key words: status epilepticus, acute encephalopathy, exanthem subitum, human herpes virus-6 and -7, HHV-6,7

Introduction

Status epilepticus (SE) is an urgent condition in pediatric emergency care because it causes diseases with sequelae, such as acute encephalopathy, bacterial meningitis, intracranial hemorrhage, and metabolic disorder.[1] However, the most common cause of seizures in children is a febrile seizure. The prevalence of febrile seizures is very high, especially in Japan where the prevalence is approximately 8%.[2] In most cases of febrile seizures, no special treatment is required after the seizures have stopped; however, cases of SE or prolonged unconsciousness tend to have poor neurological prognoses.

SE is defined as a condition in which convulsive seizures last for more than 30 minutes, or in which short seizures occur repeatedly before the patient regains consciousness.[3,4] If the seizures continue longer than 30 minutes, the probability of serious brain damage increases;[5,6] therefore, the condition is highly urgent.

Acute encephalopathy with biphasic seizures and late reduced diffusion (AESD) is the most common and important type of acute encephalopathy, which usually occurs in infancy and is characterized by a biphasic course.[7] Acute encephalopathy is caused by viral infections such as the human herpes virus-6 and -7 (HHV-6,7) or an influenza virus and develops as seizures or status epilepticus within 24 hours after a fever. Exanthem subitum tends to become complicated with seizures, with prolonged seizures and slow recovery of consciousness,

Received 30 November 2021, accepted 4 January 2022
Correspondence to: Yutaka Nonoda, Department of Pediatrics, Kitasato University School of Medicine
1-15-1 Kitasato, Minami-ku, Sagamihara, Kanagawa 252-0374, Japan
Email: nnd@med.kitasato-u.ac.jp

resulting in AESD, which is a viral eruptive infection that usually affects children younger than 2 years old and is caused by HHV-6,7,[7,8] After consciousness improves, in 3 to 6 days, short partial seizures called, "late-onset seizures," occur in clusters, and the consciousness level worsens, after which neurological sequelae can develop.[7] A characteristic feature of acute encephalopathy is the difficulty in differentiating it from febrile seizures because consciousness improves after the initial seizure. Diffusion-weighted brain magnetic resonance imaging (MRI) shows dendritic brain edema, i.e., the bright tree appearance (BTA), in the subcortical white matter at the time of the biphasic late-onset seizure;[9] however, these findings are not observed before the late-onset seizure, which makes early prediction of this disease difficult. Recent research has focused on predictive factors;[10-12] however, there is no diagnostic gold standard or established methods, and currently acute encephalopathy is usually diagnosed clinically without virological surveys. Moreover, the pathogenesis of AESD remains unknown.

In the present study, therefore, we aimed to disclose early characteristics of acute encephalopathy by comparing an AESD group (in which all patients have been virologically proven) and a non-AESD group of patients admitted to our hospital with status epilepticus.

Materials and Methods

This study was a retrospective study in a single institution and was approved by the Kitasato University Hospital Ethics Committee (B21-020). The study involved reviewing medical records from our hospital. Of the 340 patients admitted to the Department of Pediatrics at the Kitasato University Hospital for status epilepticus between May 1, 2014, and March 31, 2021, there were 29 patients with virologically proven HHV-6,7 infection among them. The definition of SE was seizures lasting longer than 30 minutes. Patients younger than 6 months of age, or older than 7 years of age, patients with afebrile seizures, and patients diagnosed with acute encephalopathy other than AESD were excluded.

AESD was defined in patients that fulfilled the following three requirements: patients with prolonged unconsciousness or lethargy, "biphasic late-onset seizure" 3－6 days after admission, and MRI findings with a BTA on diffusion-weighted brain MRI at the secondary late-onset seizure stage. The consciousness level was evaluated using the Glasgow Coma Scale. The definition of AESD was based on the Pediatric Acute Encephalopathy Guidelines 2016, supervised by the Japanese Society of Child Neurology.[13]

As a statistical method, Pearson's chi-square test was used to compare the proportions between the AESD and non-AESD groups. A *t*-test was used to compare the median values between the 2 groups. P values of <0.05 were considered to be statistically significant. Statistical analyses were performed using the statistical software JMP version 14 (SAS Institute Japan, Tokyo).

