Acute flaccid myelitis and enterovirus D68

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DOI:
10.33612/diss.704415113

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Printed by: Gildeprint Enschede
Layout: Gildeprint Enschede

Printing and publication of this thesis was financially supported by the National Institute for Public Health and the Environment (RIVM). Further support was obtained from the University of Groningen, the University Medical Center Groningen and the Research School of Behavioural and Cognitive Neurosciences (BCN).

Rijksinstituut voor Volksgezondheid en Milieu
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ACUTE FLACCID MYELITIS AND ENTEROVIRUS D68

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ter verkrijging van de graad van doctor aan de
Rijksuniversiteit Groningen
op gezag van de
rector magnificus prof. dr. C. Wijmenga
en volgens besluit van het College voor Promoties.

De openbare verdediging zal plaatsvinden op

woensdag 6 september 2023 om 14.30 uur

door

Jelte Helfferich

geboren op 8 maart 1990
te Sneek

Promotores:

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1

INTRODUCTION TO THE THESIS

INTRODUCTION

In the summer of 2016, a 3-year old boy was admitted to the pediatric intensive care unit of our university medical center, with rapidly progressive flaccid weakness of all limbs [1]. The arms were more severely affected than the legs and there was marked asymmetry of the weakness. Furthermore, there was a facial palsy on the right side. Three days before onset of these symptoms, he had a prodromal illness with headache, a cough and fever. Because of respiratory failure he was intubated soon after admission.

A preliminary diagnosis of atypical Guillain-Barré syndrome was made, requiring further investigation. Cerebrospinal fluid studies showed no abnormalities. MRI of the spinal cord and brain, six days after onset of weakness, showed a subtle hyperintensity of the entire central spinal cord and dorsal pons, as well as enhancement of the caudal roots. Electromyography showed signs of axonal loss in the affected muscles without any sensory abnormalities, which could be localized to the anterior horn or motor axons. The final diagnosis of acute flaccid myelitis (AFM) was however only made after this was suggested by the virologists, who identified enterovirus D68 (EV-D68) by PCR in a respiratory specimen and were aware of the then ongoing epidemic in the USA.

This first of documented cases of AFM associated with EV-D68 in the Netherlands provided the motivation for this thesis. The interplay between neurology and virology in the diagnosis, epidemiology and surveillance of AFM and the diagnostic dilemmas encountered when confronted with a child with acute flaccid weakness are the central themes which will be covered.

AFM and enterovirus D68

AFM was defined in 2014 as a combination of acute flaccid limb weakness and MRI abnormalities in the spinal cord, when a cluster of cases was reported in Colorado, during an outbreak of EV-D68 [2]. While the terminology was new, the clinical syndrome had long been known as poliomyelitis, associated with wild type poliovirus. Several other viruses, including enterovirus A71 (EV-A71), have been associated with AFM, but EV-D68 has remained the most important in the past years [3–5].

Since the association between AFM and EV-D68 was made, evidence for EV-D68 as a cause of AFM has been accumulating [6]. This includes the epidemiological association with

increased occurrence of AFM cases in periods of EV-D68 circulation and the identification of this virus in AFM patients in different countries [7–9]. Furthermore, injection with recent strains of EV-D68 both intramuscularly and intranasally, was able to cause limb paralysis in a mouse model of AFM, similar to the clinical picture observed in humans. After isolation of EV-D68 from the spinal cord of these mice and injection into naïve mice, these mice also developed limb weakness through anterior horn damage, thereby fulfilling Koch's postulates [10]. Also, viral material of EV-D68 was shown in anterior horn cells in autopsy material of a 5-year old patient with acute flaccid myelitis after a viral infection [11]. Similarly to poliovirus, EV-D68 is believed to travel from distal axons to the spinal cord through retrograde axonal transport and cause flaccid weakness by damaging the anterior horn cells. This mechanism is supported by evidence from *in vitro* tests and observations in the mouse model of AFM [10,12].

EV-D68, as well as wild type poliovirus, is part of the family of *Picornaviridae* and was first identified in 1962, but received limited attention until the association with AFM was made [13]. While poliovirus is mainly feco-orally transmitted and can be detected in stool samples, EV-D68 is a respiratory virus, with similarities to a rhinovirus, and is mostly found in respiratory samples [14,15]. Most patients infected with EV-D68 remain asymptomatic or will have a mild to severe respiratory infection, and only a small number will develop AFM [16].

Testing of respiratory and fecal samples in AFM patients earlier after onset of weakness is associated with a higher detection rate [17]. However, even with timely sampling, it may be difficult to identify EV-D68 in these samples. In CSF, EV-D68 is even more rarely detected. This impairs the confirmation of the diagnosis, which in turn hinders surveillance and epidemiologic studies.

Epidemiology and surveillance

Acute flaccid paralysis (AFP) was introduced as a term for the surveillance of poliomyelitis and is defined by the World Health Organization (WHO) as (1) any case of acute weakness with reduced muscle tone in a person under 15 years of age for any reason other than severe trauma, or (2) paralytic illness in a person of any age in which polio is suspected [18]. AFP has a broad differential diagnosis, including conditions affecting the spinal cord and different parts of the motor unit [19]. The surveillance for AFP, instituted by the WHO as part of the global polio eradication program, was stopped in the Netherlands in 2003. The reason to stop AFP

surveillance, was the yield of less than one case of AFP per 100.000 inhabitants younger than 15, which is one of the minimal requirements to indicate effectivity [20]. The last case of poliomyelitis in The Netherlands was reported in 1993, during an outbreak in a religious community, called the Bible Belt region, with low vaccination grades [21]. Therefore, in 2016, when the above described case of AFM was reported in The Netherlands, there was little awareness amongst Dutch clinicians and no active clinical surveillance [1].

The AFP-surveillance was replaced by EV-surveillance, in which EV-positive respiratory and fecal samples were subtyped. While he main goal was the exclusion of poliovirus, these national (TYPENED) and regional (REGIOTYPE) laboratory based surveillance systems gave insight in the epidemiology of non-polio enteroviruses, including EV-D68 [22,23]. Through these surveillance systems, upsurges of EV-D68 circulation have been reported in the Netherlands in 2010, 2014 and 2016 [23–26]. EV-D68 was mostly identified in children with mild to severe respiratory disease and only one case of AFM was reported [24,25].

In many European countries, AFP surveillance is no longer active or effective, similar to the situation in the Netherlands. This has been replaced by EV-surveillance as recommended by the WHO as part of the Global Poliovirus Elimination Action Plan, but strategies for surveillance are varied between countries [27]. Upsurges of EV-D68 circulation have been seen in different European countries, but only five cases of AFM were reported before 2016, three of which (France/Wales) were identified through EV-surveillance and two (Norway) by AFP surveillance [28–30].

In the United States of America the first cluster of AFM cases was reported in 2012, followed by a larger outbreak in 2014, which led to the institution of AFM surveillance by the Centers for Disease Control and Prevention (CDC) [31,32]. The association with EV-D68 was made in 2014 in Colorado, but the focus has remained on clinical surveillance, by which peaks of AFM cases have been identified in 2016 and 2018 [2,33].

Differential diagnosis

Especially at onset of disease it may be difficult to differentiate AFM from other causes of AFP in children. Besides AFM, the most important conditions that need to be considered in children are Guillain Barré syndrome (GBS), affecting the peripheral nerves, and transverse myelitis (TM) and acute disseminated encephalomyelitis (ADEM), both affecting the spinal cord. Similar to AFM, these diagnoses are based on clinical criteria, which include both

clinical features and findings of additional investigations, including imaging studies, electromyography and laboratory tests [34–36].

Therefore, careful history taking and examination, as well as appropriate examinations are important to make this differentiation. Most clinicians currently practicing will not have any experience with poliomyelitis, impairing proper recognition and diagnostic studies, which may lead to improper diagnoses such as atypical GBS in children with AFM [30].

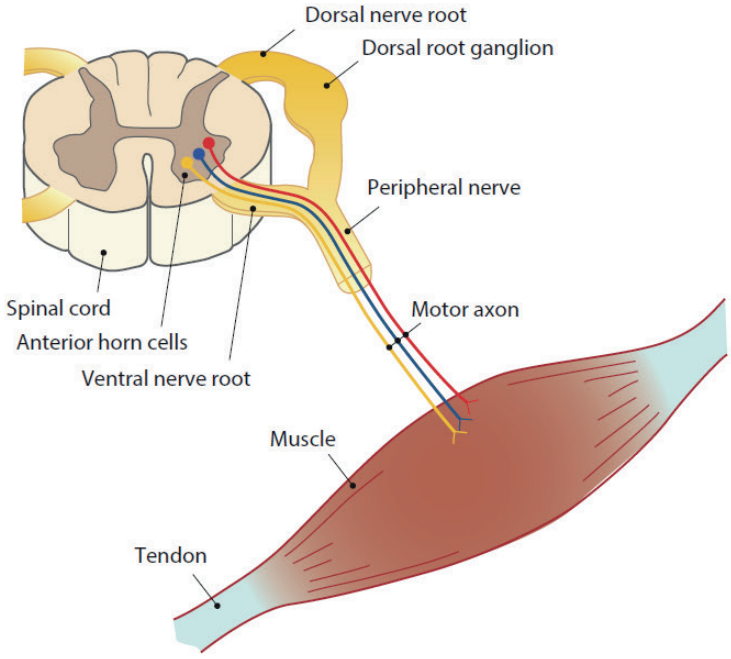

Figure 1: Picture of a motor-unit, consisting from a motor neuron in the anterior horn of the spinal cord and the associated peripheral nerve and muscle fibers.

Diagnostic work-up and criteria

As there is currently no test to confirm AFM, the diagnosis is based on a set of clinical criteria. Several sets of diagnostic criteria have been proposed for different purposes, including surveillance, clinical diagnosis and research [37–40].

Both clinical manifestations and results from imaging studies and other investigations have been important in all of these, highlighting their importance in differentiating AFM from other conditions causing AFP [9]. The evolution of these criteria indicate the increasing attention for AFM and important progress made in understanding of this disease. However, similarly to for example TM, it also shows the difficulty in creating a uniform set of diagnostic criteria in absence of a confirmative test [34,41].

AIMS AND OUTLINE OF THE THESIS

The general aim of the studies included in this thesis is to gain more insight in both the epidemiology and clinical phenotype of AFM, hoping to provide clues for an earlier and more accurate diagnosis. After a general review of the literature, the thesis is subdivided in three sections. The first section will focus on the epidemiology of AFM and EV-D68; the second will focus on the differential diagnosis and features that differentiate between AFM and other causes of AFP. In the third section the applicability of diagnostic criteria for AFM will be evaluated.

In **Chapter 2,** a review of the literature is presented to report the different outbreaks of AFM until 2018 and to describe what is known about the value of different diagnostic procedures. Based on this review, a proposal is made for the clinical work-up in a suspected AFM case**.**

Section 1: Epidemiology

In 2016, an increase of the incidence of AFM in the USA was reported and several reports of cases in Europe were published. To gain more insight in the number of cases in Europe, we collected AFM cases associated with EV-D68 through a European network, as described in **Chapter 3.** Furthermore, information on EV-D68 testing in different European labs was collected. While this was the first larger case series of AFM in Europe, the incidence of AFM in European countries remains largely unknown.

To estimate the incidence of AFM in the Netherlands and investigate the relationship with the detection of EV-D68 and EV-A71, we initiated a retrospective study together with the Dutch National Public Health Institute (RIVM), and a network of pediatric neurologists and virologists. This study is described in **Chapter 4.**

Section 2: Differential diagnosis

In **Chapter 5,** we tried to identify features differentiating AFM and GBS at onset of disease, by comparing the cohort described in Chapter 3 with a large cohort of children with GBS**.** Furthermore, we tested the diagnostic criteria of both conditions by applying them in both cohorts. The same approach was applied for a comparison between AFM and TM in **Chapter 6**, also including an evaluation of the diagnostic criteria.

Section 3: Diagnostic criteria

In **Chapter 7**, we present a review of the pathophysiology, diagnosis and management of AFM written by the international AFM working group. Also, a consensus-based proposal for diagnostic criteria was made. These criteria were evaluated in **Chapter 8** by applying them to the retrospective cohort of children with acute onset weakness described in chapter 4.

In **Chapter 9,** we summarize the results and provide a general discussion, focusing on the organization of surveillance, as well as ways to improve recognition and diagnostic accuracy of AFM.

REFERENCES

1. Helfferich J, Kingma EM, Meiners LC, Schölvinck EH, Mulder HD, Brouwer OF. Acute flaccid myelitis after a respiratory tract infection; First Dutch case related to enterovirus type D68 infection. Ned Tijdschr Geneeskd. 2017;161(35).
2. Messacar K, Schreiner TL, Maloney JA, Wallace A, Ludke J, Oberste MS, 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 (London, England). 2015 Apr;385(9978):1662–71.
3. Messacar K, Asturias EJ, Hixon AM, Leer-Buter C Van, Niesters HGM, Tyler KL, et al. Enterovirus D68 and acute flaccid myelitis-evaluating the evidence for causality. LancetInfectious Dis. 2018 Feb;
4. Jeha LE, Sila CA, Lederman RJ, Prayson RA, Isada CM, Gordon SM. West Nile virus infection: a new acute paralytic illness. Neurology. 2003 Jul;61(1):55–9.
5. Messacar K, Spence-Davizon E, Osborne C, Press C, Schreiner TL, Martin J, et al. Clinical characteristics of enterovirus A71 neurological disease during an outbreak in children in Colorado, USA, in 2018: an observational cohort study. Lancet Infect Dis. 2020 Feb;20(2):230–9.
6. Messacar K, Asturias EJ, Hixon AM, Van Leer-Buter C, Niesters HGM, Tyler KL, et al. Enterovirus D68 and acute flaccid myelitis-evaluating the evidence for causality. Lancet Infect Dis [Internet]. 2018 Aug 1;18(8):e239–47. Available from: https://doi.org/10.1016/S1473-3099(18)30094-X
7. Chong PF, Kira R, Mori H, Okumura A, Torisu H, Yasumoto S, et al. 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. Clin Infect Dis. 2017 Oct;
8. Knoester M, Helfferich J, Poelman R, Leer-Buter C Van, Brouwer OF, Niesters HGM, et al. Twenty-nine Cases of Enterovirus-D68-associated Acute Flaccid Myelitis in Europe 2016: A Case Series and Epidemiologic Overview. Pediatr Infect Dis J. 2019 Jan;38(1):16–21.
9. Messacar K, Schreiner TL, Haren K Van, Yang M, Glaser CA, Tyler KL, et al. Acute flaccid myelitis: A clinical review of US cases 2012-2015. Ann Neurol. 2016 Sep;80(3):326–38.
10. Hixon AM, Yu G, Leser JS, Yagi S, Clarke P, Chiu CY, et al. A mouse model of paralytic myelitis caused by enterovirus D68. PLoS Pathog. 2017 Feb;13(2):e1006199.
11. Vogt MR, Wright PF, Hickey WF, De Buysscher T, Boyd KL, Crowe JEJ. Enterovirus D68 in the Anterior Horn Cells of a Child with Acute Flaccid Myelitis. Vol. 386, The New England journal of medicine. United States; 2022. p. 2059–60.
12. Hixon AM, Clarke P, Tyler KL. Contemporary circulating enterovirus D68 strains infect and undergo retrograde axonal transport in spinal motor neurons independent of sialic acid. J Virol. 2019 Jun;
13. Schieble JH, Fox VL, Lennette EH. A probable new human picornavirus associated with respiratory diseases. Am J Epidemiol. 1967 Mar;85(2):297–310.
14. Elrick MJ, Pekosz A, Duggal P. Enterovirus D68 molecular and cellular biology and pathogenesis. J Biol Chem. 2021;296:100317.
15. Oberste MS, Maher K, Schnurr D, Flemister MR, Lovchik JC, Peters H, et al. Enterovirus 68 is associated with respiratory illness and shares biological features with both the enteroviruses and the rhinoviruses. J Gen Virol. 2004 Sep;85(Pt 9):2577–84.
16. Holm-Hansen CC, Midgley SE, Fischer TK. Global emergence of enterovirus D68: a systematic review. LancetInfectious Dis. 2016 May;16(5):e64-75.
17. Ayers T, Lopez A, Lee A, Kambhampati A, Nix WA, Henderson E, et al. Acute Flaccid Myelitis in the United States: 2015-2017. Pediatrics. 2019 Nov;144(5).

