

Clinical Features of Acute Flaccid Myelitis Temporally Associated With an Enterovirus D68 Outbreak: Results of a Nationwide Survey of Acute Flaccid Paralysis in Japan, August–December 2015

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Background. Acute flaccid myelitis (AFM) is an acute flaccid paralysis syndrome with spinal motor neuron involvement of unknown etiology. We investigated the characteristics and prognostic factors of AFM clusters coincident with an enterovirus D68 (EV-D68) outbreak in Japan during autumn 2015.

Methods. An AFM case series study was conducted following a nationwide survey from August to December 2015. Radiographic and neurophysiologic data were subjected to centralized review, and virology studies were conducted for available specimens.

Results. Fifty-nine AFM cases (58 definite, 1 probable) were identified, including 55 children and 4 adults (median age, 4.4 years). The AFM epidemic curve showed strong temporal correlation with EV-D68 detection from pathogen surveillance, but not with other pathogens. EV-D68 was detected in 9 patients: 5 in nasopharyngeal, 2 in stool, 1 in cerebrospinal fluid (adult case), and 1 in tracheal aspiration, nasopharyngeal, and serum samples (a pediatric case with preceding steroid usage). Cases exhibited heterogeneous paralysis patterns from 1- to 4-limb involvement, but all definite cases had longitudinal spinal gray matter lesions on magnetic resonance imaging (median, 20 spinal segments). Cerebrospinal fluid pleocytosis was observed in 50 of 59 cases (85%), and 8 of 29 (28%) were positive for antiganglioside antibodies, as frequently observed in Guillain-Barré syndrome. Fifty-two patients showed variable residual weakness at follow-up. Good prognostic factors included a pretreatment manual muscle strength test unit score >3, normal F-wave persistence, and EV-D68–negative status.

Conclusions. EV-D68 may be one of the causative agents for AFM, while host susceptibility factors such as immune response could contribute to AFM development.

Keywords. acute flaccid myelitis; enterovirus D68; nationwide survey; prognostic analysis; antiganglioside antibodies.

Acute flaccid myelitis (AFM) is a newly defined, rare, but clinically distinct syndrome of acute flaccid paralysis (AFP) with spinal motor neuron involvement of unknown etiology [1, 2]. Clusters of AFM coincided with a nationwide

outbreak of enterovirus D68 (EV-D68) in the United States during autumn 2014 [1–7]. Although a strong temporal association was noted, EV-D68 was detected in only 20% of AFM cases, mainly from respiratory specimens [2, 3]. As this was the first outbreak, and was observed without apparent viral detection from blood and cerebrospinal fluid (CSF) samples, it remains unclear whether EV-D68 is a major cause of AFM. Event-based surveillance was initiated for AFP under special provision of the Infectious Diseases Prevention Law [8] following clusters of “polio-like” paralysis reported to Japan’s National Institute of Infectious Diseases (NIID) in September 2015. During the same period, clustered detection of EV-D68 by pathogen surveillance was reported [9]. Based on this national AFP survey, we performed a case series study of AFM to clarify the symptom spectrum, examine the association with EV-D68 infection, and identify possible prognostic factors.

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METHODS

Study Population and Design

In response to AFP reports received by NIID, provisional AFP surveillance commenced 21 October 2015 [8]. All AFP cases admitted between 1 August 2015 and 31 December 2015 with new-onset neurological symptoms lasting >24 hours, but excluding those with definitive etiologies (vascular, tumor, and trauma), were included in the present study. As acute cranial nerve dysfunction with brainstem lesions (ACB) is considered one of the neurological manifestations of this syndrome and reported alongside AFM in a previous US report [4], a preliminary survey by the Japanese Society of Child Neurology included ACB cases. Following this national AFP survey, we undertook the AFM collaborative study by requesting medical data from participating clinicians. Acute flaccid myelitis was defined per criteria adopted [10]. Confirmed AFM was defined by onset of acute focal limb weakness and evidence of spinal cord lesion with predominant gray matter involvement on magnetic resonance imaging (MRI). Probable AFM was defined by acute focal limb weakness and a CSF profile showing pleocytosis with leukocyte count >5 cells/ μ L. Acute disseminated encephalomyelitis (ADEM), acute transverse myelitis, neuromyelitis optica spectrum disorders (NMOSDs), and Guillain-Barré syndrome

(GBS) were defined per the established criteria [11–14]. All MRI and neurophysiological data were sent to a panel of specialists for review. Oral or written consent was obtained from all patients or guardians, and ethical approval for this study was granted by the Ethics Committee of NIID, Japan (number 655).

Procedures

Detailed anonymized medical data were obtained from clinicians. Motor function was assessed by manual muscle strength test (MMT) and graded per the Medical Research Council scale [15]. Overall MMT score was calculated from the sums of average muscle strengths in each paralyzed extremity divided by the number of affected limbs. Motor improvement was classified using a 4-level system: complete (grade 5 on MMT score); good (2 or more grades of MMT score recovery or grade 4 on MMT score); fair (slight improvement); and poor (no improvement). All patients with neurological sequela were reevaluated after 6 months or more. Neuroimaging data were reviewed independently by a radiologist (H. M.) and a child neurologist (A. O.). Neurophysiological reports were reviewed by 2 child neurologists (H. T. and S. Y.). Discussions were held between core members (P. F. C., R. K., H. M., A. O., H. T., S. Y., K. T. T.) in uncertain cases.