HHV-6 and -7 DNA was extracted from 250－500 μl plasma, serum, and/or cerebrospinal fluid using the QIAmp MinElute Virus Vacuum kit (Qiagen, Hilden, Germany), according to the manufacturer's protocol. A volume of 50－100 μl elution buffer was used to collect the purified DNA. HHV-6 genomes were detected by quantitative real-time polymerase chain reaction (PCR) using the probe (FAM-CACCAGACGTCACACCCGAAGGAAT-MGB-3') and primers targeting the *UL67* gene, 5'-CAAAGCCAAATTATCCAGAGCG-3' and 5'-CGCTAGGTTGAGAATGATCGA-3', based on the sequence of HHV6B (Genbank AF157706).[14] Similarly, HHV-7 genomes were detected by quantitative real-time PCR using the probe (FAM-CACGGCAATAACTCTAG-MGB-3') and primers targeting the *UL100* gene, 5'-ATGTACCAATACGGTCCCACTTG-3' and 5'-AGAGCTTGCGTTGTGCATGTT-3', based on the sequence of HHV7 (Genbank AF037218).[15]

PCR reactions were performed using Taqman Universal PCR Master Mix with the CFX96 Touch Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA) at 50℃ for 2 minutes, 95℃ for 10 minutes, followed by 45 cycles at 95℃ for 15 seconds, and 58℃ for 1 minute.

Results

The patient flow diagram is shown in Figure 1. In total, there were 340 patients with eligible SE. Of those, 75 patients with afebrile seizures were excluded. An additional 12 cases were excluded because of diagnosis with other types of acute encephalopathy, such as acute necrotizing encephalopathy, hemorrhagic shock and encephalopathy syndrome, acute brain swelling, clinically mild encephalitis/encephalopathy with a reversible splenial lesion, and posterior reversible encephalopathy syndrome. Of the 253 remaining cases, 62 were tested for HHV-6,7 by PCR. Twenty-nine patients (46.8%) were positive and 33 (53.2%) were negative for HHV-6,7 PCR. Of the total 253 patients with febrile SE, there were 19 (7.5%) cases that satisfied the diagnostic criteria

of AESD; 7 (24.1%) of the 29 HHV-6,7 PCR-positive cases had AESD, and 4 (12.1%) of the 33 HHV-6,7 PCR-negative cases had AESD. The 29 HHV-6,7 PCR-positive cases were divided into an AESD or non-AESD group, and the characteristics of each group were investigated.

Patient characteristics are shown in Table 1. The median age was the same for both groups. There were no significant differences in sex between the groups. A few patients had a history of preterm birth, seizures, and developmental delay. Approximately half, 14 of 29 (48.3%), of the patients had a family history of seizures. However, there were no significant differences in those factors between the groups.

The characteristics of the patients' clinical courses and treatments are shown in Table 2. AESD is characterized by biphasic symptoms with a late-onset seizure that appears 4－6 days after the initial seizure (Table 3). The duration of the initial seizure was longer than 30 minutes in both groups. And the median number of anticonvulsants administered at the initial seizure was the same thus showing that the seizures were difficult to stop in both groups. Regarding specific treatments, all the patients in the AESD group received methylprednisolone pulse therapy as immunotherapy, and most patients in both groups received therapeutic hypothermia and mechanical ventilation, although the administration of these treatments was lower for the non-AESD group compared with that in the AESD group. There were 22 cases in the non-AESD group that were eventually diagnosed with febrile seizure and had good prognoses.