18. Suresh S, Forgie S, Robinson J. Non-polio Enterovirus detection with acute flaccid paralysis: A systematic review. J Med Virol. 2018 Jan;90(1):3–7.

19. Hardy D, Hopkins S. Update on acute flaccid myelitis: recognition, reporting, aetiology and outcomes. Arch Dis Child. 2020 Sep;105(9):842–7.

20. Surveillance Indicators for Acute Flaccid Paralysis (AFP) Surveillance. https://polioeradication.org/polio-today/polio-now/surveillance-indicators/.

21. van Wijngaarden JK, van Loon AM. The polio epidemic in The Netherlands, 1992/1993. Public Health Rev. 21(1–2):107–16.

22. Niesters HG, Rossen JW, van der Avoort H, Baas D, Benschop K, Claas EC, et al. Laboratory-based surveillance in the molecular era: the TYPENED model, a joint data-sharing platform for clinical and public health laboratories. Euro Surveill Bull Eur sur les Mal Transm = Eur Commun Dis Bull. 2013 Jan;18(4):20387.

23. Benschop KSM, Rahamat-Langendoen JC, van der Avoort HGAM, Claas ECJ, Pas SD, Schuurman R, et al. VIRO-TypeNed, systematic molecular surveillance of enteroviruses in the Netherlands between 2010 and 2014. Euro Surveill Bull Eur sur les Mal Transm = Eur Commun Dis Bull. 2016 Sep;21(39).

24. Poelman R, Scholvinck EH, Borger R, Niesters HG, van Leer-Buter C. The emergence of enterovirus D68 in a Dutch University Medical Center and the necessity for routinely screening for respiratory viruses. J Clin Virol. 2015 Jan;62:1–5.

25. Knoester M, Scholvinck EH, Poelman R, Smit S, Vermont CL, Niesters HG, et al. Upsurge of Enterovirus D68, the Netherlands, 2016. Emerg Infect Dis. 2017 Jan;23(1):10.3201/eid2301.161313.

26. Meijer A, Benschop KS, Donker GA, van der Avoort HG. Continued seasonal circulation of enterovirus D68 in the Netherlands, 2011-2014. Euro Surveill Bull Eur sur les Mal Transm = Eur Commun Dis Bull. 2014 Oct;19(42).

27. Harvala H, Jasir A, Penttinen P, Pastore Celentano L, Greco D, Broberg E. Surveillance and laboratory detection for non-polio enteroviruses in the European Union/European Economic Area, 2016. Euro Surveill Bull Eur sur les Mal Transm = Eur Commun Dis Bull. 2017 Nov;22(45).

28. Lang M, Mirand A, Savy N, Henquell C, Maridet S, Perignon R, et al. Acute flaccid paralysis following enterovirus D68 associated pneumonia, France, 2014. Euro Surveill. 2014 Nov;19(44).

29. Pfeiffer HC, Bragstad K, Skram MK, Dahl H, Knudsen PK, Chawla MS, et al. Two cases of acute severe flaccid myelitis associated with enterovirus D68 infection in children, Norway, autumn 2014. Euro Surveill. 2015 Mar;20(10):21062.

30. Williams CJ, Thomas RH, Pickersgill TP, Lyons M, Lowe G, Stiff RE, et al. Cluster of atypical adult Guillain-Barre syndrome temporally associated with neurological illness due to EV-D68 in children, South Wales, United Kingdom, October 2015 to January 2016. Euro Surveill Bull Eur sur les Mal Transm = Eur Commun Dis Bull. 2016;21(4).

31. Ayscue P, Haren K Van, Sheriff H, Waubant E, Waldron P, Yagi S, et al. Acute flaccid paralysis with anterior myelitis - California, June 2012-June 2014. MMWRMorbidity Mortal Wkly Rep. 2014 Oct;63(40):903–6.

32. Pastula DM, Aliabadi N, Haynes AK, Messacar K, Schreiner T, Maloney J, et al. Acute neurologic illness of unknown etiology in children - Colorado, August-September 2014. MMWRMorbidity Mortal Wkly Rep. 2014 Oct;63(40):901–2.

33. Lopez A, Lee A, Guo A, Konopka-Anstadt JL, Nisler A, Rogers SL, et al. Vital Signs: Surveillance for Acute Flaccid Myelitis - United States, 2018. MMWR Morb Mortal Wkly Rep. 2019 Jul;68(27):608–14.

34. Transverse Myelitis Consortium Working Group. Proposed diagnostic criteria and nosology of acute transverse myelitis. Neurology. 2002;59(4):499–505.

35. Roodbol J, de Wit MCY, van den Berg B, Kahlmann V, Drenthen J, Catsman-Berrevoets CE, et al. Diagnosis of Guillain-Barré syndrome in children and validation of the Brighton criteria. J Neurol. 2017 May;264(5):856–61.

36. Pohl D, Alper G, Haren K Van, Kornberg AJ, Lucchinetti CF, Tenembaum S, et al. Acute disseminated encephalomyelitis: Updates on an inflammatory CNS syndrome. Neurology. 2016 Aug;87(9 Suppl 2):S38-45.

37. Murphy OC, Messacar K, Benson L, Bove R, Carpenter JL, Crawford T, et al. Acute flaccid myelitis: cause, diagnosis, and management. Lancet (London, England). 2021 Jan;397(10271):334–46.

38. Kramer R, Lina B, Shetty J. Acute flaccid myelitis caused by enterovirus D68: Case definitions for use in clinical practice. Eur J Paediatr Neurol EJPN Off J Eur Paediatr Neurol Soc. 2019 Mar;23(2):235–9.

39. Elrick MJ, Gordon-Lipkin E, Crawford TO, Van Haren K, Messacar K, Thornton N, et al. Clinical Subpopulations in a Sample of North American Children Diagnosed With Acute Flaccid Myelitis, 2012-2016. JAMA Pediatr [Internet]. 2019 Feb 1;173(2):134–9. Available from: https://doi.org/10.1001/jamapediatrics.2018.4890

40. CDC. Revision to the Standardized Surveillance and Case Definition for Acute Flaccid Myelitis. 2017.

41. Hopkins SE, Elrick MJ, Messacar K. Acute Flaccid Myelitis-Keys to Diagnosis, Questions About Treatment, and Future Directions. JAMA Pediatr. 2018 Nov;Published.

2

ACUTE FLACCID MYELITIS AND ENTEROVIRUS D68: LESSONS FROM THE PAST AND PRESENT

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European Journal of Pediatrics (2019) 178:1305–1315.
DOI: 10.1007/s00431-019-03435-3.

ABSTRACT

Acute flaccid myelitis is characterized by the combination of acute flaccid paralysis and a spinal cord lesion largely restricted to the gray matter on magnetic resonance imaging. The term acute flaccid myelitis was introduced in 2014 after the upsurge of pediatric cases in the USA with enterovirus D68 infection. Since then, an increasing number of cases have been reported worldwide. Whereas the terminology is new, the clinical syndrome has been recognized in the past in association with several other neurotropic viruses such as poliovirus.

Conclusion: This review presents the current knowledge on acute flaccid myelitis with respect to the clinical presentation and its differential diagnosis with Guillain-Barré syndrome and acute transverse myelitis. We also discuss the association with enterovirus D68 and the presumed pathophysiological mechanism of this infection causing anterior horn cell damage. Sharing clinical knowledge and insights from basic research is needed to make progress in diagnosis, treatment, and prevention of this new polio-like disease.

INTRODUCTION

Acute flaccid myelitis (AFM) is a syndrome characterized by acute flaccid paralysis (AFP) and gray matter spinal cord lesions on magnetic resonance imaging (MRI). After the introduction of the term AFM in 2014, more than 500 patients, predominantly children, have been recognized both in- and outside Europe [1–4].

The Center for Disease Control and Prevention (CDC) proposed a case definition in which a definite AFM case is described as acute-onset flaccid weakness, combined with a spinal cord lesion on MRI, largely restricted to the gray matter and spanning one or more spinal segments. Acute flaccid weakness combined with cerebrospinal fluid (CSF) pleocytosis without lesions on MRI is defined as a probable case [5].

A prodromal illness, asymmetric limb weakness, and specific findings in electromyography and nerve conduction studies may further aid in distinguishing AFM from other causes of AFP such as Guillain-Barré syndrome (GBS) and acute transverse myelitis (ATM) [6].

Accumulating evidence supports an association between enterovirus D68 (EV-D68) and AFM [7, 8]. Other viruses that have been associated with outbreaks of acute flaccid weakness

and myelitis include enterovirus A71 (EV-A71), West Nile virus (WNV), Japanese encephalitis virus, and the wild-type poliovirus[9–12].

In this review, we describe the clinical syndrome of AFM, its differential diagnosis, and its association with different viruses, with the emphasis on EV-D68.

METHODS

For this review, we performed a literature search in PubMed on "flaccid myelitis" and "Enterovirus D68" from 2000 until February 2019. A total of 995 titles of articles in English were screened and selected based on relevance for epidemiology, clinical characteristics, pathophysiology, treatment, prevention, and prognosis of AFM. Only cohorts containing at least five children were selected (Table 1).

Before the term AFM was introduced, outbreaks of acute flaccid weakness and myelitis, matching the case definition for AFM, were reported in association with EV-A71, predominantly in Eastern Asia and Australia, and with WNV, causing several outbreaks in the USA in the beginning of this century Poliomyelitis also matches the case definition of AFM and can be seen as the first known cause of AFM. However, MRI was and is often not available in countries where poliomyelitis still occurs, making the definite diagnosis of AFM difficult. In 2012, the first probable cases were reported in California (USA) [6]. Since 2014, the CDC has reported over 500 cases of AFM in the USA with 2-year intervals and several cohorts of patients with AFM have been reported worldwide (Table 1)[3, 4, 6, 13–19]. A recent study reported an incidence of 1.46 per 100,000 person years, although reliable data is lacking, as AFM is notifiable in only few countries and the clinical picture is often not recognized[20].

In different cohorts of AFM patients, EV-D68 was detected in 20–40% of cases, primarily from respiratory specimens (Table 1). The variation in detection percentages might be explained by differences in timing and performance of diagnostic procedures and by selection criteria for patients [20]. Most reported patients with AFM were children under the age of 10 with a slight male preponderance. A majority were previously healthy, but asthma was seen in 12–32% of children [4, 20, 21].

Both EV-A71 and West Nile virus are still circulating and have also been detected in recent cohorts of AFM patients [17, 22, 23]. Outbreaks of poliomyelitis are currently rare, due to a global poliovirus surveillance and vaccination program [24].

Clinical features

The clinical characteristics of non-polio AFM cohorts described in literature since 2012 are summarized in Table 1. Muscle weakness typically develops over the course of several hours to days, often with a marked asymmetry. Weakness is proximally usually more severe and may be more pronounced in the upper limbs, with a spectrum of severity varying between slight paresis of a single limb to tetraplegia. Tendon reflexes are typically diminished or absent in the affected limbs. In most patients, there is a prior prodromal illness, often involving the upper respiratory tract, with a median of 5 days before onset of weakness [2, 3, 6, 21].

Weakness can be limited to the extremities, but the diaphragm and bulbar muscles may also be affected, making ventilatory support necessary in the acute phase in about 30% of cases [4, 6, 21, 25]. Cranial nerve deficits are common and may be the only finding. The facial nerve is most often affected, followed by the abducens and oculomotor nerves [21].

Associated features include severe limb pain and autonomic disturbances such as bladder dysfunction. Sensory symptoms, primarily paresthesia, are reported in up to 20% of cases [2, 3, 6, 21].

The clinical features of cohorts of AFM, described before 2012, associated with EV-A71, WNV, and poliovirus were highly similar, although poliovirus-related AFM more often affected lower limbs, with bulbar muscles usually being spared [12]. EV-A71 has also been associated with rhombencephalitis, sometimes with severe cardiorespiratory symptoms [9, 10, 12].

Differential diagnosis

AFM is included in the broad differential diagnosis of AFP. AFP is defined as a syndrome of focal weakness of peripheral origin in any part of the body with an acute onset [26].

It is important to be able to recognize AFM early in its course so that adequate diagnostic procedures can be performed and respiratory failure in the initial phase can be anticipated. Both clinical clues and findings on further investigations may help differentiate AFM from other causes of AFP.

In cases of AFM in which only one arm is affected, the initial thought may be that of synovitis or arm injury. Clinical clues that may help in distinguishing these from AFM may be

the presence of a prodromal illness, the hypo- or areflexia, and the often-associated neck weakness in AFM.

When more than one limb is affected, the differential diagnosis includes other causes of acute myelopathy, such as acute transverse myelitis (ATM), acute disseminated encephalomyelitis (ADEM), acute cord compression, and ischemic myelopathy. Furthermore, Guillain-Barré syndrome (GBS) may be suspected because of the sudden onset of flaccid weakness after a prodromal illness.

While the asymmetric weakness, the absence of encephalopathy, the paucity of sensory symptoms, and the presence of cranial nerve deficits in AFM may help in distinguishing it from other causes of AFP, further investigations are required to make the right diagnosis (table 2, figure 1-4) [6, 21, 27–29].

Table 1: Summary of cohorts of children of AFM described after 2012, showing patient characteristics, clinical findings and findings on further investigations.

