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

Pin Fee Chong,†1,‡ Ryutaro Kira,† Harushi Mori,† Akihisa Okumura,† Hiroyuki Torisu,‡ Sawa Yasumoto,§ Hiroyuki Shimizu,§ Tsuguto Fujimoto,† Nozomu Hanaoka,∥ Susumu Kusunoki,∥ Toshiyuki Takahashi,∥ Kazunori Oishi,∥ and Keiko Tanaka-Taya† for the Acute Flaccid Myelitis Collaborative Study Investigators†

†Department of Pediatric Neurology, Fukuoka Children's Hospital; ‡Department of Radiology, Graduate School and Faculty of Medicine, University of Tokyo; §Department of Pediatrics, Aichi Medical University, Nagakute; Department of Pediatrics, Fukuoka Dental College Medical and Dental Hospital; ∥Medical Education Center, Fukuoka University School of Medicine; ¶Department of Virology II and Infectious Disease Surveillance Center, National Institute of Infectious Diseases, Tokyo; †Department of Neurology, Kindai University Faculty of Medicine, Osaka-Sayama, and Department of Neurology, Tohoku University Graduate School of Medicine, Sendai, Japan

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.
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/µL. 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 sequelae 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 χ² 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
| Clinical Feature                                      | Case Number |
|------------------------------------------------------|-------------|
| 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      |
|                                                      | Female      |
|                                                      | Male        |
|                                                      | Female      |
| Time from onset of prodromal symptoms to neurological deficits, d | 1            |
|                                                      | 3           |
|                                                      | 5           |
|                                                      | 7           |
|                                                      | 5           |
|                                                      | 7           |
|                                                      | 5           |
| 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           |
| Immune compromised status                             | No          |
|                                                      | No          |
|                                                      | No          |
|                                                      | Preceding steroid usage for asthma attack | No |
|                                                      | No          |
|                                                      | No          |
|                                                      | No          |
| Final diagnosis                                       | Confirmed AFM |
|                                                      | Confirmed AFM |
|                                                      | Confirmed AFM |
|                                                      | Confirmed AFM |
|                                                      | Confirmed AFM |
|                                                      | Confirmed AFM |
|                                                      | Acute cranial nerve dysfunction with brainstem lesion |
|                                                      | Cerebellar ataxia |
| CSF pleocytosis (CSF WBCs/μL)                         | Positive (230) |
|                                                      | Positive (31) |
|                                                      | Positive (23) |
|                                                      | Negative (3) |
|                                                      | Positive (212) |
|                                                      | 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 MCS, F-wave study |
|                                                      | Abnormal MCS, F-wave study |
|                                                      | Abnormal MCS |
|                                                      | Abnormal MCS |
|                                                      | Abnormal MCS |
|                                                      | Abnormal MCS |
|                                                      | Not performed |
|                                                      | Not performed |
| Antiganglioside antibodies                             | GD1b/PA-IgG1+ |
|                                                      | GM1-IgG(I−) |
|                                                      | GM1-IgG(I−) |
|                                                      | GD1b/PA-IgG1+ |
|                                                      | Not tested |
|                                                      | Not tested |
|                                                      | GD1a/PA-IgG1+ |
|                                                      | GD1b/PA-IgG1+ |
|                                                      | GD3/PA-IgG1+ |
|                                                      | GD1b-IgG(I+)|
|                                                      | GM1-IgG(I−), GQ1b-IgG(I−) |
| Prognosis of motor symptoms                           | Fair        |
|                                                      | Fair        |
|                                                      | Fair        |
|                                                      | Fair        |
|                                                      | Fair        |
|                                                      | Poor        |
|                                                      | Poor        |
|                                                      | Poor        |
|                                                      | No motor symptoms |
|                                                      | Complete    |

**Abbreviations:** AFM, acute flaccid myelitis; CSF, cerebrospinal fluid; EV-D68, enterovirus D68; GD, ganglioside; IgG, immunoglobulin G; MCS, motor conduction study; MRI, magnetic resonance imaging; NP, nasopharynx; SCS, sensory conduction study; WBC, white blood cell.
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: 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 symptom onset, 52 cases (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 symptom onset, 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.