There were 7 cases in the AESD group, and the details of the clinical course of each case are summarized in Table 3. The age of the patients ranged from 0.8－1.6 years. The duration of the initial seizure was longer than 30 minutes in 5 of the 7 cases, although 1 case had only a cluster of repetitive short seizures of 3 minutes each. The fever lasted 2－5 days, and the time between the onset of fever and the appearance of a rash was 3－5 days. However, 2 cases had no rash at all. The period between the initial seizure and late-onset seizure was 4－6 days. The seizure types of late-onset seizures were focal clonic seizures in 3 cases, focal tonic seizure in 1 case, generalized tonic clonic seizure in 1 case, and a non-motor seizure with movement stopping and pallor in 1 case. Patient 3 had lethargy only but no seizures

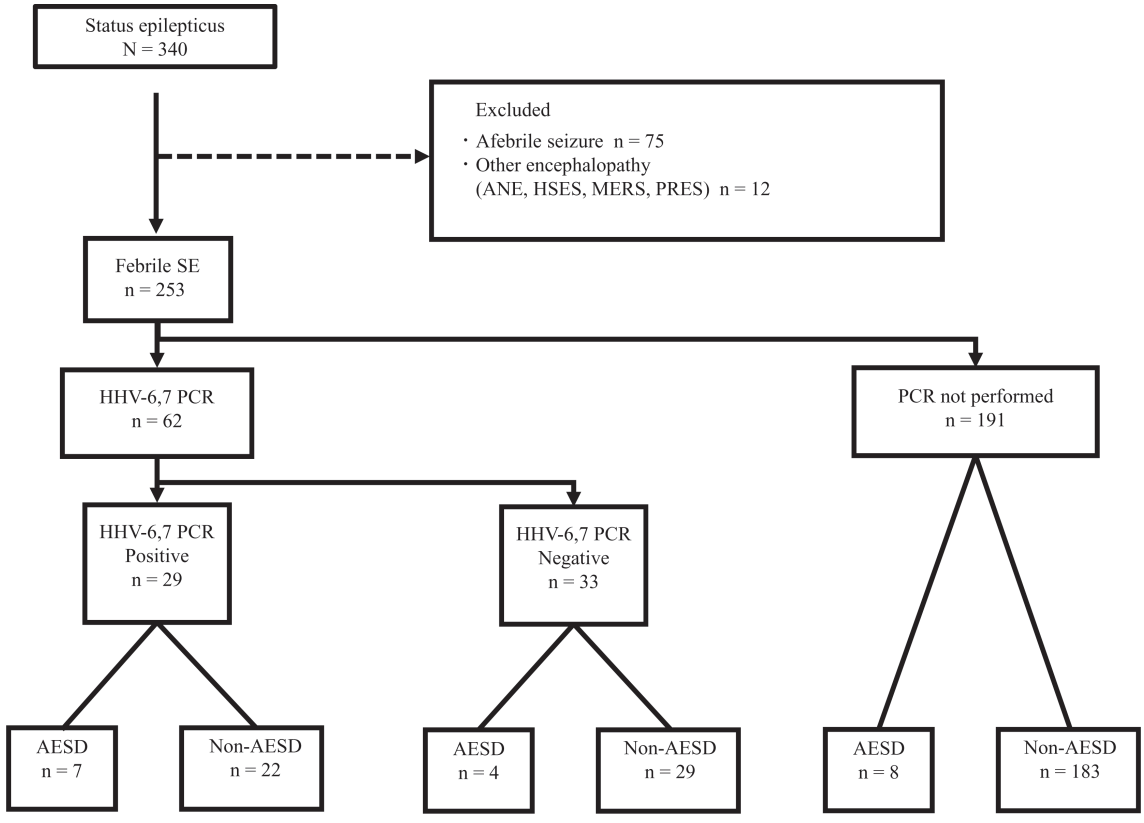

Figure 1. Patient flow diagram

Of 253 patients with febrile SE, 7 of the 29 HHV-6,7 PCR-positive cases had AESD, and 4 of the 33 HHV-6,7 PCR-negative cases had AESD.