	Author	Inclusion period	Country/ Region	No pts	EV-D68 pos (%)	Gender (% male)	Age (mean or median with range)	Prodrome (%)	Limb weakness (%)	Asymmetry (%)	Sensory involvement (%)
1	Andersen	2001-2014	Australia	8	0 (13 EV-A71)	25	Med 5	100	100	100	0
2	Messacar	2012-2015	USA	159[a]	20-45	56-91	Med 7.1 (0.4-73)	64-100	83-100	47-70	21-44
3	Elrick	2012-2016	USA	34	13	65	Med 5 (<1-15)	100	100	97	0
4	Yea	2014	Canada	25	28	64	Med 7.8 (0.8-15.0)	88	100	NS	12
5	Gordon-Lipkin	2014-2017	USA	16	23	69	Med 4 (3-6)	100	100	NS	6
6	Chong	2015	Japan	59	15	59	Med 4.4 (2.6-77)	97	100	68	20
7	Knoester	2015-2016	Europe	29	100[b]	52	Med 4 (1.6-55)	92	100	NS (usual)	7
8	Bonwitt	2016	USA	10	20 (10 EV-A71)	70	Med 6 (3-14)	80	100	NS	NS
9	Iverson	2016	USA	5	60	20	Mean 7.7 (3.5-12)	100	100	NS	NS
10	Hübner	2016	Germany	16 (7)[c]	6	50	Mean 4.6 (1.7-14.3)	100	100	86	NS
11	Ruggieri	2016	Argentina	11	36	54	Mean 3.2 (0.3-6)	100	100	81	0
12	Sarmast	2017	India	9	0	56	Med 5.5 (2-7)	100	100	100	0
13	McKay	2018	USA	80	37 (29 EV-A71)	59	Med 4 (0.7-32)	99	100	NS	NS
14	Ramsay	2018	UK	40 (16)[d]	36	53	55 under 5 yo	55	98	NS	NS

	Hyporeflexia (%)	Cranial nerve dysfunction (%)	Ventilatory support (%)	Bowel/bladder dysfunction (%)	CSF pleocytosis (%)	Protein raised in CSF (%)	MRI spine: T2 hyperintensity (%)	Nerve root enhancement (%)	Brainstem lesions (%)
1	NS	25	NS	0	85	71	100	38	25
2	80-81	18-83	9-34	18-51	64-91	45-58	90-100	20-40	35-75
3	67	>24	24	6	97	45	100	38	62
4	88	>20	28	36	72	28	100	72	32
5	63	50	31	NS	100	NS (Med 6.3g/L)	100	13	42
6	90	17	8	27	85	46	100	51	42
7	87	60	66	7	91	NS (Med 3.8g/L)	92	16	68
8	NS	30	10	50	78	NS (Med 5.8g/L)	100	0	30
9	NS	80	NS	NS	100	NS	80	NS	NS
10	NS	NS	14	NS	43	NS	86	NS	NS
11	100	45	36	0	63	18	100	NS	45
12	100	11	NS	0	89	22	100	NS	11
13	NS	NS	NS	NS	83	NS (Med 4.7g/L)	100	NS	NS
14	NS	NS	55	NS	18	NS	43	NS	NS

No pts: Number of patients, EV-D68: Enterovirus D68, EV-A71: Enterovirus A71, med: median, CSF: cerebrospinal fluid, MRI: Magnetic Resonance Imaging, USA: United States of America, UK: United Kingdom, NS: Not specified, yo: year old

[a]Combination of four US cohorts with a partial overlap in these cohorts

[b]EV-D68 had to be identified for inclusion

[c]16 registered cases, 7 of which were further described

[d]40 cases of Acute Flaccid Paralysis, of which 16 fulfilled the criteria for probable or definite AFM

Table 2: Signs, symptoms and findings on further investigations in acute flaccid myelitis, Guillain-Barré Syndrome and acute transverse myelitis.

	Acute flaccid myelitis (with EV-D68)	**Guillain-Barré syndrome**	**Acute transverse myelitis**
		Prodrome	
Type	Febrile illness often with respiratory and/or gastrointestinal symptoms	Febrile illness often with gastrointestinal symptoms and or respiratory symptoms	Commonly a preceding febrile illness
Time until onset of weakness	Usually within one week	Several weeks	Days to weeks
		Clinical details	
Neurologic deficits	Asymmetric flaccid weakness, with upper limbs often more affected, proximal>distal	Ascending weakness, lower limbs> upper limbs	Symmetric weakness, may be asymmetric initially
Reflexes	Typically low or absent	Low or absent	Usually high, can be low initially
Sensory symptoms	Typically no sensory deficits	Paresthesia and slight distal sensory symptoms (except in AMAN)	Common, often with a sensory level
Cranial nerve deficits	Bulbar weakness and asymmetric facial palsy common; sometimes oculomotor deficits	Symmetric facial weakness; oculomotor deficits in MFS	None
Other symptoms	Pain, autonomic dysfunction	Pain, autonomic dysfunction	Bowel and bladder dysfunction
Time Course	Progressive over hours to days	Progressive symptoms over several days	Progressive over 4 hours to 21 days
		Findings	
CSF	Slight pleocytosis, raised protein. May be completely normal.	Raised protein after several days, without pleocytosis (“dissociation cytoalbuminique”).	Slight pleocytosis, raised protein. May be completely normal.
Microbiology	EV-D68 in respiratory specimen	*Campylobacter jejuni* in feces; EBV, CMV, HEV, Zikavirus in blood	Usually none

	Acute flaccid myelitis (with EV-D68)	**Guillain-Barré syndrome**	**Acute transverse myelitis**
MRI Brain	Typical T2-hyperintense region in the dorsal pons, sometimes also in caudate nuclei. Cranial nerve enhancement possible.	Normal	Normal
MRI Spine	Longitudinally extensive diffuse slightly hyperintense central cord lesion, usually most pronounced in the cervical region. Sometimes cauda equina root enhancement.	Cauda equina root enhancement may be found.	Central cord focal hyperintense lesion over multiple levels affecting white and gray matter.
EMG	Findings of motor axonopathy with low CMAPs, normal NCV. Normal sensory findings.	Decreased NCV with blocks are typical. Normal sensory findings in AMAN.	Normal
		Treatment/ prognosis	
Treatment	No effective treatment, potential positive effect of IVIG	IVIG and/or plasmapheresis effective	High dose steroids, sometimes IVIG and/or plasmapheresis
Prognosis	Improvement over several months, but often significant residual weakness and muscle atrophy	Often complete recovery over the course of weeks till months	Partial recovery over the course of months till years

EV-D68: Enterovirus D68, AMAN: Acute motor axonal neuropathy, MFS: Miller Fisher Syndrome, EBV: Epstein Barr Virus, CMV: Cytomegalovirus, HEV: Hepatitis E Virus, CMAP: Compound Muscle Action Potential, NCV: Nerve Conduction Velocity, IVIG: Intravenous Immunoglobulin.

Investigations

Diagnostic tests recommended in children with suspected AFM should be directed at the identification of different microorganisms and the exclusion of other causes (Table 3) [30]. Initial investigations must be performed on blood, stool, respiratory material, and CSF, followed by MRI of the brain and spinal cord and in some cases electromyography (EMG).

Table 3: Suggested work-up for children with acute flaccid paralysis.

Blood	Routine investigations (blood count, inflammatory parameters, creatin kinase, liver and renal function tests)
	Auto-antibodies (Anti-MOG IgG, anti-AQP4, anti-GM1, Anti-GQ1b)
	Oligoclonal bands (both serum and CSF)
	Microbiology: testing for enterovirus (including poliovirus), EBV, CMV, VZV, HEV, Zikavirus*
CSF	Routine investigations (Cell count, protein, glucose)
	Oligoclonal bands (both CSF and serum)
	Microbiology: testing for enterovirus, parechovirus, HSV, VZV, EBV
Further microbiologic testing	Nasopharyngeal swab for enterovirus testing. Stool sample for enterovirus and *C. jejuni* testing
Imaging	Contrast enhanced MRI of the brain and spine
Neurophysiologic testing	EMG with motor and sensory investigation of an affected limb

MOG: Myelin-oligodendrocyte glycoprotein, AQP4: Aquaporin 4, GM1: Ganglioside M1, GQ1b: Ganglioside Q1b, EBV: Ebstain Barr Virus, CMV: Cytomegalovirus, VZV: Varicella Zoster Virus, HEV: Hepatitis E virus; CSF: Cerebrospinal Fluid. HSV: Herpex Simplex Virus, EMG: Electromyography. *For patients that have travelled to or live in countries where Zikavirus is prevalent.

Blood

General laboratory investigation of blood samples of AFM patients may show a slight leukocytosis, sometimes with raised inflammatory parameters, which is usually not helpful in the differentiation of AFM from other disorders causing AFP [21, 27].

Cerebrospinal fluid

CSF examination in AFM patients in the described cohorts since 2012 reveals a mild to moderate pleocytosis in most cases (Table 1). Protein levels are initially minimally raised in about half of AFM cases but can be completely normal. After several days, the leukocyte number tends to decrease, while protein levels rise [2, 3, 21, 31]. Oligoclonal bands in the CSF can be identified in immune-mediated conditions such as ATM, but are usually not found in AFM [28].

Interestingly, viral agents, such as EV-D68, EV-A71, and poliovirus, are only detected in the CSF in a small minority of patients with AFM [2, 3, 9, 12, 15].

Virology diagnostic testing

The viral RNA of EV-D68 is detected mostly in respiratory samples, followed to a much lesser extent by feces and can only rarely be found in blood or CSF. This in contrast to EV-A71, which is more frequently detected in blood, and poliovirus, which is routinely identified in stool samples[12, 30].

Obtaining an adequate respiratory sample is therefore indispensable for detection of EV-D68. Considering the fact that the prodromal, mostly respiratory illness is usually a few days into its natural course when a patient presents with weakness, the best chances of detecting EV-D68 is soon after onset of complaints. Several PCR tests have been described, which test either directly for EV-D68 or for enteroviruses in general [30, 32].

Magnetic Resonance Imaging

MRI of the brain and spinal cord is important in making the diagnosis of AFM and in distinguishing it from other causes of AFP (table 2 and figures 1-4) [25]. CT usually shows no abnormalities [33].

In AFM, the classical MRI feature is a longitudinally extensive slight T2-hyperintense signal in the central cord, affecting the central gray matter, often most pronounced in the cervical regions (figure 1b-cInitially, there is usually more diffuse spinal cord edema, evolving over several days to T2-hyperintensity that is restricted to the anterior horn. Enhancement of the caudal roots and sometimes of the cranial nerves can be seen (figure 1d) [33].

Initially, there is usually more diffuse spinal cord edema, evolving over several days to T2-hyperintensity that is restricted to the anterior horn. Enhancement of the caudal roots and sometimes of the cranial nerves can be seen (figure 1a), while the caudate nucleus may be involved [33]. These findings may help in securing the diagnosis, but the correlation between symptoms and radiologic findings is usually poor, making MRI unsuitable as a prognostic tool for AFM [34].

Imaging findings in earlier outbreaks of AFM, associated with WNV and poliovirus, were highly similar, while in EV-A71–associated neurological disease, these appear to be more variable and more extensive brain abnormalities may occur[9, 10, 35].

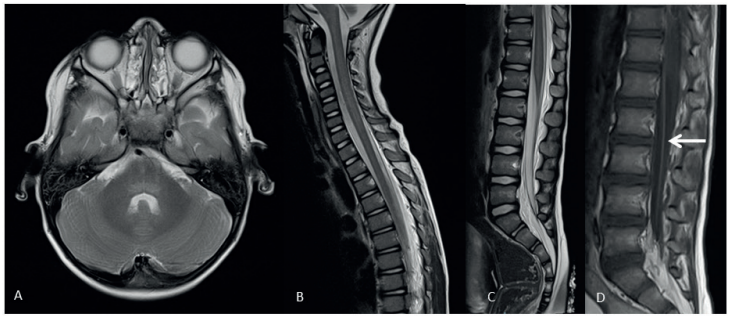

Figure 1: MRI of the neuraxis in a three-year-old boy with EV-D68 associated AFM. (republished with permission from [49]**)**

a. Brain: transverse T2-weighted image showing an area of slight hyperintensity in the dorsal pons (arrow).

b. and c. Spinal cord: sagittal T2-weighted images showing longitudinal slight hyperintensity largely restricted to the central cord, where the gray matter is situated. (arrow).

d. Spinal cord: contrast enhancement of the ventral caudal roots on a sagittal T1-weigthed image (arrow).

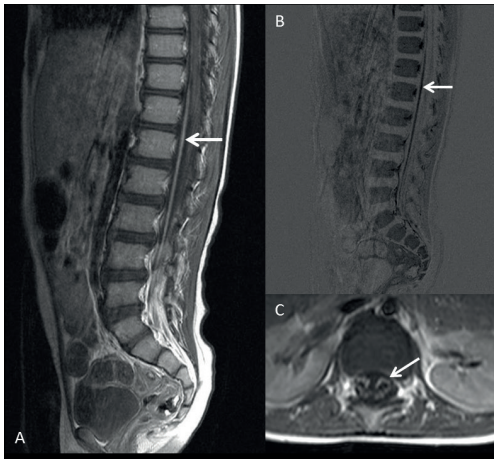

Figure 2: MRI of the spinal cord in a three-year-old boy with Guillain-Barré syndrome.

a. Sagittal contrast-enhanced T1 showing typical enhancing anterior caudal roots.

b. Subtraction of A with more clear depiction of enhancing caudal root.

c. Transverse T1 showing more clear enhancement of anterior motor roots.

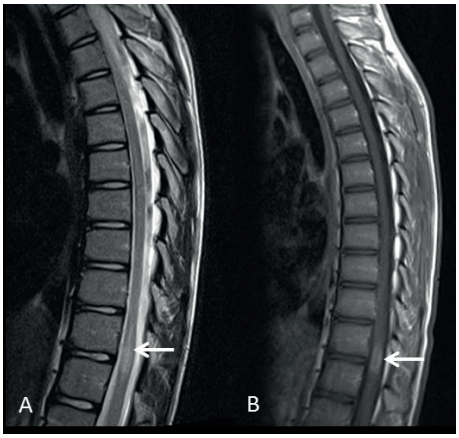

Figure 3: MRI of the spinal cord in a 15-year-old boy with acute transverse myelitis, eventually diagnosed with relapsing remitting multiple sclerosis.

a. Sagittal T2 showing focal swelling of the spinal cord at level Th11-12.

b. Sagittal T1 showing contrast enhancement of the lesion.

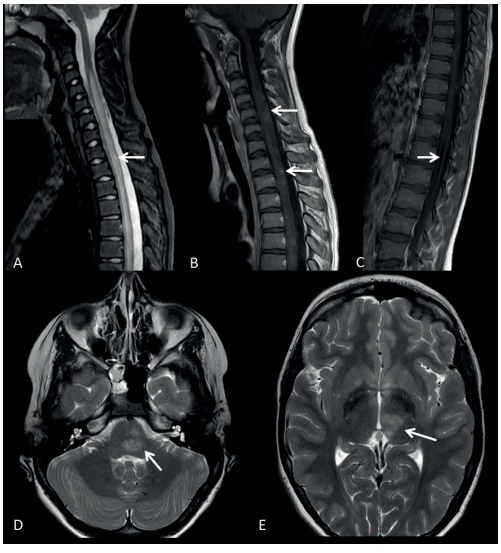

Figure 4: MRI of a 13-year-old boy with a provisional diagnosis of acute demyelinating encephalomyelitis.

a. Sagittal short tau inversion recovery (STIR) with edematous cervicothoracic spinal cord from the level of C4.

b. Sagittal T1 of the spinal cord showing diffuse areas of slight enhancement.

c. Enhancement of mainly dorsal roots in a sagittal T1 of the lumbar spine.

d. and e. Transverse T2 at the level of the pons (D) and thalamus (E) showing asymmetric hyperintense areas.