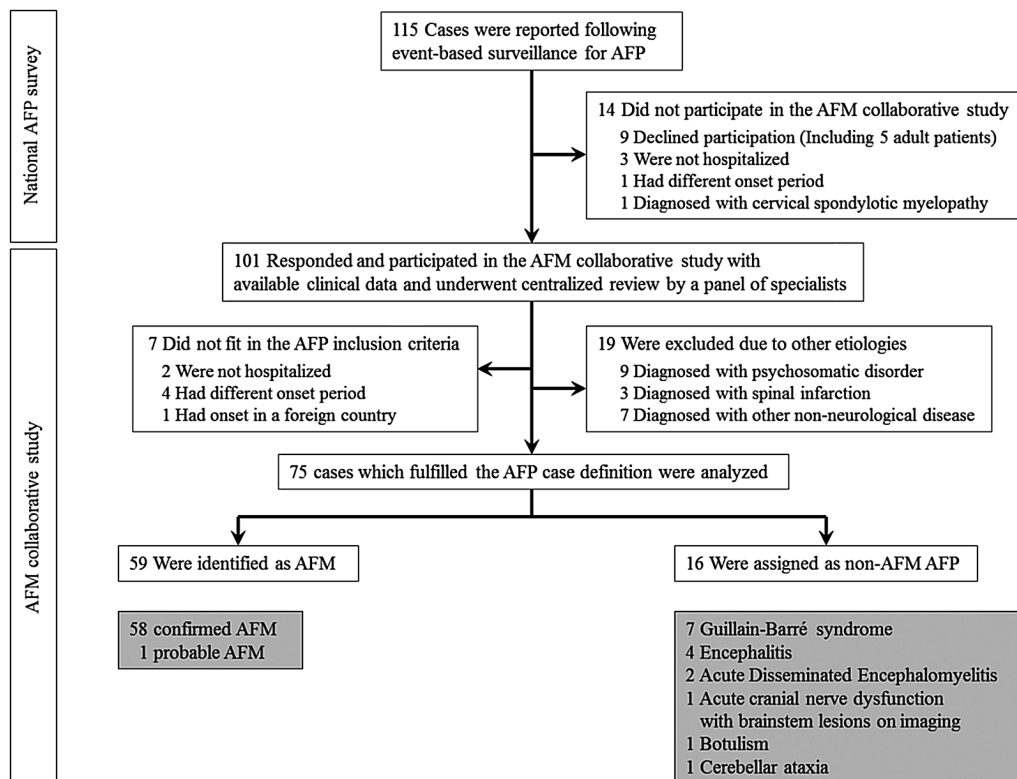


Figure 1. Overview of the study design. The first phase consisted of a national acute flaccid paralysis (AFP) survey, an event-based surveillance program initiated under special provision of the Infectious Diseases Prevention Law for the period August–December 2015. A second collaborative phase of the study aimed to clarify clinical characteristics of acute flaccid myelitis (AFM). Fifty-nine cases of AFM were identified after reviewing available clinical data. Sixteen other infectious or inflammation-related neurological diseases that did not satisfy the case definition of AFM were assigned as non-AFM AFP. Abbreviations: AFM, acute flaccid myelitis; AFP, acute flaccid paralysis.

Pathogen Detection

Clinical specimens such as serum, CSF, throat or nasopharyngeal swabs, stool, and urine were obtained for available cases. Specimens were tested by the prefectural and municipal public health institutes (PMPHIs) or NIID. At NIID, enterovirus was detected by real-time reverse-transcription polymerase chain reaction (RT-PCR) using 2 EV-D68-specific assays [3, 16], and 1 pan-enterovirus assay [17]. To confirm the accuracy of real-time RT-PCR identification, some of the PCR products were sequenced and identified by the partial VP1 sequence. Two EV-D68-positive cases were identified independently at 2 PMPHIs by sensitive, seminested RT-PCR using the consensus degenerate hybrid oligonucleotide primers [18] and partial VP1 sequencing.

Analysis of Epidemiological and Clinical Data

Associations between the AFM epidemic curve and nationwide trends in EV-D68-positive and other pathogen-positive cases from the epidemiological data of Infectious Agents Surveillance Report (IASR) were examined using the Pearson correlation. The weekly number of EV-D68-positive cases were obtained from the IASR dataset under the National Pathogen Surveillance System of Japan. The EV-D68-positive cases are voluntarily reported from the PMPHIs because EV-D68 infection is not classified as a notifiable disease [19, 20]. We used

the Student *t* test or Kruskal-Wallis test to compare continuous variables and the Pearson χ^2 test to compare categorical variables. Multiple logistic regression was applied to evaluate the multivariate-adjusted odds ratios of risk factors identified as significant by univariate analysis. A *P* value <.05 was considered statistically significant. All statistical analyses were performed using the SPSS software version 24 (IBM Corporation, Armonk, New York).

RESULTS

During the study period, 115 AFP cases from 89 hospitals were reported nationwide from event-based surveillance initiated under special provision of the Infectious Disease Prevention Law, capturing almost all of the AFP cases. Of the 101 possible cases enrolled in the AFM collaborative study, 59 satisfied the definition of AFM (58 confirmed and 1 probable case) (Figure 1), while no AFM cases satisfied the diagnostic criteria for ADEM, NMOSDs, or GBS, and only 1 AFM case fulfilled the criteria for acute transverse myelitis.

Temporal Correlation of AFM With EV-D68 Detection Rate

The AFM epidemic curve for 2015 followed a trend like that of weekly reported EV-D68-positive cases (Figure 2). Pearson correlation analysis revealed a strong association with EV-D68 trend ($r = 0.91$), an association not observed for other