Table 1. Patient characteristics

Variable	Total (n = 29)	AESD group (n = 7)	Non-AESD group (n = 22)	P value
Age (years), median (IQR)	1.3 (0.9－1.6)	1.3 (0.9－1.3)	1.2 (1.0－1.8)	0.1242
Sex				
M, n (%)	17 (58.6)	4 (57.1)	9 (40.9)	1.0000
F, n (%)	12 (41.4)	3 (42.9)	13 (59.1)	1.0000
Height (cm), median (IQR)	77.0 (73.0－82.0)	81.0 (74.5－81.5)	77.0 (73.5－85.8)	0.4201
Weight (kg), median (IQR)	10.0 (8.7－10.6)	10.4 (9.3－10.5)	10.0 (8.6－10.5)	0.3363
Premature birth, n (%)	4 (13.8)	1 (14.3)	3 (13.7)	1.0000
Developmental delay, n (%)	1 (3.4)	0 (0)	1 (4.5)	1.0000
Seizure history, n (%)	5 (17.2)	2 (28.6)	3 (13.6)	0.5688
Seizure family history, n (%)	14 (48.3)	3 (42.9)	11 (50.0)	1.0000

IQR, interquartile range

Table 2. Management of seizures

	Total (n = 29)	AESD group (n = 7)	Non-AESD group (n = 22)
Intial seizure duration (min), median (IQR)	44.0 (30.0－60.0)	30.0 (25.0－37.0)	55.0 (31.0－60.0)
Intial seizure anticonvulsants, median (IQR)	1.0 (1.0－2.0)	1.0 (1.0－2.0)	1.0 (1.0－2.0)
Glasgow Coma Scale after admission, median (IQR)	10.0 (6.0－15.0)	13.0 (8.0－14.0)	10.0 (6.0－14.8)
Treatment			
Methylprednisolone pulse, n (%)	20 (69.0)	7 (100.0)	13 (59.1)
Intravenous immunogloblin, n (%)	13 (44.8)	5 (71.4)	8 (36.4)
Therapeutic hypothermia, n (%)	18 (62.1)	6 (85.7)	12 (54.5)
Mechanical ventilation, n (%)	18 (62.1)	6 (85.7)	12 (54.5)

Table 3. Patient characteristics

Pt.	Age	Sex	Initial seizure duration (min)	Initial seizure anti-convulsants	Febrile period (days)	Postfever rash onset (days)	Initial seizure to late-onset seizure (days)
1	0.8	M	3	0	4	5	6
2	0.8	F	30	1	3	3	4
3	0.9	M	44	2	2	0	5
4	1.3	M	30	2	5	5	6
5	1.3	F	30	1	3	3	4
6	1.4	M	75	2	4	0	4
7	1.6	F	20	1	3	5	5

(Table 3). The duration of the late-onset seizures was approximately 1 minute in 4 cases, and 2 cases had long seizures lasting longer than 10 minutes. Diffusion-weighted images of the brain MRI showed brain edema images known as a BTA[9] in the dominant frontal lobe subcortical white matter in all the patients. A diffusion-weighted image of the brain MRI of patient 2 is shown in Figure 2. Three cases showed edema in the bilateral frontal lobes, 2 cases in the bilateral frontal and parietal lobes, and patient 5 showed high-intensity areas in the subcortical white matter of the right cerebral hemisphere (Table 3). In patient 7, high-intensity areas were seen not only in the subcortical white matter but also in a wide area of the bilateral frontal lobes, including the cortex (Table 3). These findings were typical findings of the BTA in AESD. These BTA findings were seen after the late-onset seizure in all the cases. Methylprednisolone pulse was given as a specific therapy in all the cases, and therapeutic hypothermia and mechanical ventilation were given to all but patient 7 (Table 3). The prognoses for the 7 cases in the AESD group included hemiplegia in 2 cases, mental retardation in 2 cases, and no sequelae in 3 cases.

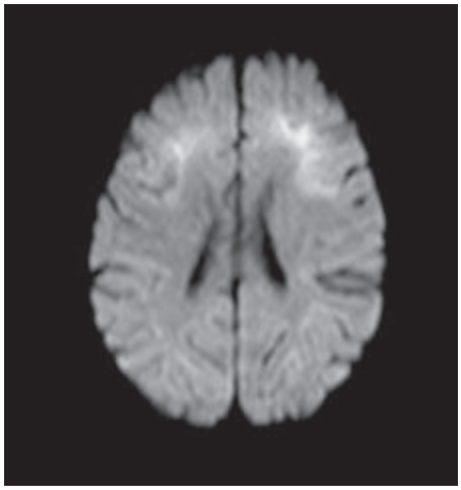

Figure 2. Diffusion-weighted image of brain MRI (patient 2)

The high intensity areas in the bilateral frontal and parietal subcortical white matter are better known as BTA (bright tree appearance).