Neurophysiological studies

While EMG findings in recent outbreaks of AFM can be normal on the first day, after several days, a pattern compatible with anterior horn disease is seen. This encompasses decreased compound muscle action potentials (CMAP) with normal conduction velocities. Sensory testing is usually completely normal [36].

After some weeks, denervation potentials can be seen, with severe ongoing denervation being a possible predictor for the gravity of residual damage. The persistence of F-waves may indicate a better prognosis [3, 21, 36].

Virology

Enteroviruses, such as EV-D68, EV-A71, and poliovirus, are small RNA viruses belonging to the picornavirus family. EV-D68 was first identified in 1962 after isolation from children with severe respiratory disease [37]. Since 2012, an increasing incidence has been recognized, with infections mostly occurring in autumn and late summer. EV-D68 appears to occur in a cyclic pattern with a 2-year interval [2, 7].

EV-D68 infection may be asymptomatic or cause respiratory disease. In hospitalized children, an asthma-like respiratory disease is most commonly seen [19]. The percentage of infected patients afflicted with paralytic disease is not yet known, but is estimated to be less than 1%, similar to poliomyelitis [12, 38].

Pathophysiology

A causal relationship between EV-D68 and AFM is supported by epidemiological and biological evidence, as was evaluated by different groups applying the Bradford Hill criteria[7, 8].

The biological evidence mainly came from mouse models, in which mice infected with contemporary circulating strains of EV-D68 develop flaccid paralysis mimicking AFM. Interestingly, neonatal or young mice are used, because older mice are not susceptible to disease [39, 40]. Pathologic examination of infected mice revealed the presence of the virus in the anterior horn with associated cell loss EV-D68 probably reaches the anterior horn by

retrograde axonal transport, as is supported by both mouse studies and in vitro studies in human motor neurons [39–41].

One study found myositis without spinal cord infection after intranasal injection of the virus in mice [39].

Although the results from mouse studies cannot simply be extrapolated to humans, these results are suggestive of a damaging effect of the virus in anterior horn cells, possibly combined with a direct damaging effect on muscles through viral myositis.

Important questions remain why only some EV-D68 infected patients develop AFM and how the variability in severity of AFM in affected patients is explained.

Treatment

There are currently no effective treatment options for AFM. Most patients are treated with intravenous immunoglobulin (IVIG), steroids, or plasmapheresis, or a combination, but no significant clinical effect of any of these interventions has been shown so far. Because of its effectiveness in the mouse model of EV-D68–associated AFM and its possible efficacy in treatment of EV-A71–associated encephalomyelitis, treatment with IVIG has been recommended [4, 21, 42, 43].

The anti-inflammatory effects of steroids may be beneficial in AFM cases with spinal cord edema or white matter involvement, but steroids are unlikely to be effective in limiting the anterior horn damage that is probably caused by a direct damaging effect of the virus. Furthermore, treatment with steroids in a mouse model of AFM associated with EV-D68 led to an increased viral load and a deterioration of motor symptoms [39, 40].

Fluoxetine, an antidepressant, is effective in inhibiting EV-D68 replication in vitro. However, treatment with fluoxetine in the mouse model of EV-D68–associated AFM did not result in reduction of the viral load or improvement of motor function. Also, no significant effect has been shown in patients with AFM, treated with fluoxetine[43, 44].

While scientific proof is still lacking, we recommend IVIG in the acute phase, combined with maximal supportive care with optimal pain control, feeding, ventilatory support, and intensive rehabilitation. Surgical procedures such as nerve and muscle transfers have been performed and cases have been described in which improvement of limb function has been achieved. Because over time degeneration of the receiving motor nerves and muscle fibers

will occur, evaluation for surgical intervention should be considered early in the disease course [45].

Prevention/ Vaccination

In the mouse model of EV-D68–associated AFM, passive immunization with pooled immune sera, if administered before injection of the virus, was effective in decreasing the rate of paralysis [43].

Arguments for vaccination as a treatment strategy arise from the development of effective vaccines against EV-A71 infections in China and the effective eradication of poliomyelitis in most of the world after introduction of vaccination [24, 46]. Recently, an experimental vaccine based on virus-like particles targeting EV-D68 has been developed. This vaccine has been proven effective in a mouse model in the prevention of AFM [47].

Prognosis

Only 5–39% of patients with AFM recover partially to completely (supplementary table 1). Most patients retain significant residual motor deficits, and prolonged need for ventilatory support is not uncommon. On follow-up, residual proximal weakness tends to be more severe than distal weakness, with severe atrophy occurring over time [3, 4, 14, 25, 38, 48]. Cranial nerve deficits usually recover well over time. Death is uncommon but has been reported in immunocompromised patients, usually because of respiratory complications [2, 21]. While not much is known about prognostic factors, more severe disability and weakness at nadir and the persistence of denervation seem to be associated with worse outcome. One study found a correlation between negative tests for EV-D68 at onset and better outcome, which made the authors speculate that viral clearance and host responses play a role in the severity of weakness in AFM [3]. Alternatively, these EV-D68–negative cases may be due to different etiologies associated with more favorable outcomes than cases confirmed to be associated with EV-D68.

CONCLUSIONS AND FUTURE PERSPECTIVES

AFM is a newly introduced term comprising AFP combined with longitudinally extensive lesions of the spinal cord on MRI. This syndrome resembles poliomyelitis and has been associated with different viruses, in particular EV-D68.

EV-D68 infection is usually asymptomatic or mildly symptomatic with respiratory illness, but it can be associated with anterior horn disease causing severe weakness, with only minimal improvement over time in most cases.

A major challenge lies in the propagation of correct diagnostic procedures, including viral testing on respiratory material in suspected AFM cases. Future research may identify risk factors for AFM in EV-D68–infected patients and will elucidate how these factors can be influenced.

We believe that worldwide collaboration between neurologists, radiologists, pediatricians, and microbiologists is necessary to make progress in preventing and treating this devastating childhood disease. Furthermore, we postulate that making AFM a notifiable disease in more countries can increase awareness among clinicians and governments.

Conflict of interest

The authors declare that they have no conflict of interest.

Informed consent

Additional informed consent was obtained from all individual participants for whom identifying information is included in this article.

REFERENCES

1. Messacar K, Schreiner TL, Maloney JA, et al (2015) A cluster of acute flaccid paralysis and cranial nerve dysfunction temporally associated with an outbreak of enterovirus D68 in children in Colorado, USA. Lancet (London, England) 385:1662–1671. https://doi.org/10.1016/S0140-6736(14)62457-0 [doi]
2. Knoester M, Helfferich J, Poelman R, et al (2019) Twenty-nine Cases of Enterovirus-D68-associated Acute Flaccid Myelitis in Europe 2016: A Case Series and Epidemiologic Overview. Pediatr Infect Dis J 38:16–21. https://doi.org/10.1097/INF.0000000000002188 [doi]
3. Chong PF, Kira R, Mori H, et al (2018) 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. Clin Infect Dis 66:653–664. https://doi.org/10.1093/cid/cix860 [doi]
4. Yea C, Bitnun A, Robinson J, et al (2017) Longitudinal Outcomes in the 2014 Acute Flaccid Paralysis Cluster in Canada. J Child Neurol 32:301–307. https://doi.org/10.1177/0883073816680770 [doi]
5. CDC (2017) Revision to the Standardized Surveillance and Case Definition for Acute Flaccid Myelitis
6. Elrick MJ, Gordon-Lipkin E, Crawford TO, et al (2019) Clinical Subpopulations in a Sample of North American Children Diagnosed With Acute Flaccid Myelitis, 2012-2016. JAMA Pediatr 173:134–139. https://doi.org/10.1001/jamapediatrics.2018.4890
7. Messacar K, Asturias EJ, Hixon AM, et al (2018) Enterovirus D68 and acute flaccid myelitis-evaluating the evidence for causality. Lancet Infect Dis 18:e239–e247. https://doi.org/10.1016/S1473-3099(18)30094-X
8. Dyda A, Stelzer-Braid S, Adam D, et al (2018) The association between acute flaccid myelitis (AFM) and Enterovirus D68 (EV-D68) - what is the evidence for causation? Euro Surveill 23:10.2807/1560-7917.ES.2018.23.3.17–00310. https://doi.org/10.2807/1560-7917.ES.2018.23.3.17-00310 [doi]
9. Huang CC, Liu CC, Chang YC, et al (1999) Neurologic complications in children with enterovirus 71 infection. N Engl J Med 341:936–942. https://doi.org/10.1056/NEJM199909233411302 [doi]
10. Glass JD, Samuels O, Rich MM (2002) Poliomyelitis due to West Nile virus. N Engl J Med 347:1280–1281. https://doi.org/10.1056/NEJM200210173471616 [doi]
11. Solomon T, Kneen R, Dung NM, et al (1998) Poliomyelitis-like illness due to Japanese encephalitis virus. Lancet (London, England) 351:1094–1097. https://doi.org/S0140-6736(97)07509-0 [pii]
12. Scheld WM, Marra CM, Whitley RJ (2004) Chapter 7: Poliomyelitis, Polio Vaccines, and the Postpoliomyelitis Syndrome. In: J G (ed) 4th ed. Wolters Kluwer, Philadelphia, pp 112–124
13. Iverson SA, Ostdiek S, Prasai S, et al (2017) Notes from the Field: Cluster of Acute Flaccid Myelitis in Five Pediatric Patients - Maricopa County, Arizona, 2016. MMWRMorbidity Mortal Wkly Rep 66:758–760. https://doi.org/10.15585/mmwr.mm6628a4 [doi]
14. Hubner J, Kruse B, Christen HJ, et al (2017) Acute Flaccid Myelitis in German Children in 2016-the Return of Polio? Dtsch Arztebl Int 114:551–557. https://doi.org/10.3238/arztebl.2017.0551 [doi]
15. Ruggieri V, Paz MI, Peretti MG, et al (2017) Enterovirus D68 infection in a cluster of children with acute flaccid myelitis, Buenos Aires, Argentina, 2016. Eur J Paediatr Neurol 21:884–890. https://doi.org/S1090-3798(17)30025-9 [pii]
16. Sarmast SN, Gowda VK, Ahmed M, et al (2018) Acute flaccid myelitis-Clustering of polio-like illness in the tertiary care centre in Southern India. J Trop Pediatr fmy052:. https://doi.org/10.1093/tropej/fmy052 [doi]
17. McKay SL, Lee AD, Lopez AS, et al (2018) Increase in Acute Flaccid Myelitis - United States, 2018. MMWRMorbidity Mortal Wkly Rep 67:1273–1275. https://doi.org/10.15585/mmwr.mm6745e1 [doi]

18. Force TUKAFPAT (2019) An increase in reports of acute flaccid paralysis (AFP) in the United Kingdom, 1 January 2018-21 January 2019: early findings. Euro Surveill 24:1900093. https://doi.org/10.2807/1560-7917.ES.2019.24.6.1900093

19. Rao S, Messacar K, Torok MR, et al (2016) Enterovirus D68 in Critically Ill Children: A Comparison With Pandemic H1N1 Influenza. Pediatr Crit Care Med 17:1023–1031. https://doi.org/10.1097/PCC.0000000000000922

20. Kane MS, Sonne C, Zhu S, et al (2019) Incidence, Risk Factors and Outcomes Among Children With Acute Flaccid Myelitis: A Population-based Cohort Study in a California Health Network Between 2011 and 2016. Pediatr Infect Dis J 38:667–672. https://doi.org/10.1097/INF.0000000000002276

21. Messacar K, Schreiner TL, Haren K Van, et al (2016) Acute flaccid myelitis: A clinical review of US cases 2012-2015. Ann Neurol 80:326–338. https://doi.org/10.1002/ana.24730 [doi]

22. Bonwitt J, Poel A, DeBolt C, et al (2017) Acute Flaccid Myelitis Among Children - Washington, September-November 2016. MMWRMorbidity Mortal Wkly Rep 66:826–829. https://doi.org/10.15585/mmwr.mm6631a2 [doi]

23. Andersen EW, Kornberg AJ, Freeman JL, et al (2017) Acute flaccid myelitis in childhood: a retrospective cohort study. Eur J Neurol 24:1077–1083. https://doi.org/10.1111/ene.13345 [doi]

24. Derrough T, Salekeen A (2016) Lessons learnt to keep Europe polio-free: a review of outbreaks in the European Union, European Economic Area, and candidate countries, 1973 to 2013. Euro Surveill 21:pii=30210. https://doi.org/10.2807/1560-7917.ES.2016.21.16.30210 [doi]

25. Gordon-Lipkin E, Munoz LS, Klein JL, et al (2018) Comparative quantitative clinical, neuroimaging, and functional profiles in children with acute flaccid myelitis at acute and convalescent stages of disease. Dev Med Child Neurol 61:366–375. https://doi.org/10.1111/dmcn.14030 [doi]

26. Marx A, Glass JD, Sutter RW (2000) Differential diagnosis of acute flaccid paralysis and its role in poliomyelitis surveillance. Epidemiol Rev 22:298–316

27. Hopkins SE, Elrick MJ, Messacar K (2018) Acute Flaccid Myelitis-Keys to Diagnosis, Questions About Treatment, and Future Directions. JAMA Pediatr Published: https://doi.org/10.1001/jamapediatrics.2018.4896 [doi]

28. Absoud M, Greenberg BM, Lim M, et al (2016) Pediatric transverse myelitis. Neurology 87:S46-52. https://doi.org/10.1212/WNL.0000000000002820 [doi]

29. Willison HJ, Jacobs BC, van Doorn PA (2016) Guillain-Barre syndrome. Lancet (London, England) 388:717–727. https://doi.org/10.1016/S0140-6736(16)00339-1 [doi]

30. Harvala H, Broberg E, Benschop K, et al (2018) Recommendations for enterovirus diagnostics and characterisation within and beyond Europe. J Clin Virol 101:11–17. https://doi.org/10.1016/j.jcv.2018.01.008

31. Greninger AL, Naccache SN, Messacar K, et al (2015) A novel outbreak enterovirus D68 strain associated with acute flaccid myelitis cases in the USA (2012-14): a retrospective cohort study. LancetInfectious Dis 15:671–682. https://doi.org/10.1016/S1473-3099(15)70093-9 [doi]

32. Poelman R, Scholvinck EH, Borger R, et al (2015) The emergence of enterovirus D68 in a Dutch University Medical Center and the necessity for routinely screening for respiratory viruses. J Clin Virol 62:1–5. https://doi.org/10.1016/j.jcv.2014.11.011 [doi]

