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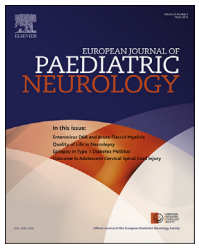

Review article

Acute flaccid myelitis caused by enterovirus D68: Case definitions for use in clinical practice

Rolf Kramer [a,b,*], *Bruno Lina* [b,c], *Jay Shetty* [d,e]

[a] *European Public Health Microbiology Training Programme (EUPHEM)*
[b] *Centre National de Référence des Enterovirus et Parechovirus, Laboratoire de Virologie, Institut des Agent Infectieux, HCL, Hôpital de la Croix-Rousse, Lyon, France*
[c] *Virpath, CIRI, Université de Lyon, INSERM U1111, CNRS 5308, ENS de Lyon, UCBL, Lyon, France*
[d] *Paediatric Neurosciences, Royal Hospital for Sick Children, Edinburgh, UK*
[e] *Child Life and Health, University of Edinburgh, Edinburgh, UK*

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ABSTRACT

Acute flaccid myelitis (AFM) was increasingly detected in recent years, coinciding with upsurges of enterovirus D68 (EV-D68) infections. We reviewed the evidence for a causal relationship between both. Based on reported cases, we provide case definitions for AFM caused by EV-D68 infections to enable a standard procedure for affected patients. Current case definitions are focussing on epidemiological aspects but clinical case definitions are still missing. We propose the following case definitions to be used in clinical practice in order to mirror clinical realities and facilitate a common systematic approach in case management: A possible case is defined as a person presenting with either acute myelitis/paralysis or Guillain-Barré Syndrome (GBS), particularly during periods of EV-D68 circulation. A probable case is defined as a person presenting with symptoms of either acute myelitis/paralysis or GBS and at least one of the following criteria: *i)* MRI abnormality representing with T2 hyperintensity in spinal cord grey matter with or without hyperintensity at dorsal brain stem, *ii)* investigations showing an axonal neuropathy including reduced compound motor action potentials with normal conduction velocities and absence of conduction blocks compatible with anterior horn cell disease or *iii)* detection of enteroviruses in a respiratory specimen obtained from the lower respiratory tract during periods of EV-D68 circulation. A confirmed case is defined as a person presenting with acute flaccid myelitis/paralysis, MRI abnormality and detection of enterovirus-D68-specific nucleic acids in a respiratory specimen using a validated PCR assay targeting the VP1 gene with subsequent sequencing and typing.

* *Corresponding author*. European Public Health Microbiology Training Programme (EUPHEM), European Centre for Disease Prevention and Control, Stockholm, Sweden.
E-mail address: kramer-rolf@gmx.de (R. Kramer).
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1. Introduction

Acute flaccid myelitis (AFM) is a poliomyelitis-like neurological disease which is often associated with significant neurodisabilty.[1] In 2016, 29 cases were reported in Europe and AFM surveillance in the USA observed 120 cases for 2014, 21 cases in 2015 and 144 cases in 2016.[2,3] AFM is defined by the following criteria: i) acute onset of focal limb weakness, ii) magnet resonance imaging (MRI) showing a spinal cord lesion largely restricted to grey matter and spanning one (or more) spinal segments, iii) cerebrospinal fluid (CSF) with pleocytosis (leukocytes >5 cells/mm^3).[4] AFM is a subtype of acute flaccid paralysis (AFP). AFP is characterised by rapid onset of flaccid weakness in a child less than 15 years of age, often associated with bulbar palsy.[5] The infectious and non-infectious causes of AFP may affect spinal cord, peripheral nerve, neuromuscular junction and muscles unlike AFM which is restricted to spinal cord gray matter. Enterovirus A71 (EV-A71) has been reported to cause AFP and other neurological manifestations like brain stem encephalitis, aseptic meningitis.[6]