Discussion

In the present study, we were able to determine the status of HHV-6,7 PCR-positive AESD patients in our acute-care hospital. The patients with HHV-6,7 PCR-positive AESD had a uniformly similar clinical course.

A schema of a typical clinical course in HHV-6,7 PCR-positive AESD is shown in Figure 3. This course was demonstrated in the present study as follows. A 1－2-year-old child with a history of seizures or a family history of seizures presents with fever and status

Late-onset seizure	Brain MRI DWI	Treatment
Recurrent tonic seizures with leftward eye deviation duration ≤1 min	High-intensity areas in the bilateral frontal subcortical white matter	Therapeutic hypothermia, mechanical ventilation, mPSL pulse
Repetitive unilateral clonic seizures ≤1 min	High-intensity areas in the bilateral frontal and parietal subcortical white matter	Therapeutic hypothermia, mechanical ventilation, mPSL pulse, IVIg, aciclovir
No seizure; lethargy	High-intensity areas in the bilateral frontal subcortical white matter	Therapeutic hypothermia, mechanical ventilation, mPSL pulse, IVIg
Upper limb clonic seizures with blinking eyes repeated ≤1 min	High-intensity areas in the right dominant bilateral frontal subcortical white matter	Therapeutic hypothermia, mechanical ventilation, mPSL pulse, IVIg, aciclovir
Clonic seizures on the left upper and lower extremities ≤10 min with Todd's Paralysis	High-intensity areas in the subcortical white matter on the right hemisphere	Therapeutic hypothermia, mechanical ventilation, mPSL pulse, IVIg, aciclovir
Generalized tonic-clonic seizure ≤15 min	High-intensity areas in the bilateral frontal and parietal subcortical white matter	Therapeutic hypothermia, mechanical ventilation, mPSL puse
Stopping motion and pale for ≤30 sec	Widespread high-intensity area mainly in the subcortical white matter and the surrounding cortex of bilateral frontal lobesg	mPSL pulse, IVIg

epilepticus lasting more than 30 minutes. The initial seizure is difficult to stop, as in febrile SE, and the fever lasts for 2－5 days when a rash appears 3－5 days subsequent to the fever. The patient regains consciousness; however, 4－6 days after the initial seizure, late-onset seizures with repetitive short focal seizures of about 1 minute in duration occur. Diffusion-weighted images of the brain MRI after the late-onset seizure show a BTA in the subcortical white matter with bilateral frontal lobe predominance. To our knowledge, this is the first time such data have accurately established the clinical image of virologically proven, HHV-6,7 related AESD.

The largest epidemiological survey on AESD in Japan was the 2010 nationwide survey conducted by Mizuguchi et al.[16] Therefore, we compared the present study and that nationwide survey to determine any similarities and differences. In the nationwide survey, AESD was found to be the most common subtype of acute encephalopathy. In the present study, 19 cases satisfied the diagnostic criteria for AESD, which accounted for 61% of the total 31 cases diagnosed with any type of acute encephalopathy. Moreover, the rate of AESD was higher than the other types of acute encephalopathies. Regarding age, the median age of AESD patients was 1.7 years old in the nationwide survey, which was significantly consistent with that of 1.3 years in the present study. The nationwide survey also reported that HHV-6,7 was the most common causative pathogen of AESD (38%).[16-18] The frequency of HHV-6,7 infection in the present study is also consistent with that finding. Exanthem subitum diagnosis is usually based on the appearance of a rash without virological evidence. Conversely, in the present study, HHV-6,7 PCR was more useful for the accurate diagnosis of exanthem subitum because several cases of the PCR-positive in the present study did not develop a rash. We purport that the most important factor when comparing nationwide surveys with previous and/or current reports is ensuring that the clinical course and imaging findings are consistent as they are in the present study.