33. Maloney JA, Mirsky DM, Messacar K, et al (2014) MRI Findings in Children with Acute Flaccid Paralysis and Cranial Nerve Dysfunction Occurring during the 2014 Enterovirus D68 Outbreak. Am J Neuroradiol 36:245–250. https://doi.org/10.3174/ajnr.A4188

34. Okumura A, Mori H, Fee Chong P, et al (2018) Serial MRI findings of acute flaccid myelitis during an outbreak of enterovirus D68 infection in Japan. Brain Dev 41:443–451. https://doi.org/10.1016/j.braindev.2018.12.001

35. Choudhary A, Sharma S, Sankhyan N, et al (2010) Midbrain and spinal cord magnetic resonance imaging (MRI) changes in poliomyelitis. J Child Neurol 25:497–499. https://doi.org/10.1177/0883073809340918 [doi]

36. Hovden IA, Pfeiffer HC (2015) Electrodiagnostic findings in acute flaccid myelitis related to enterovirus D68. Muscle Nerve 52:909–910. https://doi.org/10.1002/mus.24738 [doi]

37. Schieble JH, Fox VL, Lennette EH (1967) A probable new human picornavirus associated with respiratory diseases. Am J Epidemiol 85:297–310

38. Martin JA, Messacar K, Yang ML, et al (2017) Outcomes of Colorado children with acute flaccid myelitis at 1 year. Neurology 89:129–137. https://doi.org/10.1212/WNL.0000000000004081 [doi]

39. Morrey JD, Wang H, Hurst BL, et al (2018) Causation of Acute Flaccid Paralysis by Myelitis and Myositis in Enterovirus-D68 Infected Mice Deficient in Interferon alphabeta/gamma Receptor Deficient Mice. Viruses 10:10.3390/v10010033. https://doi.org/E33 [pii]

40. Hixon AM, Yu G, Leser JS, et al (2017) A mouse model of paralytic myelitis caused by enterovirus D68. PLoS Pathog 13:e1006199. https://doi.org/10.1371/journal.ppat.1006199 [doi]

41. Hixon AM, Clarke P, Tyler KL (2019) Contemporary circulating enterovirus D68 strains infect and undergo retrograde axonal transport in spinal motor neurons independent of sialic acid. J Virol. https://doi.org/10.1128/JVI.00578-19

42. Wang SM, Lei HY, Huang MC, et al (2006) Modulation of cytokine production by intravenous immunoglobulin in patients with enterovirus 71-associated brainstem encephalitis. J Clin Virol 37:47–52. https://doi.org/S1386-6532(06)00199-5 [pii]

43. Hixon AM, Clarke P, Tyler KL (2017) Evaluating Treatment Efficacy in a Mouse Model of Enterovirus D68-Associated Paralytic Myelitis. J Infect Dis 216:1245–1253. https://doi.org/10.1093/infdis/jix468 [doi]

44. Messacar K, Sillau S, Hopkins SE, et al (2018) Safety, tolerability, and efficacy of fluoxetine as an antiviral for acute flaccid myelitis. Neurology 92:e2118–e2126. https://doi.org/10.1212/WNL.0000000000006670 [pii]

45. Saltzman EB, Rancy SK, Sneag DB, et al (2018) Nerve Transfers for Enterovirus D68-Associated Acute Flaccid Myelitis: A Case Series. Pediatr Neurol 88:25–30. https://doi.org/S0887-8994(17)31136-0 [pii]

46. Zhu F, Xu W, Xia J, et al (2014) Efficacy, safety, and immunogenicity of an enterovirus 71 vaccine in China. N Engl J Med 370:818–828. https://doi.org/10.1056/NEJMoa1304923 [doi]

47. Dai W, Zhang C, Zhang X, et al (2018) A virus-like particle vaccine confers protection against enterovirus D68 lethal challenge in mice. Vaccine 36:653–659. https://doi.org/S0264-410X(17)31818-2 [pii]

48. Kirolos A, Mark K, Shetty J, et al (2018) Outcome of paediatric acute flaccid myelitis associated with enterovirus D68: a case series. Dev Med Child Neurol 61:376–380. https://doi.org/10.1111/dmcn.14096 [doi]

49. Helfferich J, Meiners LC, Brouwer OF (2017) Acute flaccid weakness associated with enterovirus D68. Eur J Paediatr Neurol 21:594–595. https://doi.org/10.1016/j.ejpn.2017.02.001

SUPPLEMENTARY FILES

Supplementary Table

Author	No pts	EV-D68 pos	Follow-up duration (months)	Outcome	Positive prognostic factors	Negative prognostic factors
Messacar	159	20-45%	Med 4.2-12	Persistent motor deficits in 75-95%		
Chong	59	15%	Med 8,5	39% good or complete improvement	Higher pre-treatment muscle strength, normal F-wave persistence, negative EV-D68 identification	
Gordon-Lipkin	16	23%	Med 4	38% good recovery (GFMCS I or II), 57% wheelchair bound		More severe disability at nadir
Martin	10	13%	12	33% full recovery		Possibly persistent denervation on follow-up EMG (mean 10.5 months after onset)
Yea	25	28%	3-18	8% full recovery, median EDSS 3		Initial EDSS score>4
Knoester	29	100%		11% full recovery, 75% partial recovery		
Kirolos	5	100%	18	20% full recovery		

Studies showing longer term outcome; in Acute Flaccid Myelitis
EDSS: Expanded Disability Status Scale, EMG: Electromyography, EV-D68: Enterovirus D68, med=median, GFMCS= Gross Motor Function Classification System

SECTION 1:

EPIDEMIOLOGY

3

TWENTY-NINE CASES OF ENTEROVIRUS-D68–ASSOCIATED ACUTE FLACCID MYELITIS IN EUROPE 2016. A CASE SERIES AND EPIDEMIOLOGIC OVERVIEW

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The Pediatric Infectious Disease Journal 38(1):p 16-21, January 2019. DOI10.1097/INF.0000000000002188

ABSTRACT

Background

Enterovirus-D68 (EV-D68) is a respiratory virus within the genus Enterovirus and the family of Picornaviridae. Genetically, it is closely related to rhinovirus that replicates in the respiratory tract and causes respiratory disease. Since 2014, EV-D68 has been associated with the neurologic syndrome of acute flaccid myelitis (AFM).

Methods

In October 2016, questionnaires were sent out to a European network including 66 virologists and clinicians, to develop an inventory of EV-D68–associated AFM cases in Europe. Clinical and virologic information of case patients was requested. In addition, epidemiologic information on EV testing was collected for the period between March and October 2016.

Results

Twenty-nine cases of EV-D68–associated AFM were identified, from 12 different European countries. Five originated from France, 5 from Scotland and 3 each from Sweden, Norway and Spain. Twenty-six were children (median age 3.8 years), 3 were adults. EV-D68 was detected in respiratory materials (n = 27), feces (n = 8) and/or cerebrospinal fluid (n = 2). Common clinical features were asymmetric flaccid limb weakness, cranial nerve deficits and bulbar symptoms. On magnetic resonance imaging, typical findings were hyperintensity of the central cord and/or brainstem; low motor amplitudes with normal conduction velocities were seen on electromyography. Full clinical recovery was rare (n = 3), and 2 patients died. The epidemiologic data from 16 European laboratories showed that of all EV-D68–positive samples, 99% was detected in a respiratory specimen.

Conclusions

For 2016, 29 EV-D68–related AFM cases were identified in mostly Western Europe. This is likely an underestimation, because case identification is dependent on awareness among clinicians, adequate viral diagnostics on respiratory samples and the capability of laboratories to type EVs.

INTRODUCTION

Enterovirus-D68 (EV-D68) is a member of the genus Enterovirus, which belongs to the Picornaviridae family. The genus Enterovirus consists of many species, including human EV-A, B, C, D and human rhinovirus A, B, C, which can be further classified in different genotypes. Examples of EV genotypes are coxsackievirus, enterovirus A71, poliovirus and echovirus. These viruses are associated with a range of clinical symptoms, such as myocarditis, hand–foot–mouth disease, acute flaccid paralysis (AFP) and aseptic meningitis. The majority of enteroviruses replicate in the gastrointestinal tract and can be detected in stool samples. EV-D68 and rhinovirus, however, replicate in the upper airways and are best detected in respiratory samples.

Since 2014, EV-D68 has gained interest after causing a large respiratory disease outbreak in North America [1]. Symptoms varied in severity from a common cold to respiratory failure requiring mechanical ventilation. Most hospitalized patients were children and severe cases often had underlying pulmonary conditions, such as asthma [2]. The concurrent circulation of EV-D68 in Europe was shown by a joint effort of the European Society of Clinical Virology (ESCV) – European Centre for Disease Prevention and Control (ECDC) EV-D68 study group: 16,332 respiratory samples were screened for EV-D68 and 343 (2.1%) were positive [3].

During this 2014 epidemic, 120 cases of acute flaccid myelitis (AFM) in children were identified in the United States, with the most common virus detected in upper respiratory tract specimens being EV-D68 [4]. AFM is a polio-like neurologic condition, characterized by an acute onset of asymmetric multifocal limb weakness with spinal cord lesions evident on magnetic resonance imaging (MRI)[5]. An epidemiologic link was made between the AFM upsurge and the concurrent EV-D68 outbreak in the United States [4,6]. During the same period, 4 AFM patients with respiratory EV-D68 infections were reported in Europe [7–9].

In the winter of 2015/2016, 2 AFM cases with concurrent EV-D68 infection were identified in Wales [10], and in July 2016, a severe case of EV-D68–related AFM in a 4-year-old boy was identified in the Netherlands [11]. Subsequently, through an e-mail alert to the previously established ESCV-ECDC EV-D68 study group network, more cases of EV-D68–related AFM were rapidly identified. An intense collaboration between virologists and clinicians (ie, pediatric neurologists and infection disease specialists) from across Europe was established. In this article, we present the clinical and virologic data of the 29

cases that were identified through this network, to illustrate the clinical picture and to improve future patient identification.

The 2014 ESCV-ECDC collaborative work showed that only limited data were available on the epidemiology of respiratory EV infections. This was especially, but not exclusively, the case for Eastern and Southern European countries, among others because of a lack of diagnostic testing and typing of EVs in respiratory samples. In line with our study in 2014, we collected epidemiologic data for Europe in 2016. We present data on EV and EV-D68 testing and positivity rates, to emphasize the impact of adequate diagnostics, and on notification regulations of AFM in the various European countries.

MATERIALS AND METHODS

Members of the 2014 ESCV-ECDC EV-D68 study group, mostly virologists, were contacted by the coordinating center (University Medical Center, Groningen, The Netherlands) through an e-mail alert. Additionally, EV reference laboratories in Eastern Europe were informed of the initiative, as they were underrepresented in the study group. Finally, through this network and in reply to scientific presentations or publications on the subject, we contacted clinicians who diagnosed or treated a patient with EV-D68–related AFM. The collaborating centers and clinicians (from this point on referred to as the 2016 EV-D68 AFM Working Group, with 66 members were sent a questionnaire by which they were asked to report the number of EV-D68–related AFM cases diagnosed in 2016. For each case, information was inquired regarding age, gender, prodromal phase, neurologic abnormalities (mental status, signs of nuchal rigidity, cranial nerve dysfunction, limb weakness, tendon reflexes and sensory disturbances), virologic diagnostics, neurologic investigations [cerebrospinal fluid (CSF) analysis, MRI, electromyography (EMG)] and clinical follow-up.

Additionally, information on diagnostic EV testing was collected via the questionnaire. For the period between March and October 2016, we requested the number of EV tests performed on all clinical specimens (respiratory, CSF, feces and blood), the number of EV-positive tests and the number of EV-D68–positive tests. Twenty-one laboratories responded, including both diagnostic and reference laboratories. The national reference center of Bulgaria reported that EV detection was performed in their institution, but no further typing was done for non-polio EVs. Furthermore, the data from 3 laboratories that

tested less than 100 samples for EV (from Portugal, Czech and Finland) and from 1 laboratory that could not detect EV-D68 with the current techniques (from Estonia) were excluded.

Finally, the notification status of AFM was questioned per country.

Case Definition EV-D68–related AFM

AFM is a specific form of AFP. Its definition was stated in 2015 and adapted since by the Centers for Disease Control and Prevention [5]. The 3 key components of the EV-D68–related AFM case definition are: (1) Acute onset of focal limb weakness, (2) MRI showing a spinal cord lesion largely restricted to the grey matter and spanning 1 or more spinal segments and (3) Detection of EV-D68 in a respiratory, fecal, blood or CSF specimen using a validated polymerase chain reaction (PCR) assay for EV-D68, or a validated PCR assay for EVs in general and subsequent sequencing and typing. If MRI is not performed, or findings are normal, and the CSF shows pleocytosis, the patient is considered a “probable” case.

Typing and Phylogenetic Mapping

To compare the viral sequences, the collaborating centers were asked to share the sequencing files of their EV-D68 cases (both of respiratory and AFM cases). Alternatively, samples could be sent to one of the participating laboratories for sequencing. Typing was performed using the standard method described by Nix et al [12], which consists of partial sequencing of the viral protein 1. Phylogenetic analysis was performed using BioNumerics Software version 6.6 (Applied Maths, Sint-Martens-Latem, Belgium).

Ethics Approval

The research ethics committee of the coordinating center confirmed exemption from the Medical Research Involving Human Subjects Act (Decree M17.207412). Local ethics approval and informed consent from participating patients or their parents were obtained according to individual institutional requirements.

RESULTS

EV-D68 related AFM cases

We received the clinical data from 29 EV-D68–related AFM cases, from 12 different countries. Table 1 shows the clinical data of these cases (more extensive descriptions can be found in the Table (Supplementary Table 1). The distribution of cases over Europe is shown in Supplementary Figure 1. Twenty-six children were affected, with a median age of 3.8 years (range 1.6–9.0) and 3 adults were included in this series. Gender was equally distributed. EV-D68 was detected in a respiratory sample of 27 patients, in the feces of 8 patients and in the CSF of 2 patients. Only 1 child was coinfected with another neurotropic virus, EV-A71.

Medical history was nonsignificant, except for an adult patient who received an allogeneic hematopoietic stem cell transplantation for Non-Hodgkin B-cell lymphoma 2 years earlier.[13] A prodromal phase with fever (n = 24) and/or respiratory symptoms (n = 26) preceded weakness by a median period of 2 days. Weakness was flaccid and usually asymmetric, with decreased or absent reflexes. Upper limbs were more frequently and often more severely affected than lower limbs. Cranial nerve deficits were common (n = 17). Nineteen patients needed ventilatory support. Information on duration of ventilator dependency was scarce, but at least 7 children needed tracheostomy for long-term ventilator support.

CSF analysis frequently showed a moderate pleocytosis (normal value <5 leukocytes/μL), while protein levels were mostly normal or slightly raised (normal value <0.55 g/L, dependent on age and center).