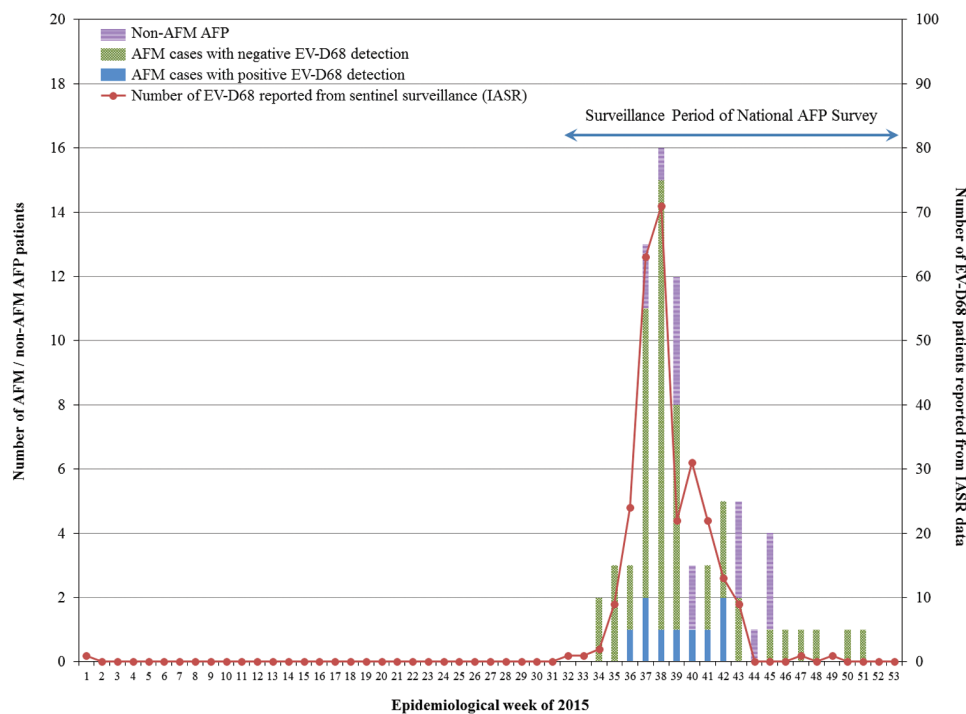


Figure 2. Cases of enterovirus D68 (EV-D68) infection and acute flaccid myelitis (AFM) in Japan during August–December 2015. The national acute flaccid paralysis (AFP) survey was commenced on 21 October 2015, for the surveillance period of August–December 2015. Epidemic curves of AFM cases positive for EV-D68, AFM cases negative for EV-D68, and AFP cases not meeting the definition of AFM (non-AFM AFP) are shown in parallel with EV-D68 infection cases reported to Japan's National Institute of Infectious Diseases through surveillance data of the Infectious Agents Surveillance Report. Although data for all of 2015 are shown, correlation analysis was conducted for the surveillance period only. Abbreviations: AFM, acute flaccid myelitis; AFP, acute flaccid paralysis; EV-D68, enterovirus D68; IASR, Infectious Agents Surveillance Report.

Table 1. Clinical Features and Laboratory Results of Patients Who Tested Positive for Enterovirus D68

Clinical Feature	Case Number													
	1	2	23	33	42	47	50	53	54	64	93			
Age	0 y 11 mo	3 y 7 mo	10 y 7 mo	6 y 10 mo	1 y 2 mo	3 y 9 mo	4 y 0 mo	7 y 5 mo	28 Y	33 Y	2 y 4 mo			
Sex	Male	Male	Male	Female	Male	Female	Male	Female	Female	Male	Female			
Time from onset of prodromal symptoms to neurological deficits, d	1	3	5	7	5	7	5	8	7	5	7			
Biological specimens tested positive for EV-D68	NP	NP	NP	Tracheal aspiration/NP/serum	Stool	Stool	NP	NP	CSF	Blood	NP			
Time from onset of neurological symptoms to specimen sampling, d	0	4	5	-5/12/23	6	3	1	11	1	2	3			
Immunocompromised status	No	No	No	Preceding steroid usage for asthma attack	No	No	No	No	No	No	No			
Final diagnosis	Confirmed AFM	Confirmed AFM	Confirmed AFM	Confirmed AFM	Probable AFM (clinically diagnosed radiculoneuritis)	Confirmed AFM	Confirmed AFM	Confirmed AFM	Confirmed AFM	Acute cranial nerve dysfunction with brainstem lesion	Cerebellar ataxia			
CSF pleocytosis (CSF WBCs/ μ L)	Positive (230)	Positive (31)	Positive (23)	Negative (3)	Positive (212)	Positive (68)	Positive (84)	Positive (41)	Positive (45)	Positive (116)	Negative (3)			
Limb paralysis	Lower limbs	Left upper limb	Left lower limb	Four limbs	Right lower limb	Right upper limb	Lower limbs	Left lower limb	Upper limbs			
Cranial nerve dysfunction	Bulbar weakness	...	Facial weakness	Bulbar weakness	Facial and bulbar weakness	...			
Localization of T2 high-intensity lesion on spinal gray matter	Medulla-L1	C2-C7	T6-L1	C2-C7, T3-L1	...	Medulla-L1	Medulla-L1	Medulla-L1	Medulla-T1	Pons and medulla	...			
No. of vertebral levels of spinal cord affected	20	6	8	17	...	20	20	20	8			
Other neuroradiological findings	Gd enhancement in right ventral nerve root	Atrophy of thoracic spine in follow-up MRI	Gd enhancement in ventral root of thoracic and lumbar spine, and cauda equina	Gd enhancement in cauda equina	Gd enhancement in ventral root of cervical spine	T2 high intensity in pons and medulla	...			
Neurophysiological study	Abnormal MCS, F-wave study	Abnormal MCS, F-wave study	Abnormal F-wave study	Abnormal MCS, SCS, F-wave study	Abnormal MCS	Abnormal F-wave study	Abnormal MCS	Abnormal MCS	Abnormal SCS, F-wave study	Not performed	Not performed			
Antianglioside antibodies	GD1b/PA-IgG(1+)	GM1-IgG(-), GO1b-IgG(-)	GM1-IgG(-), GO1b-IgG(-)	Not tested	GD1a/PA-IgG(1+), GD1b/PA-IgG(1+), GD3/PA-IgG(1+)	Not tested	Not tested	Not tested	GD1b-IgG(1+)	GM1-IgG(-), GO1b-IgG(-)	Not tested			
Prognosis of motor symptoms	Fair	Fair	Fair	Fair	Fair	Fair	Poor	Fair	Poor	No motor symptoms	Complete			

Abbreviations: AFM, acute flaccid myelitis; CSF, cerebrospinal fluid; EV-D68, enterovirus D68; Gd, gadolinium; IgG, immunoglobulin G; MCS, motor conduction study; MRI, magnetic resonance imaging; NP, nasopharynx; SCS, sensory conduction study; WBC, white blood cell.

viruses (Supplementary Figure 1 and Supplementary Table 4). Enterovirus A71 and poliovirus, important causative agents of AFP, were not detected during this period.