Early prediction of AESD is important because the disease can develop into neurological sequelae. However, it is difficult to distinguish between febrile seizures and AESD in the early stages of the disease. In the present study, it was also difficult to distinguish between the two diseases because of the duration of SE and the difficulty in stopping seizures with anticonvulsants. Therefore, to make such a diagnostic judgement, physicians and researchers have recently focused on cytokines,[19] biomarkers,[20,21] and predictive scoring.[10-12] One of the most widely known predictive scoring methods is the AESD predictive score described by Tada et al.[10] A comparison of the findings of the present study with that scoring method showed similar trends regarding age of onset and use of mechanical ventilation.

A limitation of the present study was that there were some patients who had a rash with HHV-6,7 for whom PCR was not performed. These patients did not undergo PCR testing for several reasons, one of which was the short hospitalization period. Therefore, the number of HHV-6,7 patients might have been larger than that described.

In conclusion, we described the clinical features of HHV-6,7 PCR-positive AESD. HHV-6,7 infection is an important factor in childhood status epilepticus because it is easily complicated with acute encephalopathy. The cases presented in this study had similar epidemiological

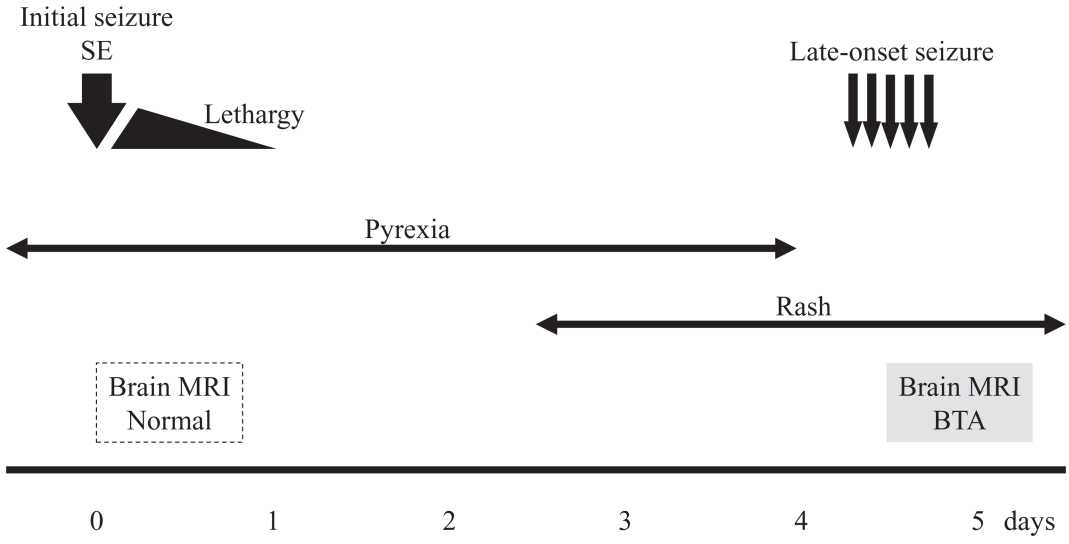

Figure 3. Schema of the typical clinical course in HHV-6,7 positive AESD

characteristics as those in previous reports and nationwide surveys of AESD caused by various infections. Therefore, it must be important to keep in mind the clinical course for early diagnosis.

Acknowledgments

The authors thank all the staff members who helped treat patients at Kitasato University Hospital and Ms. Keiko Nomoto and Ms. Ayumi Saito for conducting virological examinations. We also thank Georgia Lenihan-Geels, PhD, of Edanz, Fukuoka, for editing a draft of this manuscript.