MRI was reported to be abnormal in 25 cases, with hyperintensity in the central grey matter of the cervical and/or thoracic spine in 23 patients and hyperintensity of the dorsal brainstem in 17 patients. Figure 1 shows the typical AFM lesions on MRI for 2 patients. EMG was performed in 11 patients and generally revealed decreased amplitude of compound motor action potentials with normal conduction velocities and absence of conduction blocks, compatible with anterior horn cell disease. Spontaneous muscle fiber activity, either positive sharp waves or fibrillations, was found in the affected muscles when needle EMG was performed after sufficient time (range: 7 days to 3 months).

Most patients were treated with intravenous immunoglobulins (n = 20), steroids (n = 17) or both (n = 15), with typically only partial recovery over time. Follow-up time ranged from 0.5 to 12 months. Two patients, who both showed EV-D68 in the CSF, died. One was a 55-year-old immunocompromised woman, who died of respiratory failure. The second was a 3.5-year-old child, who died of severe ventilation acquired pneumonia with septic shock.

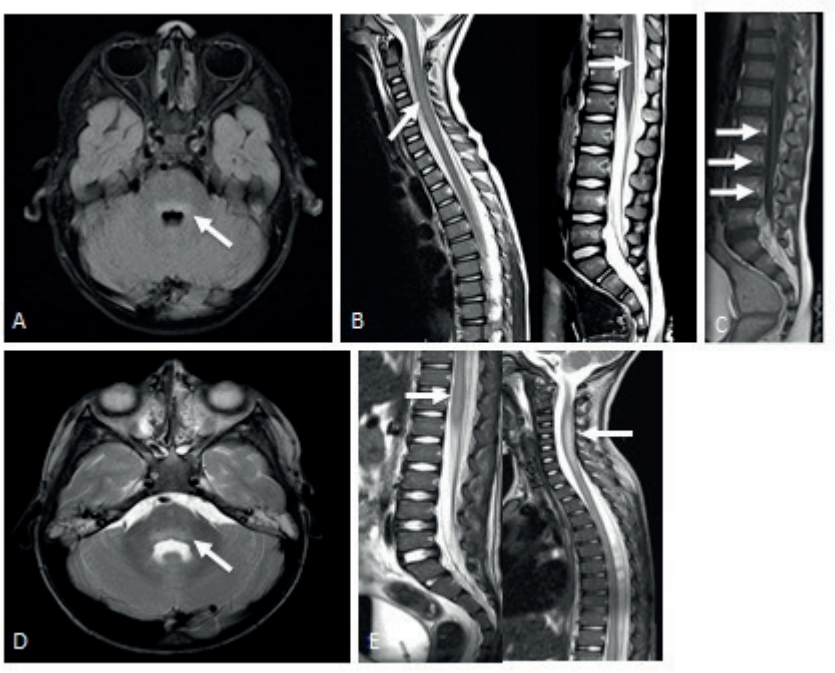


Figure 1: Typical magnetic resonance imaging findings in patients with enterovirus-D68–associated acute flaccid myelitis.

Magnetic resonance images of 2 cases (case a: A–C; case b: D–E), dating from the first week after the start of neurologic symptoms, showing typical imaging features of AFM. A + D, Transversal T2-weighted images of the brain show a slight hyperintensity (arrow) in the dorsal pons. B + E, Sagittal T2-weighted images show hyperintensity (arrow) of the central gray matter in the cervical and thoracic regions in both patients. C, A sagittal T1-weighted image with gadolinium shows enhancement (arrows) of the caudal roots.

Epidemiology and Diagnostics

From March to October 2016, 21,875 EV tests were reported by 16 European laboratories, as shown in the Table (Supplementary Table 2). This table does not contain the data from EV-D68 AFM cases that were reported by clinicians without epidemiologic data from the diagnostic laboratory, so it has only a partial overlap with Table 1. Of the 21,875 EV tests reported on all clinical specimens, 2111 were EV positive (10%; excluding those EV-positive samples for which no denominator was given). Of the total number of 2381 EV-positive samples, 416 were EV-D68 (17%). Taking a closer look at respiratory samples, 10,226 EV tests were performed, with 987 (10%) EV-positive samples. Of the total amount of 1067 EV-positive samples in respiratory specimens, 414 were EV-D68 positive (39%). Only 1 of 558 EV-positive CSF samples (0.18%) and 1 of 711 EV-positive feces samples (0.14%) was positive for EV-D68 (data not shown in the table).

Table 1. Clinical description of 29 enterovirus-D68 related acute flaccid myelitis cases, Europe 2016.

		Data available for *n*=	No. (percentage or range)
Demographics			
	Median age (years)	29	4 (1.6-55)
	Male sex	29	15 (52%)
Prodromal symptoms			
	Fever	26	24 (92%)
	Respiratory symptoms	29	26 (90%)
	Gastrointestinal symptoms	29	7 (24%)
	Median days of fever until onset of weakness	22	2 (0-8)
Neurologic symptoms			
	Cranial nerves affected	28	17 (60%)
	Facial nerve palsy		8 (29%)
	Dysphagia		4 (14%)
	Bulbar symproms		9 (32%)
	Eye movement disorders		5 (18%)
	Ventilatory support needed	29	19 (66%)

Table 1. Clinical description of 29 enterovirus-D68 related acute flaccid myelitis cases, Europe 2016.

	Limb weakness	29	29 (100%)
	1 limb		3 (10%)
	2 limbs		8 (28%)
	3 limbs		0 (0%)
	4 limbs		16 (55%)
	Limbs not specified		2 (7%)
	Hyporeflexia/areflexia	22	20 (87%)
Other symptoms		29	Autonomic dysfunction, n=3
			Acute respiratory distress syndrome, n=1
			Generalized convulsions, n=1
			Limb ataxia, n=1
			Limb pain, n=4
			Neurogenic bladder dysfunction, n=1
			Paresthesia, n=2
			Pneumonia, n=4
			Transient myocardial dysfunction, n=1
CSF analysis			
	Pleocytosis (CSF cell count >5 leukocytes/µl)	22	20 (91%)
	Median CSF cell-count (leukocytes/µL)	20	79 (3-416)
	Median CSF protein level (g/L)	17	0.38 (0.21-1.6)
MRI abnormalities			
	MRI: Hyperintensity central cord	25	23 (92%)
	Location (if specified)	20	Cervical, n=10
			Cervical/thoracic, n=6
			Thoracic/lumbar, n=1
			Entire spinal cord, n=3
	MRI brain: Hyperintensity dorsal pons/medulla	25	17 (68%)
	Other MRI abnormalities	6	Enhancing roots, n=4
			Meningeal enhancement, n=1
			Hyperintensity dentate nuclei, dorsal medulla, n=1
Electromyography		11	
	Low motor amplitudes		10 (91%)

Table 1. Clinical description of 29 enterovirus-D68 related acute flaccid myelitis cases, Europe 2016.

	Reduced conduction velocities		1 (9%)
	Spontaneous muscle fiber activity		5 (45%)
Treatment		24	
	Intravenous immunoglobulin		20 (83%)
	Intravenous steroids		17 (71%)
	Plasmapheresis		5 (21%)
	Other		Fluoxetine, n=1; Rituximab, n=1
Follow-up		28	
	Full recovery		3 (11%)
	Partial recovery		21 (75%)
	No recovery		2 (7%)
	Death		2 (7%)
	Median follow-up time (months)	24	4 (0.5-12)
Virology			
EV-D68 positive in:	Any sample	29	29 (100%)
	Cerebrospinal fluid	25	2 (8%)
	Respiratory sample	28	27 (96%)
	Fecal sample	22	8 (36%)

Two patients were diagnosed in December 2015. Sixteen cases were previously reported in separate publications (references: [10,11,13–18] Abbreviations: CSF: cerebrospinal fluid, MRI: magnetic resonance imaging

Sequence data of the viral protein 1 region were available for 6 of 29 AFM patients, and together with many other EV-D68 strains of 2014 and 2016, these were used for sequence analysis. The Figure (Supplementary Figure 2) shows the dominance of the B3 clade in 2016, irrespective of respiratory or neurologic symptoms.

Notification Regulations

The following information was obtained regarding notification regulations: AFP/AFM is a reportable disease in all European countries within the scope of polio eradication. Only in Norway, also non-polio AFP/AFM cases are notifiable and the requirement of a respiratory sample for testing was added after the EV-D68 outbreak in 2014. In Germany and France, non-polio AFP/AFM is voluntarily reported. In Norway, Sweden, Ireland, Italy, France and Slovenia, (entero-) (viral) meningitis/encephalitis is reportable. In Denmark, EV meningitis

and paralysis are reportable and recently the required specimens for testing were expanded with a respiratory sample.[19] For the remaining countries, no clear regulations exist for non-polio AFP/AFM cases.

DISCUSSION

The association between EV-D68 and AFM has become clear since 2014, although causality was not yet proven [4,6,20]. The recent publication of a mouse model [21], in which mice that had been inoculated with EV-D68 developed symptoms of myelitis, added important evidence supporting causality. Furthermore, using the Bradford-Hill criteria, 2 groups evaluated both the epidemiologic and biologic evidence linking EV-D68 to AFM [22,23]. Several case reports and small case series have been published from the United States, Canada, South America, Australia, Asia and Europe, describing patients with EV-D68–related AFM [7,8,10,11,13–18,24–30]. In this article, we presented the first comprehensive EV-D68 AFM case series and an epidemiologic overview for Europe in 2016.

Clinical Manifestations and Treatment

In children, the median age of 3.8 years at onset of AFM was in line with the median age in a Japanese EV-D68–related AFM upsurge in 2015 (4.4 years)[30], but somewhat lower than the median age of those affected in 2014 in the United States (7.1 years)[4]. If this is a true difference, it would be interesting to investigate if lower serologic protection rates in the 4-year-olds in 2016 could have caused this shift. We included the 3 adult cases in our series to point out that EV-D68–related AFM is not restricted to childhood age.

The clinical presentation of the affected patients in Europe 2016 resembled that of patients from other parts of the world regarding prodromal symptoms and neurologic manifestations, with asymmetric flaccid limb weakness, sometimes accompanied by pain, cranial nerve deficits and bulbar symptoms [26]. It may be difficult to distinguish AFM clinically from other neurologic diseases, such as Guillain–Barre syndrome, acute transverse myelitis, Miller Fisher syndrome or acute disseminated encephalomyelitis. Additionally, mild cases can be easily missed. The case definition provides descriptions of specific MRI lesions along the spinal cord. Additionally, in the literature, lesions in the grey matter of the anterior horn and in the brainstem are described, as well as contrast enhancement of nerve

roots [31]. When MRI is not performed or these specific MRI lesions are not (yet) visible, patients may meet the criteria of a probable case when they show a mild CSF pleocytosis [5,26], as did 1 of our patients. Two patients strictly did not fulfill the criteria of the case definition, as MRI results and CSF analyses were lacking. They were nevertheless included in this study, based on the clinical picture of AFM with respiratory insufficiency and/or bulbar symptoms, and the detection of EV-D68 in respiratory samples.

If feasible in the young child, EMG findings can be of great value in supporting the diagnosis of AFM. Thus far, children with EV-D68–associated AFM generally showed low amplitude compound muscle action potentials, most often with normal conduction velocity, without signs of sensory nerve conduction abnormalities. In a later stage of disease, spontaneous muscle fiber activity can be found in the affected muscles [26,32].

Although an attempt was made to capture the features of EV-D68–related AFM in a case definition, it should be emphasized that EV-D68 is not the only virus that can cause AFM. For example, West-Nile virus and other EVs, such as EV-A71, should be considered as causative agents. Neither is AFM the only neurologic disorder that is associated with EV-D68 infection; EV-D68 has been found in patients with rhombencephalitis [24] and, in this cases series, 1 child from France was submitted with a Guillain–Barre syndrome and a concurrent EV-D68 infection (data not shown).

Various treatment regimens were prescribed in this case series. It is unfortunately not possible to deduce any positive or negative effects from these data. Similarly, in other series, no therapeutic intervention seemed to have significantly improved outcome. However, with a mouse model, Hixon et al [21] showed that EV-D68 immune-sera protected mice from development of paralysis and death when administered before viral challenge. Furthermore, recent data using this mouse model showed a favorable effect of intravenous immunoglobulin administered after infection as well; high-dose corticosteroids, however, had a negative effect on motor function and mortality [33]. Because of these findings, treatment protocols with corticosteroids as a first-line treatment may be subject to discussion.

In the literature, full neurologic rehabilitation has occurred only in a minority of patients after a 12- to 18-month period of follow-up, although MRI lesions may disappear [34,35]. The 2016 EV-D68 AFM Working Group aims at a standardized follow-up of the European patients beyond 12 months after the onset of illness, to get more insight in the natural course of the disease and to further improve education of patients and parents on the prognosis of EV-D68–related AFM.

In this series, the 2 patients who showed EV-D68 in the CSF did not survive. This may imply more severe disease, but larger studies are needed to evaluate this.

Epidemiology and Diagnostics

Our data showed a wide range of both EV positivity rates and EV-D68 positivity rates between the laboratories. This is likely explained by differences in non-polio EV-surveillance strategies and testing and typing algorithms in Europe, as mapped out by Harvala et al [36]. A way to overcome these differences would be the intensification of non-polio EV-surveillance, such as initiated in Denmark and by the European non-polio EV network [19,37].

Standard EV diagnostics, as well as poliovirus surveillance, generally relies on testing in feces, as poliovirus and the majority of other EV serotypes can indeed be detected in fecal samples. However, our epidemiologic data show that 99% of EV-D68–positive samples were respiratory specimens. This underlines that EV-D68 has a predominant respiratory tropism and respiratory specimens are required for identification of the virus [9,26,35]. The near absence of EV-D68 in fecal samples is in line with a previous study [38]. However, in our case series, EV-D68 was more frequently detected in feces and CSF than was expected based on our epidemiologic data. This difference likely reflects the widespread occurrence of EV-D68 respiratory disease, with the virus being present in respiratory specimens, and the rarity of EV-D68–related AFM, with the virus potentially present in multiple compartments, plus a more thorough microbiologic investigation in AFM patients because of disease severity.

Recently, the World Health Organization and the Pan American Health Organization have released an epidemiologic alert to include testing for EV-D68 on respiratory samples in cases of AFP/AFM, both for case management and for surveillance purposes [39]. It is important to note that not all respiratory PCR panels include EV as a target. Second, not all molecular tests that target EVs are able to detect EV-D68 or distinguish EV-D68 from rhinoviruses. Communication between clinicians and virologists is therefore essential to optimize diagnostics.

Sequence analysis showed that most of the EV-D68 strains in 2014 clustered with clades A1, A2, B1 and B2.3 [40]. In 2016, however, nearly all strains belonged to subclade B3 in Europe as well as in the United States. The clinical importance of this shift is yet unclear.

This study reveals that crucial information is often not (timely) available, among others by a lack of non-polio AFP/AFM notification regulations, and therefore the overview is by no

means complete. By activating the 2016 EV-D68 AFM Working Group network, we were able to identify 29 EV-D68–related AFM cases in Europe in 2016, but these probably represent only the tip of the iceberg. All cases were reported by countries that had also joined in the 2014 initiative. Clearly, these countries were already interested in EV-associated diseases and were therefore more prompted to identify cases when confronted with paralyzed patients. Additional AFM cases that may have been due to EV-D68 but did not have etiology confirmed because of late or absent sampling and testing, likely have been missed.