Pathogen Detection

This strong temporal correlation prompted us to test for EV-D68 in available biological samples of AFM cases at NIID. Among 20 cases tested, 7 (35%) were EV-D68 positive: 3 from respiratory, 2 from stool, 1 from CSF, and 1 from nasopharyngeal, serum, and tracheal aspiration samples (a pediatric case with preceding steroid usage) [21]. Remarkably, EV-D68 was detected in a CSF sample collected 1 day after the AFM onset from an adult AFM case (Table 1, case 54) by EV-D68-specific real-time RT-PCR [22]. Two additional AFM cases with EV-D68-positive nasopharyngeal samples were identified at PMPHIs, for a total of 9 EV-D68-positive cases (15%) (Table 2). We also tested for EV-D68 in non-AFM AFP patients and identified 2 additional cases, an ACB patient with positive serum and a cerebellar ataxia patient with a positive nasopharyngeal sample. Most EV-D68-positive samples were collected within 1 week after AFM onset; however, EV-D68 was detected in 1 case from several samples obtained at different times.

Clinical Characteristics of AFM

Table 3 shows the demographic features of the AFM patient group. Median age was 4.4 years (interquartile range [IQR], 2.6–7.7 years). Most cases were children, but 4 adults (7%) satisfied the inclusion criteria (Figure 3A). No known comorbidities before symptom onset were noted in 41 patients (69%), whereas 10 (17%) had asthma. Before neurological symptom onset, 52 patients (88%) had fever and experienced a prodromal illness with respiratory (75%) or gastrointestinal (19%) symptoms. Fever occurred 3.5 days (IQR, 1.0–5.3) before neurological symptoms and lasted about 4 days, with a peak body temperature of 38.5°C.

Most cases (78%) exhibited rapid onset with neurological symptoms progressed rapidly within 48 hours. All patients

Table 3. Demographic, Clinical, and Laboratory Findings of Patients With Acute Flaccid Myelitis (n = 59)

Characteristic	No. (%)
Demographics	
Median age, y (IQR)	4.4 (2.6–7.7)
Male sex	35 (59)
Prodromal symptoms before neurological onset	
Fever	52 (88)
Respiratory symptoms	44 (75)
Gastrointestinal symptoms	11 (19)
Median duration of fever, d (IQR)	4 (3–6)
Median period of fever before onset of limb weakness, d (IQR)	3.5 (1.0–5.3)
Neurological symptoms in acute stage	
Limb paralysis	59 (100)
1 limb	22 (37)
2 limbs	23 (39)
3 limbs	3 (5)
4 limbs	11 (19)
Asymmetric limb weakness	40 (68)
Hyporeflexia/areflexia	53 (90)
Cranial neuropathy	10 (17)
Focal paresthesia	12 (20)
Neurogenic bladder or bowel	16 (27)
Neck stiffness	7 (12)
Headache	7 (12)
Altered mental status	7 (12)
Lesions on brain MRI	
Cortical gray matter	0 (0)
Subcortical white matter	1 (2)
Basal ganglia	1 (2)
Cerebellum	0 (0)
Brainstem (any)	25 (42)
Midbrain	1 (2)
Pons	4 (7)
Medulla oblongata	25 (42)
Lesions on spine MRI	
T2 hyperintensity in spinal parenchyma ^a	59 (100)
Lesions localized in anterior horn gray matter	10 (17)
Median length of spinal lesion, No. of vertebral levels (IQR) ^b	20 (8–20)
Gadolinium enhancement (any)	
Spinal parenchyma	3 (5)
Spinal nerve root	9 (15)
Spinal nerve of cauda equina	30 (51)
Neurophysiological study	
Motor nerve conduction study, no./total no. of cases (%)	
Diminished motor conduction velocity	4/51 (8)
Absent compound muscle action potential	8/51 (16)
Diminished compound muscle action potential	31/51 (61)
Abnormal waveform	3/51 (6)
Sensory nerve conduction study, no./total no. of cases (%)	
Diminished sensory conduction velocity	1/30 (3)
Diminished sensory nerve action potential	7/30 (23)
F-wave study, no./total no. of cases (%)	
Decreased persistence	30/41 (73)
Results of CSF analysis, no./total no. (%)	
Pleocytosis, initial sample (WBC count >5 cells/ μ L)	50/59 (85)
Pleocytosis in samples taken within 0–5 d	40/42 (95)
Protein elevation, initial sample (>45 mg/dL)	27/59 (46)

Table 2. Detection of Enterovirus D68 From Different Biological Samples

No. of Samples Tested Positive for EV-D68	Types of Biological Sample				
	Respiratory	CSF	Serum	Stool	Urine
Samples collected within 1 wk after neurological onset, no. (%)	5/27 (19)	1/40 (3)	0/36 (0)	2/20 (10)	0/15 (0)
Samples collected in >1 wk after neurological onset, no. (%)	2/13 (15)	0/16 (0)	1/13 (8)	0/24 (0)	0/12 (0)
Total number of EV-D68 samples identified, no. (%)	7/40 (18)	1/56 (2)	1/49 (2)	2/44 (5)	0/27 (0)

Fifty-seven of 59 patients with acute flaccid myelitis underwent virological testing. Results included testing done in the National Institute of Infectious Diseases and prefectural and municipal public health institutes.

Abbreviations: CSF, cerebrospinal fluid; EV-D68, enterovirus D68.

Table 3. Continued

Characteristic	No. (%)
Elevated myelin basic protein (>102 pg/mL)	4/44 (9)
Elevated IgG index (>0.85)	7/36 (19)
Serum immunological study, no./total no. (%)	
Positive anti-aquaporin 4 antibody	0/27 (0)
Positive antimyelin oligodendrocyte glycoprotein antibody	0/8 (0)
Positive antiganglioside antibody	8/29 (28)
Positive GM1-IgG and GQ1b-IgG (conducted at community laboratories)	0/13 (0)
Positive antiganglioside antibodies after a full panel analysis	8/16 (50)
Median sampling day from neurological onset (IQR)	2.5 (1.8–3.0)
Treatment administered (any)	
Intravenous steroid	44 (75)
Intravenous immunoglobulin	46 (78)
Plasmapheresis	3 (5)
Mechanical ventilation	5 (8)
Clinical outcome	
Good outcome	
Complete improvement	7 (12)
Good improvement	10 (17)
Poor outcome	
Fair improvement	32 (54)
Poor improvement	10 (17)

Data are presented as no. (%) unless otherwise indicated.