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

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. 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) |
| Prodomal symptoms before neurological onset | 57 (97) |
| 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 (80) |
| 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* | 59 (100) |
| Lesions localized in anterior horn gray matter | 10 (17) |
| Median length of spinal lesion, No. of vertebral levels (IQR)* | 20 (8–20) |
| Gadolinium enhancement (any) | 36 (61) |
| 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/μL) | 50/59 (85) |
| Pleocytosis in samples taken within 0–5 d | 40/42 (95) |
| Protein elevation, initial sample (>45 mg/dL) | 27/59 (46) |

Acute Flaccid Myelitis and EV-D68 • CID 2018:66 (1 March) • 657
displayed flaccid paralysis, with hyporeflexia or areflexia in 53 patients (90%). Type and severity of limb paralysis varied, with upper limb monoparesis in 14 (24%), lower limb monoparesis 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 anti-myelin 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...
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 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.
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%...
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 levels on MRI) suggest EV-D68 as a critical AFM-causing agent [34]. The best option for disease control if further evidence implicates EV-D68 as a critical AFM-causing agent [35].

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.

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 | PValue | Odds Ratio | 95% CI Lower Bound | 95% CI Upper Bound | PValue |
| 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   |            |                   |                   |        |
| Poliomyelgia                                  | 1.932               | .719                 | 5.194              | .237   | 0.918      | .121              | 6.968              | .934   |
| No respiratory failure                        | 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   | 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   | 13.813     | 1.886             | 101.160            | .010   |
| Negative EV-D68 identification                | ND                  | ND                   | ND                 | .048   |            |                   |                   |        |
| 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              | .966   |
| 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.

*Thirty-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).

All 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.
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 occurring 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.

Acknowledgments. We thank the patients, their families, and members of the Japanese Society of Child Neurology for participation; Takao Takahashi, Akira Oka, Shinji Saitoh, Kenji Okada, Mitsuaki Hosoya, Masato Yashiro, Tsuneo Morishima, and Toshiro Hara for valuable advice; and Harutaka Katano, Hitomi Kinoshita, Hideo Okuno, Hiroshi Satoh, Satoru Arai, and Tomimasa Sunagawa for their valuable comments and technical supports. We also thank epidemiologists and laboratory experts at the prefectural and municipal health centers, local public health institutions, Japan association of prefectural and municipal public health institutes, and Ministry of Health, Labour and Welfare for providing information, laboratory data, and clinical samples under provisional AFP surveillance from August to December 2015. The authors also thank Enago (www.enago.jp) for the English language review.

Financial support. This work was supported by the Health and Labor Sciences Research Grant from the Ministry of Health, Labour and Welfare of Japan (grant number H25-Shinko-Shitei-006 to K. T. T. and H28-Shinkokogyosei-Ippan-007 to K. T. T., R. K., H. M., A. O., H. T., S. Y., H. S.), the Research Program on Emerging and Re-emerging Infectious Diseases from the Japan Agency for Medical Research and Development (AMED) (grant numbers 40104400 to H. S. and 40104402 to T. F.), the Ministry of Education, Culture, Sports, Sciences, and Technology of Japan (grant numbers 16H05200 and 15H04845 to S. K.), and Fukuoka Children’s Hospital Research Fund (to R. K.).