As EV-D68 has shown a cyclic pattern since 2010 [11,29,41], it is conceivable that the virus might reappear in the very near future. As no major changes have occurred in making AFM reportable in Europe, a new outbreak may go largely undetected by the health authorities. In the short term, we might benefit most from an e-mail alert system, by which clinicians and laboratories inform each other on the start of the EV season, the upsurge of rare types and on special EV-associated syndromes, such as AFM.

Conflict of interest

The authors have no funding or conflicts of interest to disclose.

REFERENCES

1. Messacar K, Abzug MJ, Dominguez SR. 2014 outbreak of enterovirus D68 in North America. J Med Virol. 2016 May;88(5):739–45.
2. Midgley CM, Watson JT, Nix WA, Curns AT, Rogers SL, Brown BA, et al. Severe respiratory illness associated with a nationwide outbreak of enterovirus D68 in the USA (2014): a descriptive epidemiological investigation. LancetRespiratory Med. 2015 Nov;3(11):879–87.
3. Poelman R, Schuffenecker I, Van Leer-Buter C, Josset L, Niesters HGM, Lina B. European surveillance for enterovirus D68 during the emerging North-American outbreak in 2014. J Clin Virol Off Publ Pan Am Soc Clin Virol. 2015 Oct;71:1–9.
4. Sejvar JJ, Lopez AS, Cortese MM, Leshem E, Pastula DM, Miller L, et al. Acute Flaccid Myelitis in the United States, August-December 2014: Results of Nationwide Surveillance. Clin Infect Dis. 2016 Sep;63(6):737–45.
5. Centers for Disease Control and Prevention. Acute flaccid myelitis. Case definitions. [Internet]. [cited 2018 Jan 15]. Available from: https://www.cdc.gov/acute-flaccid-myelitis/hcp/case-definition.html.
6. Aliabadi N, Messacar K, Pastula DM, Robinson CC, Leshem E, Sejvar JJ, et al. Enterovirus D68 Infection in Children with Acute Flaccid Myelitis, Colorado, USA, 2014. Emerg Infect Dis. 2016 Aug;22(8):1387–94.
7. Pfeiffer HC, Bragstad K, Skram MK, Dahl H, Knudsen PK, Chawla MS, et al. Two cases of acute severe flaccid myelitis associated with enterovirus D68 infection in children, Norway, autumn 2014. Euro Surveill. 2015 Mar;20(10):21062.
8. Lang M, Mirand A, Savy N, Henquell C, Maridet S, Perignon R, et al. Acute flaccid paralysis following enterovirus D68 associated pneumonia, France, 2014. Euro Surveill. 2014 Nov;19(44).
9. Varghese R, Iyer A, Hunter K, Cargill JS, Cooke RPD. Sampling the upper respiratory tract for enteroviral infection is important in the investigation of an acute neurological illness in children. Vol. 19, European journal of paediatric neurology : EJPN : official journal of the European Paediatric Neurology Society. England; 2015. p. 494–5.
10. Williams CJ, Thomas RH, Pickersgill TP, Lyons M, Lowe G, Stiff RE, et al. Cluster of atypical adult Guillain-Barre syndrome temporally associated with neurological illness due to EV-D68 in children, South Wales, United Kingdom, October 2015 to January 2016. Euro Surveill Bull Eur sur les Mal Transm = Eur Commun Dis Bull. 2016;21(4).
11. Knoester M, Scholvinck EH, Poelman R, Smit S, Vermont CL, Niesters HG, et al. Upsurge of Enterovirus D68, the Netherlands, 2016. Emerg Infect Dis. 2017 Jan;23(1):10.3201/eid2301.161313.
12. 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 Aug;44(8):2698–704.
13. Giombini E, Rueca M, Barberi W, Iori AP, Inghilleri M, Castilletti C, et al. Enterovirus D68-Associated Acute Flaccid Myelitis in Immunocompromised Woman, Italy. Emerg Infect Dis. 2017 Oct;23(10):1690–3.
14. Cabrerizo M, Garcia-Iniguez JP, Munell F, Amado A, Madurga-Revilla P, Rodrigo C, et al. First Cases of Severe Flaccid Paralysis Associated with Enterovirus D68 Infection in Spain, 2015-2016. Pediatr Infect Dis J. 2017 Jun;
15. Dyrdak R, Grabbe M, Hammas B, Ekwall J, Hansson KE, Luthander J, et al. Outbreak of enterovirus D68 of the new B3 lineage in Stockholm, Sweden, August to September 2016. Euro Surveill Bull Eur sur les Mal Transm = Eur Commun Dis Bull. 2016 Nov;21(46).
16. Esposito S, Chidini G, Cinnante C, Napolitano L, Giannini A, Terranova L, et al. Acute flaccid myelitis associated with enterovirus-D68 infection in an otherwise healthy child. Virol J. 2017 Jan;14(1):0–4.

17. Stacpoole SRL, Molyneux A, Baumer D. Acute segmental poliomyelitis-like flaccid paralysis in an adult in the UK, associated with enterovirus D68. Pract Neurol. 2017 Aug;17(4):297–301.

18. Kirolos A, Mark K, Waugh C, Shetty J, McCallum A, Templeton K, et al. Cluster of acute flaccid paralysis in children following enterovirus D68 infection in Scotland: Amir Kirolos. Eur J Public Health [Internet]. 2017 Nov 1;27(suppl_3):ckx187.697. Available from: https://doi.org/10.1093/eurpub/ckx187.697

19. Barnadas C, Midgley SE, Skov MN, Jensen L, Poulsen MW, Fischer TK. An enhanced Enterovirus surveillance system allows identification and characterization of rare and emerging respiratory enteroviruses in Denmark, 2015-16. J Clin Virol Off Publ Pan Am Soc Clin Virol. 2017 Aug;93:40–4.

20. Messacar K, Schreiner TL, Maloney JA, Wallace A, Ludke J, Oberste MS, 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 (London, England). 2015 Apr;385(9978):1662–71.

21. Hixon AM, Yu G, Leser JS, Yagi S, Clarke P, Chiu CY, et al. A mouse model of paralytic myelitis caused by enterovirus D68. PLoS Pathog. 2017 Feb;13(2):e1006199.

22. Dyda A, Stelzer-Braid S, Adam D, Chughtai AA, MacIntyre CR. The association between acute flaccid myelitis (AFM) and Enterovirus D68 (EV-D68) - what is the evidence for causation? Euro Surveill. 2018 Jan;23(3):10.2807/1560-7917.ES.2018.23.3.17-00310.

23. Messacar K, Asturias EJ, Hixon AM, Leer-Buter C Van, Niesters HGM, Tyler KL, et al. Enterovirus D68 and acute flaccid myelitis-evaluating the evidence for causality. LancetInfectious Dis. 2018 Feb;

24. Antona D, Kossorotoff M, Schuffenecker I, Mirand A, Leruez-Ville M, Bassi C, et al. Severe paediatric conditions linkedwithEV-A71andEV-D68,France,MaytoOctober2016.EuroSurveill.2016Nov;21(46):10.2807/1560-7917.ES.2016.21.46.30402.

25. Engelmann I, Fatoux M, Lazrek M, Alidjinou EK, Mirand A, Henquell C, et al. Enterovirus D68 detection in respiratory specimens: Association with severe disease. J Med Virol. 2017 Jul;89(7):1201–7.

26. Messacar K, Schreiner TL, Haren K Van, Yang M, Glaser CA, Tyler KL, et al. Acute flaccid myelitis: A clinical review of US cases 2012-2015. Ann Neurol. 2016 Sep;80(3):326–38.

27. Ruggieri V, Paz MI, Peretti MG, Rugilo C, Bologna R, Freire C, et al. Enterovirus D68 infection in a cluster of children with acute flaccid myelitis, Buenos Aires, Argentina, 2016. Eur J Paediatr Neurol. 2017 Jul;21(6):884–90.

28. Crone M, Tellier R, Wei XC, Kuhn S, Vanderkooi OG, Kim J, et al. Polio-Like Illness Associated With Outbreak of Upper Respiratory Tract Infection in Children. J Child Neurol. 2016 Mar;31(4):409–14.

29. Levy A, Roberts J, Lang J, Tempone S, Kesson A, Dofai A, et al. Enterovirus D68 disease and molecular epidemiology in Australia. J Clin Virol Off Publ Pan Am Soc Clin Virol. 2015 Aug;69:117–21.

30. Chong PF, Kira R, Mori H, Okumura A, Torisu H, Yasumoto S, et al. 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. Clin Infect Dis. 2018 Feb;66(5):653–64.

31. Maloney JA, Mirsky DM, Messacar K, Dominguez SR, Schreiner T, Stence N V. MRI Findings in Children with Acute Flaccid Paralysis and Cranial Nerve Dysfunction Occurring during the 2014 Enterovirus D68 Outbreak. Am J Neuroradiol [Internet]. 2014 Nov 20;36(2):245–50. Available from: http://www.ajnr.org/content/early/2014/11/20/ajnr.A4188.abstract

32. Hovden IA, Pfeiffer HC. Electrodiagnostic findings in acute flaccid myelitis related to enterovirus D68. Muscle Nerve. 2015 Nov;52(5):909–10.

33. Hixon AM, Clarke P, Tyler KL. Contemporary circulating enterovirus D68 strains infect and undergo retrograde axonal transport in spinal motor neurons independent of sialic acid. J Virol. 2019 Jun;

34. Martin JA, Messacar K, Yang ML, Maloney JA, Lindwall J, Carry T, et al. Outcomes of Colorado children with acute flaccid myelitis at 1 year. Neurology. 2017 Jul;89(2):129–37.

35. Yea C, Bitnun A, Robinson J, Mineyko A, Barton M, Mah JK, et al. Longitudinal Outcomes in the 2014 Acute Flaccid Paralysis Cluster in Canada. J Child Neurol. 2017 Mar;32(3):301–7.

36. Harvala H, Jasir A, Penttinen P, Pastore Celentano L, Greco D, Broberg E. Surveillance and laboratory detection for non-polio enteroviruses in the European Union/European Economic Area, 2016. Euro Surveill Bull Eur sur les Mal Transm = Eur Commun Dis Bull. 2017 Nov;22(45).

37. Harvala H, Broberg E, Benschop K, Berginc N, Ladhani S, Susi P, et al. Recommendations for enterovirus diagnostics and characterisation within and beyond Europe. J Clin Virol. 2018 Apr;101:11–7.

38. Van Leer-Buter CC, Poelman R, Borger R, Niesters HGM. Newly Identified Enterovirus C Genotypes, Identified in the Netherlands through Routine Sequencing of All Enteroviruses Detected in Clinical Materials from 2008 to 2015. J Clin Microbiol. 2016 Sep;54(9):2306–14.

39. Pan American Health Organization. Epidemiological alert: acute flaccid myelitis associated with enterovirus D68.

40. Wang G, Zhuge J, Huang W, Nolan SM, Gilrane VL, Yin C, et al. Enterovirus D68 Subclade B3 Strain Circulating and Causing an Outbreak in the United States in 2016. Sci Rep. 2017 Apr;7(1):1242.

41. Rahamat-Langendoen J, Riezebos-Brilman A, Borger R, van der Heide R, Brandenburg A, Schölvinck E, et al. Upsurge of human enterovirus 68 infections in patients with severe respiratory tract infections. J Clin Virol Off Publ Pan Am Soc Clin Virol. 2011 Oct;52(2):103–6.

SUPPLEMENTARY FILES

Supplementary Table 1: Clinical description of 29 enterovirus-D68 related acute flaccid myelitis cases, Europe 2016

		Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9
Characteristics	Age group at onset (years)	5-9	0-4	5-9	0-4	0-4	0-4	5-9	>20	0-4
	Gender	F	M	M	F	F	F	M	F	M
	Medical history	Asthma								
Clinical	Prodrome	Resp/GI	Resp/GI	Resp	Resp	Resp	Resp	Resp	Resp	Resp
	Fever	Yes	Yes	Yes	Yes	No	X	X	X	Yes
	No. days until onset of weakness	2	6	6	2	0	X	X	X	2
	Cranial nerves affected	No	No	No	No	No	Bulbar symptoms	Dysphagia	Diplopia, Dysphagia	Bulbar symptoms
	Ventilatory support	Yes	No	No	Yes	No	Yes	Yes	Yes	No
	Limb weakness							Yes		
	Arms affected	2	0	1	1	0	2	X	2	2
	Legs affected	2	2	0	0	2	2	X	2	0
	Reflexes decreased or absent	Yes	Yes	Yes	X	Yes	X	X	Yes	Yes
	Other symptoms	Back pain	Headache			Neurogenic bladder dysfunction, dysesthesia legs, pneumonia			Headache	
Investigations	CSF cell-count (leukocytes/µL)	141	50	169	89	9	X	X	X	90
	CSF protein level (g/L)	0,48	0,49	X	0,48	0,36	X	X	X	0,21

		Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9
	MRI: Hyperintensity central cord	Yes	No	No	Yes	Yes	X	X	Yes	Yes
	Location (if specified)	From C3 till conus			Cervical and thoracic spine	From T10 till L1				From C1 till C7
	MRI brain: Hyperintensity dorsal pons/ medulla	No	No	No	Yes	No	X	X	X	Yes
	Other MRI abnormalities					Enhancing roots				
	EMG	Low motor amplitudes	Normal at onset; Reduced conduction velocities after 2 days	Low motor amplitudes and spontaneous muscle fiber activity after 2 weeks	X	Low motor amplitudes	X	X	X	X
Treatment	IVIG	Yes	Yes	Yes	Yes	Yes	X	X	X	Yes
	Steroids	Yes	No	No	Yes	Yes	X	X	X	Yes
	Other	Fluoxetine								
Outcome	Recovery	Partial	Full	Partial	Partial	Partial	No	Partial	Partial	Partial
	Follow-up time (months)	4	2	2	4	1	X	X	X	5
Virology	EV-D68 positive in sample:	Resp	Resp	Resp	Feces	Resp	Resp	Resp	Resp	Resp
Remarks										

Patient 10	Patient 11	Patient 12	Patient 13	Patient 14	Patient 15	Patient 16	Patient 17	Patient 18	Patient 19
0-4	0-4	0-4	0-4	>20	0-4	0-4	0-4	0-4	>20
M	F	F	M	F	F	F	M	M	M
		Possible asthma		Lymphoma			Prematurity		
Resp	Malaise	Resp/GI	Resp	Fever	Resp	Resp	Resp	Resp/GI	Resp
Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
2	X	3	2	2-3	4	1	X	2	2-14
Right facial nerve palsy	Facial droop, dysathria, eye deviation	Bulbar symptoms	Right facial nerve palsy, dysphagia	Bulbar symptoms	No	Facial and bulbar weakness	X	Dysphagia	Bilateral abducens palsy
Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes
2	2	2	2	2	1	2	2	2	2
2	0	2	2	0	1	2	2	2	0
Yes	Yes	Yes	Yes	X	Yes	Yes	Yes	X	Yes
Headache	Generalized convulsions	Autonomic disturbance	Pneumonia				Irritability		
3	27	63	80	130	175	X	142	Raised	28
0,35	0,3	1,6	0,76	0,34	0,61	X	X	Raised	0,38
Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	X	Yes
Cervical and thoracic spine	Cervical and thoracic spine	Cervical spine	Cervical and thoracic spine	Cervical spine	From C2 till C7	Cervical and thoracic spine	Cervical and thoracic spine		C2 till C7
Yes	Yes	Yes	Yes	No	No	Yes	X	Yes	No
Enhancing roots		Hyperintensity dentate nuclei, dorsal medulla	Enhancing roots				Meningeal enhancement		