Abbreviations: CSF, cerebrospinal fluid; IgG, immunoglobulin G; IQR, interquartile range; MRI, magnetic resonance imaging; WBC, white blood cell.

^aIncluded 1 case with undetermined predominant spinal gray matter involvement.

^bThe length of spinal lesions could be determined in 47 patients.

displayed flaccid paralysis, with hyporeflexia or areflexia in 53 patients (90%). Type and severity of limb paralysis varied, with upper limb monoplegia in 14 (24%), lower limb monoplegia in 8 (13%), 2-limb involvement in 23 (39%), triplegia in 3 (5%), and quadriplegia in 11 (19%). Asymmetric limb weakness was the prominent feature in 40 patients (68%), including 10 diplegic and 4 quadriplegic patients. Ten patients (17%) had cranial nerve dysfunction, 16 (27%) neurogenic bladder or bowel, 12 (20%) focal paresthesia, and 7 (12%) altered mental status. All patients with impaired mental status showed improvement and eventually returned to baseline status (Supplementary Table 2). In addition, 40 patients (68%) developed muscle atrophy in the paralyzed limbs during the clinical course. Clinical features of patients who tested positive for EV-D68 are summarized in Table 1.

Neuroradiological Features

All 59 patients underwent at least 1 spinal MRI, and 56 patients underwent brain MRI. Abstracted data are summarized in Table 3 and Figure 3B, and representative images are shown in Figure 4. Consistent with previous reports, all confirmed AFM patients had longitudinal cord lesions spanning a median of 20 vertebral levels (length determinable in 47 patients). Brainstem lesions were observed in 25 (42%) cases. Gadolinium was administered in 39 patients, revealing parenchymal enhancement in

3 cases (5%), ventral nerve root enhancement in 9 (15%), and cauda equina enhancement in 30 (51%). Predominant spinal gray matter involvement could not be determined in the only probable AFM patient (Table 1), who exhibited enhancement in cauda equina and both thoracic and lumbar ventral roots, clinically diagnosed as radiculoneuritis.

Neurophysiological Features

Neurophysiological investigation results are summarized in Table 3. Most studies were conducted within 2 weeks of neurological onset. Abnormal motor conduction was detected in 42 of 51 patients (82%), while 30 of 41 (73%) exhibited abnormal F-waves (Supplementary Table 5).

Cerebrospinal Fluid Features

Eighty-nine CSF specimens were obtained from 59 patients. Pleocytosis was present in 50 patients (85%), and CSF protein level was elevated in 27 (46%) at initial lumbar puncture. When all CSF specimens were considered, earlier lumbar puncture after neurological symptom onset yielded higher leukocyte count (Figure 5). In the 42 samples tested 0–5 days after onset, 40 (95%) showed pleocytosis.

Serum Immunology

None of the patients tested positive for anti-aquaporin 4 (AQP4) antibody or antimyelin oligodendrocyte glycoprotein (MOG) antibody. Anti-AQP4 antibody is a major pathogenic antibody detectable and is included as a diagnostic criteria in NMOSDs [13], while anti-MOG antibody has been associated with demyelinating disease of the central nervous system, including multiple sclerosis, NMOSDs, and ADEM [23]. Antiganglioside antibodies were detected in 8 of 29 patients (28%), with a higher positive detection rate (8/16 [50%]) using a full panel analysis (Supplementary Table 6) [24, 25], including 3 EV-D68–positive cases (Table 1). Median sampling time for antiganglioside antibodies from neurological onset was 2.5 days (IQR, 1.8–3.0 days). Leukocyte count and C-reactive protein level during admission were 9700 cells/μL (IQR, 7700–11600 cells/μL) and 0.09 mg/dL (IQR, 0.02–0.17 mg/dL), respectively.

Treatment, Outcome, and Prognostic Factors

Most patients received immunomodulation therapy consisting of high-dose intravenous immunoglobulin (IVIG) (11/59 [19%]), pulse methylprednisolone (mPSL) (9/59 [15%]), or combined IVIG and mPSL (35/59 [59%]). Seventeen patients (29%) received acyclovir because of unknown etiology, and 3 (5%) underwent plasmapheresis. Five patients (8%) required temporary mechanical ventilation because of respiratory failure. Despite treatment, only 7 patients (12%) showed complete recovery of limb weakness. The median follow-up period was 8.5 months (IQR, 6.9–9.6 months). Treatment response was evaluated by comparing MMT scores at pretreatment baseline and final checkup. Patients showing complete or good improvement