Enteroviruses are small single stranded RNA viruses, divided into four species EV-A, EV-B, EV-C and EV-D. Several out of >100 serotypes of enteroviruses are known to cause neurological diseases, such as meningitis, encephalitis and paralysis, e.g. Poliovirus and EV-A71.[7] In recent years, AFM was increasingly associated with EV-D68 which is a serotype of enterovirus D and was first isolated in 1962 from children with pneumonia and bronchiolitis.[8] Until 2008, EV-D68 was rarely reported and represented only approximately 0.1% of all clinical EV isolates in the US.[9] Between 2008 and 2014, small outbreaks were observed worldwide and retrospective phylogenetic analyses revealed the emergence of three different clades representing previously unseen genetic dynamics of EV-D68 during the past two decades.[10] In 2014, a total of 1152 cases were reported between August and December in 49 US states and approximately 40% of tested respiratory specimens were positive for EV-D68. Patients developed respiratory symptoms like asthma and bronchiolitis. Oermann et al. also reported 94 children presenting AFM with onset in the period of the EV-D68 outbreak.[11] Upsurges of EV-D68 infections appear cyclical with a biennial pattern like it was observed for AFM.[12] Clinicians often are the first to see the upsurges of AFM cases. Since 2014, increasing numbers of EV-D68-infected children presenting severe respiratory illnesses and AFM have been described[13,14]. Various therapeutic intervention were applied but no clear positive or negative effect could be deduced from available data on case management.[3] Different treatment options are currently evaluated in mouse models which may contribute to future guidelines in case management.[15] However, with or without clear guidelines, early detection of cases is essential for optimal care. Current case definitions are focussing on epidemiological aspects but clinical case definitions are still missing. Nonetheless, clinical definitions are critical as a trigger for early investigation because this virus is not routinely tested. Based on the clinical pictures of previous cases, we propose definitions for possible, probable and confirmed cases which will improve early detection, appropriate management and disease surveillance. Despite convincing evidence on causality, an under-appreciation of the importance of this emerging threat is still common. These newly proposed case definitions may facilitate to recognize the threat.

2. Causal association between EV-D68 and acute flaccid myelitis

On the basis of the Bratford Hill criteria,[16] reasoning for causality was recently reviewed.[17,18] Although limitations were given due to small numbers of available studies, all criteria were met and are briefly presented below. *Temporality*: During the US outbreak 2014, EV-D68 infections coincided with an upsurge of AFM cases[19,20]. *Analogy*: Most notably, EV-A71, a known cause for hand-foot-and-mouth disease with neurological complications, is repeatedly reported in association with acute flaccid paralysis[21,22]. *Strength of the association*: A retrospective case-control study observed a 10-fold increased chance being EV-D68 positive for AFM patients during the US outbreak 2014 when compared to children 12 months to 18 years of age admitted with respiratory symptoms at the time of the outbreak and undergone routine testing for circulating pathogens.[23] *Consistency*: In recent years, several studies worldwide reported AFM in paediatric patients positive for EV-D68, e.g. 2005 and 2008 in USA, 2010 in Australia, 2014 in Norway, France and USA, 2016 in Scotland and elsewhere in Europe.[3,11,13,14,24–27] *Biological plausibility*: Although detection of EV-D68 in CSF was only reported in a few cases, its presence indicates the virus is capable of causing infection of the central nervous system[9,24,26]. *Coherence*: Other EVs are already known to be neurotropic and capable of damaging the central nervous system (CNS),

adding EV-D68 does not conflict with current scientific concepts. *Animal experiments*: Using a mouse model, Hixon et al. showed that four out of five EV-D68 strains isolated during the US outbreak 2014 induced neurological diseases in neonatal mice. Infectious virus, virion particles, and viral genome were found in spinal cords of paralysed mice.[28] *Specificity*: AFM is not specific to EV-D68 and can be caused by different exposures. However, clusters of cases indicate that children with EV-D68 infection are more often affected by AFM than other population groups.[3,19,29] *Dose–response relationship (biological gradient)*: It remains unclear if such a relationship does apply for neurological complications of EV infections or if other factors, e.g. virus genetic, host or environmental factors, are associated with severity and diseases progression. However, early experiments from 1967 in suckling mice provided evidence for a biological gradient.[8] After inoculation with four strains of EV-D68, mice were observed for 2 weeks post-inoculation and muscle as well as brain tissue subsequently harvested before passaged to new mice. Mice inoculated with one specific strain developed limb tremor and weakness when that strain was passaged twice in mice and died after the same strain was passaged three times.

In Europe, upsurges of EV-D68 infections were observed to occur in 2-year intervals[12,30]. Mainly causing respiratory diseases, AFM appears to be a rare event in EV-D68 infections. Well established Polio and EV surveillance systems in most European countries do not include EV-D68 and respiratory specimens are not routinely tested for EV infections. In Europe and beyond, AFP surveillance is focussing on stool samples for virus detection which may have contributed to the underappreciation of the threat constituted by respiratory viruses and outbreaks may have been missed. Notably also for Poliovirus, infections of the nervous system were considered a rare event (in 1–2% of cases) and it circulated in human populations at low levels for most of the 1800s without causing large outbreaks of Poliomyelitis until early 1900s.[31]

3. Clinical picture and case definitions of AFM caused by EV-D68

EV-D68 infection with AFM was observed presenting in children with a short prodromal illness, acute flaccid limb weakness (proximal muscle weakness worse than distal) with or without bulbar palsy and respiratory weakness, CSF pleocytosis as well as MRI showing typical spinal grey matter abnormality with T2 hyperintensity (dorsal brainstem in some cases) and motor neuropathy on neurophysiological testing representing reduced compound motor action potentials with normal conduction velocities and absence of conduction blocks compatible with anterior horn cell disease. Typical presentation of MRI abnormality is shown in Fig. 1. Clinical features of patients may include but are not limited to 2–7 days of prodromal illness followed by sudden onset of weakness (poor neck control, breathing difficulties, change in voice, feeding difficulties), severe pain, asymmetric weakness (upper limb > lower limb; proximal > distal) and cranial nerve involvement.[1,3]