Conflicts of Interest: None

References

1. Nishiyama I, Ohtsuka Y, Tsuda T, et al. An epidemiological study of children with status epilepticus in Okayama, Japan: Incidence, etiologies, and outcomes. *Epilepsy Res* 2011; 96: 89-95.
2. Tsuboi T. Epidemiology of febrile and afebrile convulsions in children in Japan. *Neurology* 1984; 34: 175-81.
3. Guidelines for epidemiologic studies on epilepsy. Commission on Epidemiology and Prognosis, International League Against Epilepsy. *Epilepsia* 1993; 34: 592-6.
4. Trinka E, Cock H, Hesdorffer D, et al. A definition and classification of status epilepticus--Report of the ILAE Task Force on Classification of Status Epilepticus. *Epilepsia* 2015; 56: 1515-23.
5. Aicardi J, Chevrie JJ. Convulsive status epilepticus in infants and children. A study of 239 cases. *Epilepsia* 1970; 11: 187-97.
6. Moriyama Y, Hayashi K, Matsuo E, et al. Epidemiology of Status Epilepticus in Childhood. *J Japan Pediatr Soc* 2014; 118: 1336-41 (in Japanese).
7. Maegaki Y, Kondo A, Okamoto R, et al. Clinical characteristics of acute encephalopathy of obscure origin: a biphasic clinical course is a common feature. *Neuropediatrics* 2006; 37: 269-77.
8. Yoshikawa T, Ohashi M, Miyake F, et al. Exanthem subitum-associated encephalitis: nationwide survey in Japan. *Pediatr Neurol* 2009; 41: 353-8.
9. Takanashi J, Oba H, Barkovich AJ, et al. Diffusion MRI abnormalities after prolonged febrile seizures with encephalopathy. *Neurology* 2006; 66: 1304-9.
10. Tada H, Takanashi J, Okuno H, et al. Predictive score for early diagnosis of acute encephalopathy with biphasic seizures and late reduced diffusion (AESD). *J Neurol Sci* 2015; 358: 62-5.
11. Nishiyama M, Ishida Y, Yamaguchi H, et al. Prediction of AESD and neurological sequelae in febrile status epilepticus. *Brain Dev* 2021; 43: 616-25.
12. Yokochi T, Takeuchi T, Mukai J, et al. Prediction of acute encephalopathy with biphasic seizures and late reduced diffusion in patients with febrile status epilepticus. *Brain Dev* 2016; 38: 217-24.
13. Japanese Society of Child Neurology. *Pediatric Acute Encephalopathy Guidelines 2016*. Tokyo: Shindan to Chiryo Sha; 2016; 92-100 (in Japanese).
14. Ogawa H, Suzutani T, Baba Y, et al. Etiology of severe sensorineural hearing loss in children: independent impact of congenital cytomegalovirus infection and GJB2 mutations. *J Infect Dis* 2007; 195: 782-8.
15. Fernandez C, Boutolleau D, Manichanh C, et al. Quantitation of HHV-7 genome by real-time polymerase chain reaction assay using MGB probe technology. *J Virol Methods* 2002; 106: 11-6.
16. Mizuguchi M. National Survey on Acute Encephalopathy. Research Group for Elucidation of Etiology and Establishment of Medical Treatment for Severe and Intractable Acute Encephalopathy (Research Grant for Overcoming Intractable Diseases) 2010 (in Japanese).
17. Hoshino A, Saitoh M, Oka A, et al. Epidemiology of acute encephalopathy in Japan, with emphasis on the association of viruses and syndromes. *Brain Dev* 2012; 34: 337-43.
18. Mizuguchi M, Ichiyama T, Imataka G, et al. Guidelines for the diagnosis and treatment of acute encephalopathy in childhood. *Brain Dev* 2021; 43: 2-31.
19. Ichiyama T, Ito Y, Kubota M, et al. Serum and cerebrospinal fluid levels of cytokines in acute encephalopathy associated with human herpesvirus-6 infection. *Brain Dev* 2009; 31: 731-8.
20. Tanuma N, Miyata R, Kumada S, et al. The axonal damage marker tau protein in the cerebrospinal fluid is increased in patients with acute encephalopathy with biphasic seizures and late reduced diffusion. *Brain Dev* 2010; 32: 435-9.
21. Shiihara T, Miyake T, Izumi S, et al. Serum and cerebrospinal fluid S100B, neuronspecific enolase, and total tau protein in acute encephalopathy with biphasic seizures and late reduced diffusion: a diagnostic validity. *Pediatr Int* 2012; 54: 52-5.