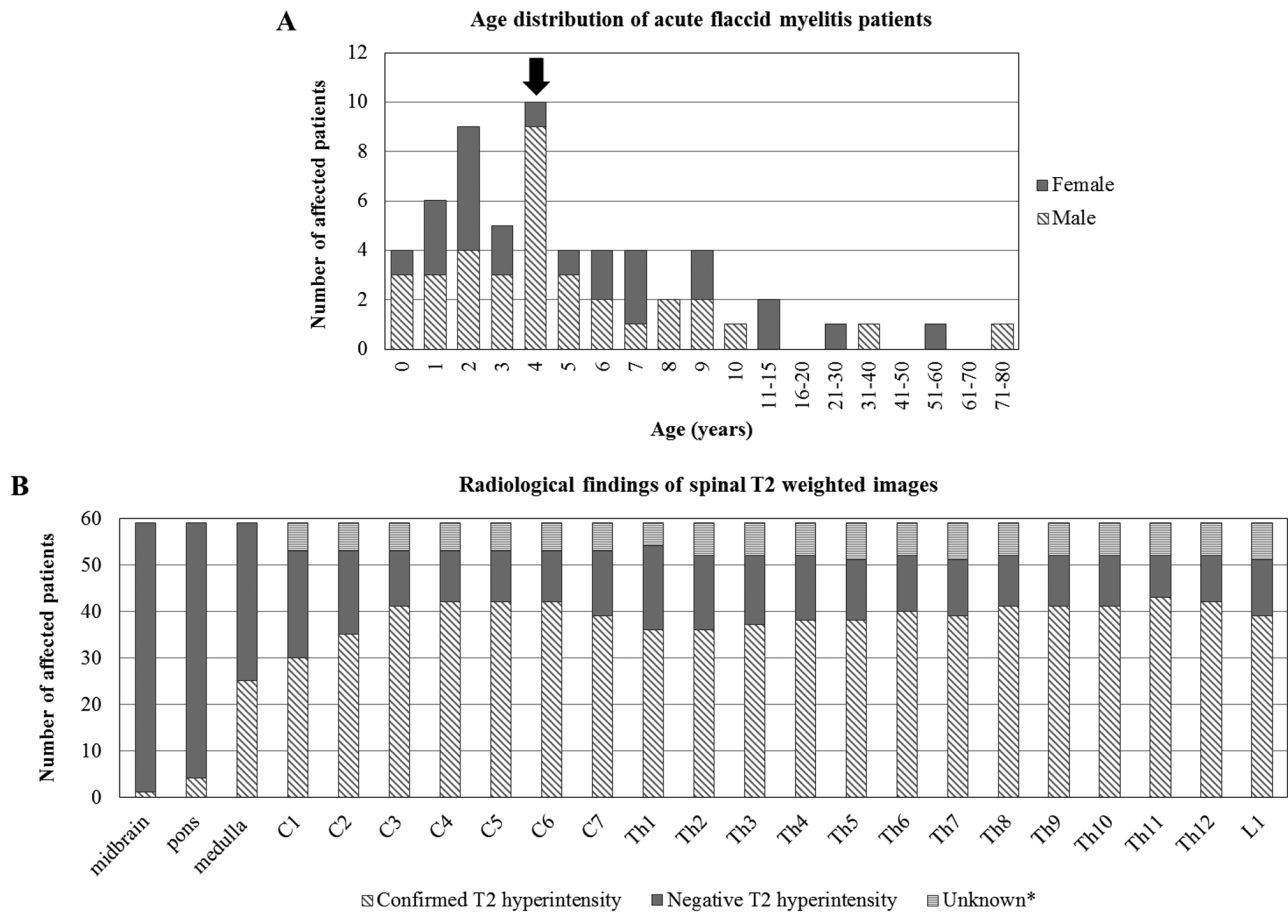


Figure 3. Age distribution and distribution of spinal T2 lesions of patients with acute flaccid myelitis (AFM). *A*, Median age at onset of patients with AFM was 4.4 y (arrow) with an interquartile range of 2.6–7.7 y. A total of 35 male and 24 female patients, including 4 adult patients, was reported in this period. *B*, Extensive longitudinal lesions were observed in most of the cases. *Unknown due to incomplete data (total spinal magnetic resonance imaging not done) or poor radiographic images.

were defined as having good outcome, and those with fair or poor improvement as having poor outcome. Most patients treated with IVIG or mPSL, even with early initiation after neurological onset, showed poor outcome. Cases with pretreatment MMT scores >3 ($P = .013$) and negative for EV-D68 ($P = .048$) showed good motor prognosis (Table 4). However, multiple regression analysis identified normal F-wave persistence as the only significant independent factor for good prognosis ($P = .010$). Other neurological symptoms usually had good outcome, with only 1 patient exhibiting residual facial paralysis, 2 paresthesia, and 3 neurogenic bladder at follow-up (Supplementary Table 2).

DISCUSSION

Although EV-D68 infections are mainly associated with respiratory illness, previous North American reports from 2014 suggested a strong epidemiological association with AFM [2, 4, 6]. In the present study, we detected EV-D68 in 9 AFM patients, including in the CSF (1/9) and blood (1/9) specimens of patients. Additional supporting evidence for a link between EV-D68 infection and AFM included a strong temporal correlation, with both incidences peaking within a 2- to 3-week

period. Furthermore, no such associations were observed for other viruses. This sudden increase in AFM cases during an EV-D68 outbreak and a second such occurrence, separated by 1 year and in a distant geographical area from the first, strongly suggests that EV-D68 is a major causative agent for AFM.

Core symptoms of AFM had been described as motor dominant, consistent with involvement of anterior horn neurons [1]. Although limb paralysis varied in type and severity, most cases showed extensive longitudinal spinal involvement (median of 20 spinal segments), and CSF pleocytosis (95% in samples taken within 0–5 days of onset), further confirming AFM as a defined clinical syndrome, although these traits were more pronounced than in previous US reports [2–4]. Alternatively, no AFM case fulfilled the diagnostic criteria for ADEM or NMOSDs with negative test results for anti-AQP4 and anti-MOG antibodies, indicating largely nonoverlapping clinical features. On the other hand, brainstem, and spinal root lesions were observed in 42% and 15% of radiographically investigated cases, respectively. Neurophysiological studies also confirmed peripheral nerve involvement in >80% of tested cases. Further, EV-D68 was detected in 1 ACB case without apparent spinal lesion involvement (Table 1, case 64), and 1