Potential conflicts of interest. All authors: No potential conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References
1. Messacar K, Schreiner TL, Van Haren K, et al. Acute flaccid myelitis: a clinical review of US cases 2012–2015. Ann Neurol 2016; 80:326–38.
2. Van Haren K, Ayseuc P, Wauhunt E, et al. Acute flaccid myelitis of unknown etiology in California, 2012-2015. JAMA 2015; 314:2663–71.
3. Sejvar JJ, Lopez AS, Cortese MM, et al. Acute flaccid myelitis in the United States, August–December 2014: results of nationwide surveillance. Clin Infect Dis 2016; 63:737–45.
4. Messacar K, Schreiner TL, Maloney JA, et al. A cluster of acute flaccid paralysis and cranial nerve dysfunction temporally associated with an outbreak of enterovirus D68 in children in Colorado, USA. Lancet 2015; 385:1662–71.
5. Greninger AL, Naccache SN, Messacar K, et al. A novel outbreak enterovirus D68 strain associated with acute flaccid myelitis cases in the USA (2012-14): a retrospective cohort study. Lancet Infect Dis 2015; 15:671–82.
6. Alahadi N, Messacar K, Pastula DM, et al. Enterovirus D68 infection in children with acute flaccid myelitis, Colorado, USA, 2014. Emerg Infect Dis 2016; 22:1387–94.
7. Nelson GR, Bonkowski JL, Doll E, et al. Recognition and management of acute flaccid myelitis in children. Pediatr Neurol 2016; 55:17–21.
8. Ministry of Health, Labour and Welfare, Japan. About the survey of cases with acute flaccid paralysis (request of cooperation), released on October 21, 2015. Available at: http://www.jispd.or.jp/news/1510_afp.pdf. Accessed 21 October 2015.
9. Infectious Agents Surveillance Report. Weekly reports of enterovirus 68 isolation/detection, 2015 and 2016. Available at: http://www.nih.go.jp/niss/ideas/iasr/arc/ott/2016/data2016.109e.pdf. Accessed 26 October 2016.
10. Council of State and Territorial Epidemiologists. Standardized case definition for acute flaccid myelitis, 2015. Available at: http://cymcdn.com/sites/www.cste.org/resource/resmgr/2015FS/2015FSfinal/15-ID-01.pdf. Assessed 20 October 2015.
11. Krupp LB, Tardieu M, Amato MP, et al. International Pediatric Multiple Sclerosis Study Group criteria for pediatric multiple sclerosis and immune-mediated central nervous system demyelinating disorders: revisions to the 2007 definitions. Mult Scler 2013; 19:1261–67.
12. Transverse Myelitis Consortium Working Group. Proposed diagnostic criteria and nosology of acute transverse myelitis. Neurology 2002; 59:499–505.
13. Wingerchuk DM, Banwell B, Bennett JL, et al; International Panel for NMO Diagnosis. International consensus diagnostic criteria for neuromyelitis optica spectrum disorders. Neurology 2015; 85:177–89.
14. Asbury AK, Cornblath DR. Assessment of current diagnostic criteria for Guillain-Barré syndrome. Ann Neurol 1990; 27(Suppl):S21–4.
15. Hilsop HJ, Montgomery I, eds. Daniels and Worthingham’s muscle testing techniques of manual examination. Philadelphia, PA: W.B. Saunders Company, 2002.
16. Wylie TN, Wylie KM, Buller RS, Cannella M, Storch GA. Development and evaluation of an enterovirus D68 real-time reverse transcription PCR assay. J Clin Microbiol 2015; 53:2641–7.
17. Nijhuis M, van Maarseveen N, Schuurman R, et al. Rapid and sensitive routine detection of all members of the genus enterovirus in different clinical specimens by real-time PCR. J Clin Microbiol 2002; 40:3666–70.
APPENDIX