To avoid exclusion of cases or adult patients, no specific epidemiological criteria in terms of time and person were defined. Clinical criteria are defined as any person with flaccid myelitis/paralysis at any given time or any person with Guillain-Barré Syndrome (GBS) during a period of high incidence of EV-D68 respiratory infections. In the absence of systematic surveillance, respective alerts from national or regional Centres for Disease Control and Prevention (CDC) may indicate periods of increased EV-D68 circulation. In Europe, those periods were observed to occur biennially in late summer/autumn.[12] Atypical GBS may present similar to AFM associated with EV-D68 and vice versa. However MRI abnormality of spinal grey matter is supportive of AFM. Laboratory criteria comprise detection of EV-D68-specific nucleic acids in a respiratory specimen using a validated real-time reverse transcriptase polymerase chain reaction (PCR) assay targeting the

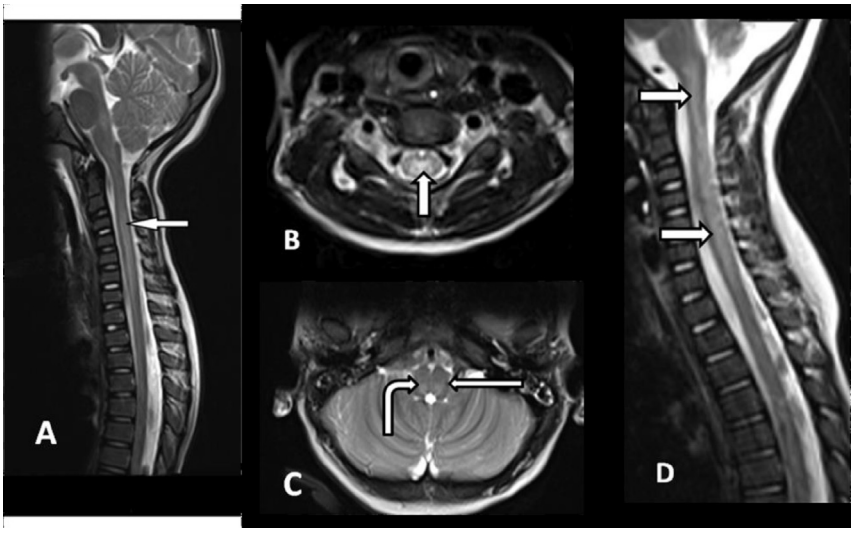

Fig. 1 – Typical Magnetic Resonance Imaging (MRI) abnormality in AFM due to EV D68.[1] The Saggital T2 weighted sequences (A&D) showing longitudinal hyper-intense signal and the Axial T2-Weighetd sequences (B&C) showing hyper-intensity in spinal cord Grey matter(B) and dorsal brainstem(C).

Table 1 – Case classifications of acute flaccid myelitis caused by enterovirus D68.

Suspected	A possible case is defined as a person presenting with symptoms of either acute myelitis/paralysis or Guillain-Barré Syndrome, particularly during periods of EV-D68 circulation indicated by epidemiological alerts or systematic surveillance.
Probable	A probable case is defined as a person presenting with symptoms of either acute myelitis/paralysis or Guillain-Barré Syndrome and at least one of the following criteria: - MRI abnormality representing with T2 hyperintensity in spinal cord grey matter with or without hyperintensity at dorsal brain stem - investigations showing an axonal neuropathy including reduced compound motor action potentials with normal conduction velocities and absence of conduction blocks compatible with anterior horn cell disease - detection of enteroviruses in a respiratory specimen obtained from the lower respiratory tract during periods of EV-D68 circulation.
Confirmed	A confirmed case is defined as a person presenting with the following criteria: - acute flaccid myelitis/paralysis - MRI abnormality representing with T2 hyperintensity in spinal cord grey matter with or without hyperintensity at dorsal brain stem - detection of enterovirus-D68-specific nucleic acids in a respiratory specimen using a validated PCR assay targeting the VP1 gene with subsequent sequencing and typing

VP1 gene with subsequent sequencing and typing. Lower respiratory samples or nasopharyngeal aspirates should be preferred and respiratory samples need to be taken as soon as possible in a suspected case.

If local diagnostic laboratories cannot perform the specific PCR assays, samples should be sent to the national reference laboratory for enteroviruses. Detection of viruses in CSF is not required because negative results do not exclude AFM caused by EV-D68. Case classifications are summarised in Table 1.

Conflicts of interest

None declared.

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