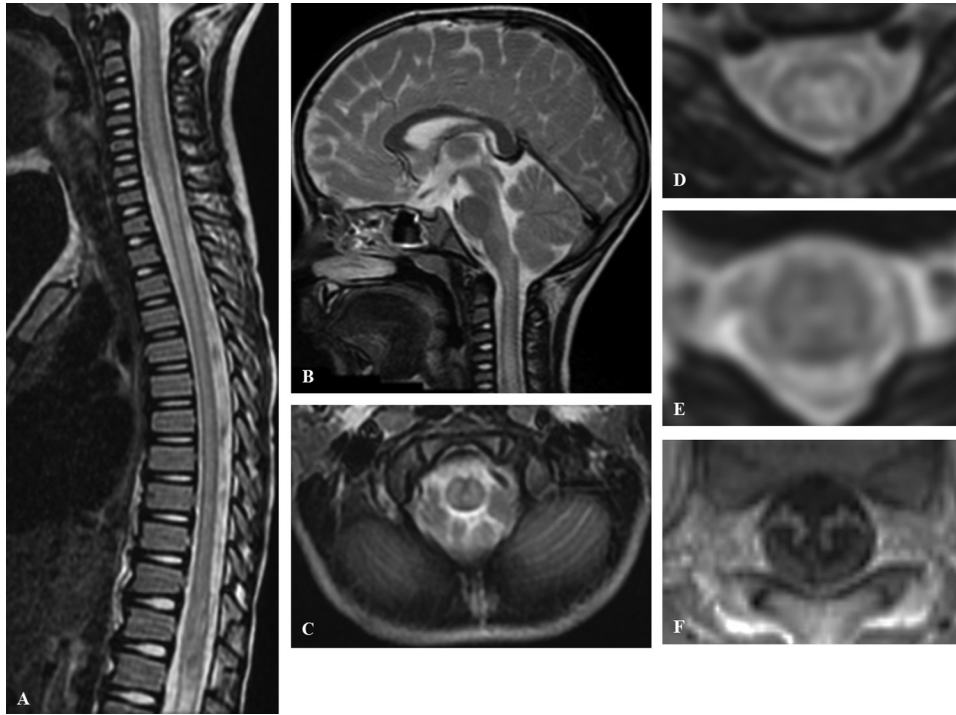


Figure 4. Representative magnetic resonance imaging findings. *A–E*, T2-weighted images of a 5-y-old child 2 d after the onset of acute flaccid myelitis (AFM). *A*, Longitudinally extensive hyperintense lesion involving the entire spinal cord was observed. *B*, This patient also had brainstem lesions ranging from midbrain to medulla oblongata. *C*, Axial images of medulla showed hyperintensities mainly in pyramids and around the obex. *D* and *E*, Axial images of spinal cord demonstrated hyperintensities in gray matter and surrounding white matter (at C5 level in *D*, and at the Th11/12 level in *E*). *F*, Panel shows gadolinium-enhanced T1-weighted images of a 4-y-old child 20 d after the onset of AFM. Enhancement was observed in anterior roots of cauda equina.

clinically diagnosed radiculoneuritis (Table 1, case 42), suggesting that neurological complications of EV-D68 infection can extend to or specifically target brainstem and peripheral nerves with myelitis as the predominant feature.

While previous reports suggested predominant neurological manifestations, not all EV-D68 infections have been linked to AFM [26]. Indeed, Japanese IASR data during the 2015 outbreak detected EV-D68 mainly from patients with respiratory symptoms [27]. Like poliovirus and hand, foot, and mouth disease (HFMD), only a small fraction EV-D68 infection cases manifested neurological symptoms [26]. Apparent polio cases are identified among <1% of the infected individuals and the case-severity rate among HFMD cases was reported to be 1.1% [28, 29].

Only 3 cases, 1 adult with AFM [22], 1 adult with ACB, and a pediatric AFM patient with early steroid usage [21], had EV-D68 in blood or CSF specimens, suggesting that host susceptibility factors increase the risk of neurological manifestations. Administration of steroids for asthma in the pediatric patient 5 days before neurological onset may have induced transient immunosuppression and persistent viremia. Differences in the immune responses in adults may have resulted in prolonged viremia and CSF presence, or increased viral load. Among paralytic cases due to poliomyelitis, the case-severity rate in adults has been reported to be higher than in infants [28]. Clinical features also differed between adult and pediatric

cases in that adults more frequently exhibited subacute onset (75% with neurological symptoms progressing in >72 hours) and extramotor symptoms (75% with cranial neuropathy; 50%

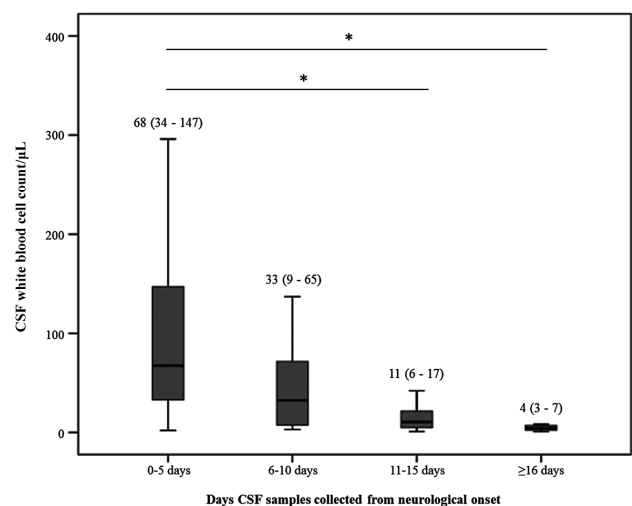


Figure 5. Cerebrospinal fluid (CSF) white blood cell (WBC) count during clinical course of acute flaccid myelitis (AFM). Boxplot of CSF WBC count taken during 0–5 d, 6–10 d, 11–15 d, and ≥16 d after neurological onset from patients with AFM. The Kruskal-Wallis rank-sum test with significant values adjusted by the Bonferroni correction for multiple tests was used. The median WBC count with interquartile range indicated in bracket of each group was shown. * $P < .05$. Abbreviation: CSF, cerebrospinal fluid.

Table 4. Predictors of Good Clinical Outcome in Patients With Acute Flaccid Myelitis