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

Saitama Citizens Medical Center: Tsutsui Toyofuku; Nagano Children's Hospital: Tetsumi Fukuyama; Nagasaki University Hospital: Tatsumaru Sato; Nagaoka Red Cross Hospital: Yuya Takahashi; Kochi Health Sciences Center: Akane Kanazawa; Okinawa Prefectural Southern Medical Center and Children's Medical Center: Masato Hiyane; Niigata Prefectural Shibata Hospital: Takao Fukushima; Kitasato University: Taira Toki; Osaka Medical Center and Research Institute for Maternal and Child Health: Ryoko Hayashi; Nihon University Itabashi Hospital: Sonoko Kubota, Wakako Ishii; Iwate Medical University: Manami Akasaka, Haruna Miyazawa; Shinsu University Hospital: Mitsuo Motobayashi; Nagano Municipal Hospital: Mari Asaoka; Gunma Children's Medical Center: Takashi Shiihara; Matsudo City Hospital: Yoshitaka Miyoshi, Tomohiko Tsuru, Kenta Ikeda; Fukuoka Children's Hospital: Masaru Matsukura, Ryoko Nakamura; Tokyo Medical and Dental University: Kengo Moriyama; Soka Municipal Hospital: Yuji Sugawara; Japanese Red Cross Society Himeji Hospital: Yuichi Takami; Fukuoka University Hospital: Takako Fujita; Akita University Hospital: Tamami Yano; The University of Tokyo Hospital: Mariko Kasai; Hachinohe City Hospital: Takashi Uchida, Masashi Fujita; Tohoku University Hospital: Mitsugu Uematsu; Kitano Hospital, Tazuke Kofukai Medical Research Institute: Atsuo Hata, Hideto Ogata; Yokosuka General Hospital Uwamachi: Tomoyuki Miyamoto, Kataraha Sumi; Tokyo Medical University Hospital: Yu Ishida; National Center Hospital, National Center of Neurology and Psychiatry: Eri Takeshita, Tomoya Kawaoe; Ishikawa Prefectural Central Hospital: Takayoshi Kawabata; Nippon Medical School Chiba Hokusoh Hospital: Chiharu Miyatake; National Center for Child Health and Development: Akiko Yakuwa, Yu Kakimoto, Hiroshi Terashima, Masaya Kubota; Saitama Medical University: Yuichi Abe, Michiaki Nagura, Hideo Yamanouchi; Matsue Red Cross Hospital: Satomi Mori; Kagawa University: Yukihiko Konishi; Tokai University Hachioji Hospital: Mariko Ikeyama; Yoko Tomonaga; Kanagawa Children's Medical Center: Yumiko Takashima, Kazushi Ickihara; Hitachi, Ltd. Hitachinaka General Hospital: Nobuko Moriyama; Hirakata City Hospital: Chizu Oba, Mitsuru Kashiwagi; Osaka Rosai Hospital: Susuke Yoshikawa; Banbuntane Hotokukai Hospital, Fujita Health University: Kenichi Tanaka; University of Fukui Hospital: Genrei Ohta; Nagoya City University Hospital: Ayako Hattori, Daisy Eida; Kawasaki Medical School Hospital: Sahoko Ono; Yokkaichi Municipal Hospital: Tomoshige Tomina, Kyoko Ban; Tokai University: Nobuyoshi Sugiyama; Japanese Red Cross Society Kyoto Daichi Hospital: Nozomi Kouzan, Yuki Yamada; National Hospital Organization Kanazawa Medical Center: Mika Inoue, Kenichi Sakajiri; Toyoohashi Municipal Hospital: Ken Ohyama; Japan Community Health Care Organization Osaka Hospital: Miho Yamamuro; Hamamatsu Medical Center: Hidetoshi Ishigaki; Niigata City General Hospital: Azusa Seino, Shuichi Igarashi; Hospital of the University of Occupational and Environmental Health: Takahito Nakamoto, Kanae Sugimoto, Mitsuhiro Ochi; Nara Medical University Hospital: Eri Hamana; Hyogo Prefectural Amagasaki General Medical Center: Kazuki Ohi, Hidefumi Kawasaki, Masahiko Nishitani; Nippon Koukan Fukuyama Hospital: Hiroshi Uno; Okayama Red Cross General Hospital: Masaru Inoue, Tokyo Metropolitan Children's Medical Center: Maid Okuyama; Saiseikai Yokohamashi Tobu Hospital: Ayako Yamamoto; Kannon Medical Center: Ryota Sato; Tokyo Women's Medical University Medical Center

Acute Flaccid Myelitis and EV-D68 • CID 2018:66 (1 March) • 663

18. Nix WA, Oberste MS, Pallansch MA. Sensitive, seminested PCR amplification of VP1 sequences for direct identification of all enterovirus serotypes from original clinical specimens. J Clin Microbiol 2006; 44:2698–704.
19. Taniguchi K, Hashimoto S, Kawado M, et al. Overview of infectious disease surveillance system in Japan, 1999–2005. J Epidemiol 2007; 17(Suppl):S3–13.
20. Infectious Disease Surveillance Center. Pathogen surveillance system in Japan and Infectious Agents Surveillance Report (IASR). Available at: http://idsc.nih.go.jp/iasr/31/361/tpc361.html. Accessed 30 October 2016.
21. Mori S, Endo M, Uchida Y, et al. Six cases of enterovirus D68 infection including acute flaccid paralysis (in Japanese). J Jpn Pediatr Soc 2016; 120:1495–501.
22. Kimura K, Fukushima T, Katada N, et al. Adult case of acute flaccid paralysis with enterovirus D68 detected in the CSF. Neuroul Clin Prac 2016. doi:10.1212/CPCJ.1.311.
23. Reindl M, Di Pauli F, Rostasy K, Berger T. The spectrum of MOG auto-body-associated demyelinating diseases. Nat Rev Neurol 2013; 9:455–61.
24. Kusunoki S, Chiba A, Kon K, et al. N-acetylglactosaminyl GD1α as a target molecule for serum antibody in Guillain-Barré syndrome. Ann Neurol 1994; 35:570–6.
25. Kusunoki S, Morita D, Ohminami S, Hitoshi S, Kanazawa I. Binding of immunoglobulin G antibodies in Guillain-Barré syndrome sera to a mixture of GM1 and a phospholipid: possible clinical implications. Muscle Nerve 2003; 27:302–6.
26. Holm-Hansen CC, Midgley SE, Fischer T. Global emergence of enterovirus D68: a systematic review. Lancet Infect Dis 2016; 16:664–75.
27. Korematsu S, Nagashima K, Sato Y, et al. ‘Spike’ in acute asthma exacerbations during enterovirus D68 epidemic in Japan: a nation-wide survey. Allergol Int 2017. doi:10.1016/j.alit.2017.04.003.
28. Nathanson N, Kew OM. From emergence to eradication: the epidemiology of polioviruses deconstructed. Am J Epidemiol 2010; 172:1231–29.
29. Xing W, Liao Q, Viboud C, et al. Hand, foot, and mouth disease in China, 2008–12: an epidemiological study. Lancet Infect Dis 2014; 14:308–18.
30. Notes from the field: acute flaccid myelitis among persons aged </=21 years—United States, August 1–November 13, 2014. MMWR Morb Mortal Wkly Rep 2015; 63:1243–4.
31. Choudhary A, Sharma S, Sankhyan N, et al. Midbrain and spinal cord magnetic resonance imaging (MRI) changes in poliomyelitis. J Child Neurol 2010; 25:497–9.
32. Petersen JR, Brault AC, Nasri RS. West Nile virus: review of the literature. JAMA 2013; 310:308–15.
33. Teoh HL, Mohammad SS, Britton PN, et al. Clinical characteristics and functional motor outcomes of enterovirus 71 neurological disease in children. JAMA Neurol 2016; 73:300–7.
34. Kaida K, Kusunoki S. Antibodies to gangliosides and ganglioside complexes in Guillain-Barré syndrome and Fisher syndrome: mini-review. JAMA 2016; 310:308–15.
35. Teoh HL, Mohammad SS, Britton PN, et al. Clinical characteristics and functional motor outcomes of enterovirus 71 neurological disease in children. JAMA Neurol 2016; 310:308–15.
36. Li R, Liu L, Mo Z, et al. An inactivated enterovirus 71 vaccine in healthy children. N Engl J Med 2014; 370:829–37.
37. Funahashi S, Nagano A, Sano M, Ogihara H, Omura T. Restoration of shoulder function and elbow flexion by nerve transfer for poliomyelitis-like paralysis caused by enterovirus 71 infection. J Bone Joint Surg Br 2007; 89:246–8.
38. Satbhai NG, Doi K, Hattori Y, Sakamoto S. Restoration of prehensile function for motor function in Hopkins syndrome: case report. J Hand Surg Am 2014; 39:312–6.
39. Shimizu H. Development and introduction of inactivated poliovirus vaccines derived from Sabin strains in Japan. Vaccine 2016; 34:1975–85.
40. Horner LM, Poulter MD, Benton JN, Turner RB. Acute flaccid paralysis associated with novel enterovirus C105. Emerg Infect Dis 2015; 21:1858–60.
East: Norihiko Azuma, Sakiko Mabuchi, Yoko Shida; Kyushu University Hospital: Yu Hashimoto, Motoi Yoshimura; Gifu Prefectural General Medical Center: Yuki Matsuhisa; Saiseikai Yokohamashi Nanbu Hospital: Kotaro Nakano; Yokohama Municipal Citizen’s Hospital: Yukio Yamashita; Nerima Hikarigaoka Hospital: Eriko Kikuchi; Kyorin University Hospital: Asuka Yamamoto; Juntendo University Nerima Hospital: Naru Igarashi, Noboru Yoshida; Tokyo Metropolitan Cancer and Infectious Diseases Center Komagome Hospital: Shingo Nishiki; National Tokyo Medical Center: Daisuke Yasutomi; Kindai University: Nobuyoshi Kusano; PL Hospital: Ryohei Wakahara; Okitama Public General Hospital: Masayuki Furuyama; Iwate Prefectural Central Hospital: Hitoshi Mikami; Sagamihara National Hospital: Hiroaki Taniguchi; Nippon Koukan Hospital: Yasuhiro Yoshii; Kawasaki Municipal Kawasaki Hospital: Atsushi Narabayashi; Yonezawa National Hospital: Toshiyuki Takahashi; The Research Foundation for Microbial Diseases of Osaka University (BIKEN): Tomofumi Nakamura; Saitama City Institute of Health Science and Research: Yasuo Kaburagi; Kyoto City Institute of Health and Environmental Sciences: Akiko Nagasao; Kindai University: Motoi Kuwahara; National Defense Medical College: Kenichi Kaida.