Variable	Univariate Analysis				Multivariate Analysis			
	Relative Risk	95% CI Lower Bound	95% CI Upper Bound	P Value	Odds Ratio	95% CI Lower Bound	95% CI Upper Bound	P Value
Fever >3 d	2.217	.723	6.801	.218	2.086	.164	26.605	.571
Fever >38.5°C	1.582	.697	3.590	.389				
Age <10 y	1.010	.290	3.512	1.000				
Adult	1.833	.628	5.349	.571				
Female sex	1.296	.583	2.880	.569				
No concomitant allergic disease	1.427	.539	3.779	.545				
Quadriplegia	1.343	.541	3.333	.713				
Polyplegia	1.932	.719	5.194	.237	0.918	.121	6.968	.934
No respiratory failure ^a	ND	ND	ND	.308				
Altered mental status	2.286	1.031	5.065	.176				
Pretreatment MMT score >3	3.030	1.509	6.084	.013 ^b	4.183	.497	35.198	.188
CSF WBC <60 cells/μL	1.050	.464	2.376	1.000				
CSF protein ≥45 mg/dL	2.030	.865	4.765	.149	1.223	.185	8.080	.834
No brainstem lesion on MRI	2.229	.826	6.016	.143	4.615	.479	44.451	.186
Spinal lesions <10 vertebral levels on MRI	1.391	.628	3.083	.557				
Normal amplitude of M-wave	1.064	.353	3.205	1.000				
Normal persistence of F-wave	5.250	1.934	14.249	.002 ^b	13.813	1.886	101.160	.010 ^b
Negative EV-D68 identification ^c	ND	ND	ND	.048 ^b				
Intravenous steroids	1.108	.426	2.880	1.000				
Steroids started in <3 d from neurological onset	1.500	.609	3.693	.496				
Intravenous immunoglobulin	1.930	.884	4.216	.166	0.980	.113	8.532	.986
Immunoglobulin started within <3 d from onset	1.056	.279	3.987	1.000				

Abbreviations: CI, confidence interval; CSF, cerebrospinal fluid; EV-D68, enterovirus D68; MMT, manual muscle strength test; MRI, magnetic resonance imaging; ND, not determined; WBC, white blood cell count.

^aThirty-one percent of cases without respiratory failure had good outcome. All 5 cases with respiratory failure had poor outcome; hence, relative risk was not determined. *P* value was calculated by 2-sided Fisher exact test.

^bStatistically significance (*P* < .05).

^cAll 9 EV-D68-positive cases had poor outcome, whereas 34% of EV-D68-negative cases had good outcome. Thus, relative risk by univariate analysis and odds ratio by multivariate analysis could not be performed.

with neurogenic bladder, focal paresthesia, and altered mental status). Considering the poorer prognosis of EV-D68-positive cases, it is plausible that the balance between host responses to EV-D68 and viral clearance contributes to disease susceptibility.

Acute flaccid myelitis is a new clinical entity originally defined during a 2014 EV-D68 outbreak [30], but the pathomechanisms are still uncertain because many cases are without detectable EV-D68 infection. Clinical and radiographic patterns appear like those of poliovirus, enterovirus A71, and West Nile virus, suggesting a common pathophysiology [31–33]. However, active surveillance in the United States during 2015–2016 failed to consistently detect EV-D68 in sporadic AFM cases. Inability to detect EV-D68 in CSF makes direct neuroinvasion as the sole infection route unlikely. Selective localization to spinal motor neurons, which typically occurs during the acute stage of an infection, and detection of antiganglioside antibodies (median of 2.5 sampling days from onset) as seen in GBS point to an immune-mediated process following a parainfectious profile.

Antiganglioside antibodies targeted gangliosides in the peripheral nervous system causing immune-mediated polyradiculoneuropathy, including GBS, through molecular mimicry, usually after an infectious event [34]. It is also possible that several mechanisms, ranging from infectious to parainfectious, contribute to the disease spectrum of AFM.

To our knowledge, none of the earlier reports on AFM investigated prognostic factors. In the present study, severe pretreatment limb weakness (MMT score ≤3) was associated with poor outcome, and immunomodulation therapy failed to ameliorate neurological consequences irrespective of therapeutic agent or timing. This treatment failure could be caused by the rapid progression of neurological symptoms. Considering these poor neurological outcomes, vaccine development might offer the best option for disease control if further evidence implicates EV-D68 as a critical AFM-causing agent [35]. Preserved F-waves and other electrophysiological metrics are good predictors of clinical prognosis. Successful restoration of upper

limb function was reported in AFP patients following nerve and muscle transfer [36, 37]; therefore, these procedures may be therapeutic options for patients with predicted poor outcome.

There are some limitations to our study, including a lack of recent comparative data. There had been no routine surveillance for AFP in Japan since the last poliomyelitis case owing to wild poliovirus in 1980 [38], and the actual numbers of AFP/AFM cases and associated infectious agents were not officially monitored before the introduction of provisional AFP surveillance in October 2015. Second, infectious agent surveillance protocols for AFP/AFM, including timely sample collection, and laboratory diagnosis systems for EV-D68 and other possible agents have not been established and standardized. Third, the possibility of other pathogens causing AFM via similar pathomechanisms cannot be excluded as we only focused on EV-D68 detection because of the limited sample volume. Indeed, during the 2014 US outbreak, enterovirus C105, influenza virus, and other pathogens were detected in patients with AFM [2, 3, 5, 7, 39].

Our study clarifies the clinical characteristics of AFM and expands the spectrum of neurological involvement observed during an EV-D68 outbreak. Clusters of AFM cases were temporally correlated with reported EV-D68 infections, and detection of EV-D68 in CSF and serum specimens provided further evidence that EV-D68 may be one of the major causative agents for AFM. However, the relatively low EV-D68 detection rate and the many sporadic cases without detectable EV-D68 infection suggest that factors such as host susceptibility predispose to AFM. With no effective treatment identified in the acute stage, studies are required to elucidate the pathophysiology and pathomechanisms of AFM and to develop both preventive measures and novel therapeutic interventions.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Author contributions. P. F. C., R. K., and K. T. T. conceived of and designed the study. P. F. C. and R. K. analyzed the clinical data and wrote the first draft of the manuscript. H. M. and A. O. analyzed neuroradiological data of all AFP cases. H. T. and S. Y. reviewed all neurophysiological data. P. F. C., R. K., H. M., A. O., H. T., S. Y., and K. T. T. conducted centralized review of clinical data to determine AFM cases. H. S., T. F., and N. H. collected biological samples, coordinated and did laboratory testing for enterovirus D68. S. K. and T. T. collected samples and analyzed immunological studies for autoantibodies. K. O. coordinated and analyzed pathogen surveillance data. P. F. C. and H. T. performed statistical analysis. All authors read and approved the final manuscript. R. K. and K. T. T. acted as the guarantors of the manuscript.

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APPENDIX

In addition to the authors, the following investigators and institutions participated in the acute flaccid myelitis collaborative study:

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