Low motor amplitudes; positive sharp waves/ fibrillations after two months	Low motor amplitudes	Low motor amplitudes	X	X	X	X	X	X	X	Low motor amplitudes; frequent fibrillations and positive sharp waves after 3 months
Yes	Yes	Yes	Yes	No	X	Yes	Yes	No	Yes	Yes
No	Yes	Yes	Yes	Yes	X	Yes	Yes	No	Yes	Yes
	Plasmapheresis	Plasmapheresis	Plasmapheresis			Plasmapheresis				
Partial	Partial	Partial	Partial	Death after 14 days	Partial	Partial	Partial	Partial	Partial	Partial
12	11	1	1	1	0,5	5	2	7	12	
Resp	Feces	Resp	Resp	CSF/Resp	Resp	Resp/Feces	Resp/Feces	Resp/Feces	Resp	
	Dec 2015							Dec 2015		

	Patient 21	Patient 22	Patient 23	Patient 24	Patient 25	Patient 26	Patient 27	Patient 28	Patient 29
0-4	5-9	5-9	0-4	0-4	0-4	0-4	0-4	5-9	0-4
M	F	M	F	M	F	F	M	M	M
Mild Wheeze									
Resp/GI	Resp	Resp	Resp/GI	Resp/GI	Otitis/skin	Resp	Resp	No	Resp
Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes
4	2	7	1	1	3	1	X	X	2
Facial and bulbar weakness	Facial and bulbar weakness	No	Facial and abducens palsy	No	No	Strabismus, ptosis, facial palsy, bulbar dysfunction	No	Bulbar weakness	No
Yes	Yes	No	No	No	No	Yes	No Yes	Yes	Yes
2	2	2	2	2	X	2	X	2	1
2	2	2	2	2	2	2	X	2	0
Yes	Yes	Yes	Yes	Yes	Increased	Yes	X	No	X
Pain, autonomic instability	Pain, autonomic instability		Feeding difficulties		Ataxia, pain	Pain, paresthesia	Pneumonia	Acute respiratory distress syndrome	Severe pneumonia, transient myocardial dysfunction
78	X	X	X	19	38	416	<5	Raised	369
0,35	X	X	X	0,27	0,24	0,44	Normal	X	0,59
Yes	Yes	Not clear; movement artefacts	Yes	Yes	No	Yes	Yes	Yes	Yes

	Patient 21	Patient 22	Patient 23	Patient 24	Patient 25	Patient 26	Patient 27	Patient 28	Patient 29
C2 till C5	C2 till C7		C2 till C7	Entire spinal cord; Maximum T1-2		Entire spinal cord			C3 till C7
Yes	Yes	Yes	Yes	Yes	Yes	Yes Caudal root enhancement	No	Yes	No
Low motor amplitudes; frequent fibrillation potentials after 7 days	Low motor amplitudes; widespread fibrillation potentials	X	X	X	X	Low motor amplitudes	X	X	X
Yes	Yes	No	Yes	Yes	Yes	Yes	No	X	Yes
Yes	No	No	No	Yes	Yes	Yes Plasmapheresis, Rituximab	Yes	X	Yes
Partial	Partial	Almost full recovery	Partial	Partial	Full	Death after 3 months	X	Partial	Partial
12	12	12	12	12	1	X	X	3	3
Resp	Resp	Resp	Resp	Resp	Resp/Feces Also EV-A71	Resp/CSF/Feces	Resp/Feces	Resp	Resp

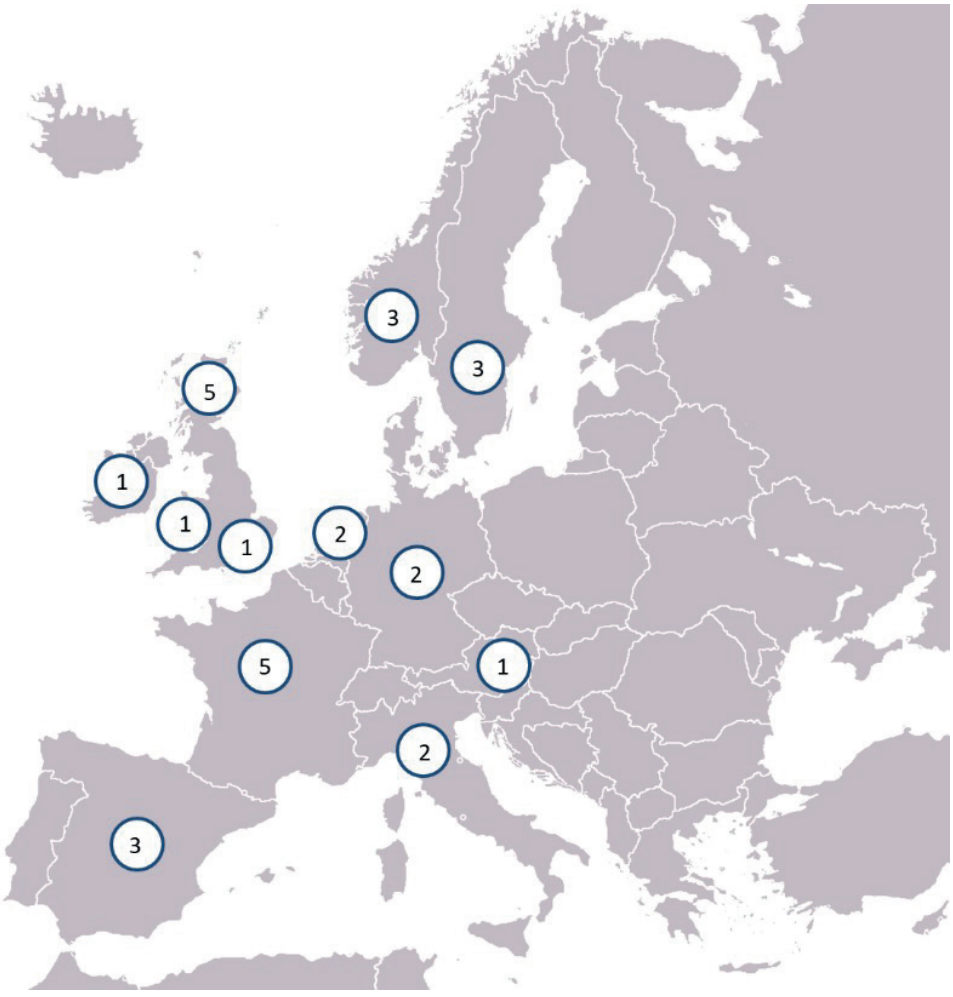
Patient no. 11 and 18 were diagnosed in December 2015. Patient no. 25 had a co-infection with enterovirus A71. Sixteen cases were previously reported in separate publications references: [10,11,13–18] Abbreviations: CSF: cerebrospinal fluid, EMG: electromyography, F: female, GI: gastro-intestinal, IVIG: intravenous immunoglobulins, M: male, MRI: magnetic resonance imaging, Resp: respiratory, X: unknown

Supplementary Table 2: Results of enterovirus and enterovirus-D68 testing as reported by 16 European laboratories, from March to October 2016, for all materials (respiratory, CSF, feces and blood) and respiratory specimens separately.

Country		***n* EV tests on ALL clinical samples (respiratory, CSF, feces, blood)**				
Diagnostic laboratories		***n* EV tests**	***n* EV POS**	***n* EV-D68 POS**	**% EV POS**	**% EV-D68 POS**
1	Iceland	610	8	**8**	1%	100%
2	Norway	1236	240	**146**	19%	61%
3	Netherlands	2508	114	**36**	5%	32%
4	Sweden	4162	252	**74**	6%	29%
5	Germany		73	**19**		26%
6	Germany	790	52	**11**	7%	21%
7	Italy	198	24	**2**	12%	8%
8	Austria	1845	64	**4**	3%	6%
9	Austria	1046	74	**1**	7%	1%
10	Spain	160	37	**0**	23%	0%
Reference laboratories						
11a	Netherlands	458	11	**8**	2%	73%
11b	Netherlands	297	211	**26**	71%	12%
12	Slovenia	1058	100	**24**	9%	24%
13	Wales	3570	312	**52**	9%	17%
14	Denmark		197	**4**		2%
15	Hungary	297	44	**0**	15%	0%
16	Ireland	3640	568	**1**	14%	<1%
Total		21875	2381	**416**		17%
Country		***n* EV tests ONLY on respiratory samples**				
Diagnostic laboratories		***n* EV tests**	***n* EV POS**	***n* EV-D68 POS**	**% EV POS**	**% EV-D68 POS**
1	Iceland	331	24	**8**	7%	33%
2	Norway	1090	221	**146**	20%	66%
3	Netherlands	1327	55	**36**	4%	65%

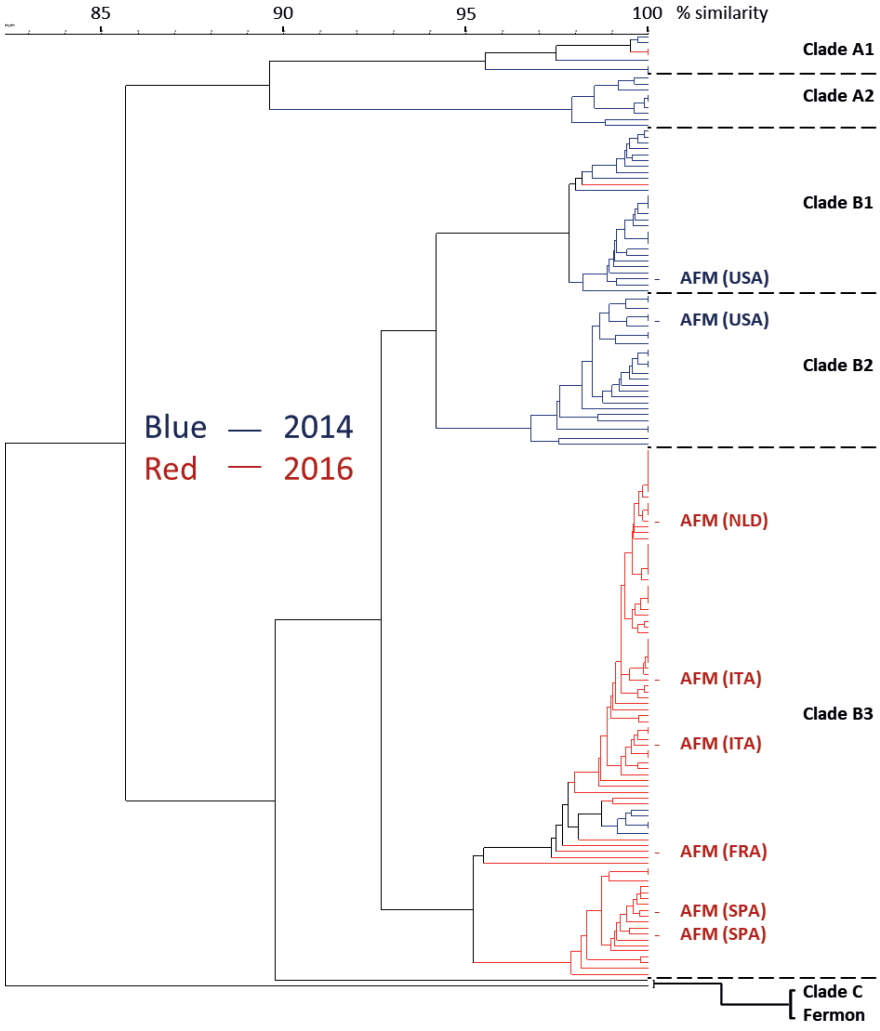
Country		*n* EV tests on ALL clinical samples (respiratory, CSF, feces, blood)				
4	Sweden	2025	135	**74**	7%	55%
5	Germany		61	**19**		31%
6	Germany	456	25	**10**	5%	40%
7	Italy	12	4	**1**	33%	25%
8	Austria	561	11	**4**	2%	36%
9	Austria	125	7	**1**	6%	14%
10	Spain	46	11	**0**	24%	0%
Reference laboratories						
11a	Netherlands	458	11	**8**	2%	73%
11b	Netherlands	94	54	**26**	57%	48%
12	Slovenia	1054	99	**24**	9%	24%
13	Wales	1819	153	**52**	8%	34%
14	Denmark		19	**4**		21%
15	Hungary	58	10	**0**	17%	0%
16	Ireland	770	167	**1**	22%	1%
Total		10226	1067	**414**		39%

1. National University Hospital Reykjavik, 2. Trondheim University Hospital/Norwegian University of Science and Technology, 3. University Medical Center Groningen, 4. Karolinska Institutet/Karolinska University Hospital, 5. Medical Center – University of Freiburg, 6. University of Bonn Medical Center, 7. National Institute for Infectious Diseases, 8. Medical University of Vienna, 9. Medical University of Vienna, regional hospitals, 10. Marqués de Valdecilla University Hospital Santander, 11. National Institute for Public Health and the Environment (a. surveillance of respiratory tract infections in general practice; b. enterovirus surveillance for polio eradication), 12. National Laboratory of Health, Environment and Food, 13. Public Health Wales, 14. Statens Serum Institute, 15. National Public Health Institute, 16. University Center Dublin, National Virus Reference Laboratory



Supplementary Figure 1: Distribution of enterovirus-D68 related acute flaccid myelitis cases over Europe in 2016, as inquired by the 2016 EV-D68 AFM Working Group.

Numbers indicate the number of cases per country.



Supplementary Figure 2: Phylogenetic tree including 72 enterovirus D68 strains from 2014 (blue) and 88 from 2016 (red).

The majority of 2016 strains clustered in clade B3, a subclade of B1. Cases of acute flaccid myelitis are marked as AFM, with the following GenBank accession numbers: KM851225 (USA [MO]), KM851230 (USA [IL]), KX685078 (NLD), MF061604 (ITA), MH118296 (ITA), KX949560 (SPA), KX949563 (SPA), MH138302 (FRA). GenBank accession numbers of respiratory cases: KM851225-31, KX957754-58, KP090456-59, KP122208, KM975347, KP153538-41, KP153543-46, CF211059, CF253080, CF254007, CF266150, CF298012, KX685066-77, KX685079-84, KX710328, KT803594, KT803598-99, KP240936, KP196362-3, KP196367, KP196378, KP745730-2, KP745734-6, KX830887-929, KP729103-5, KP729108-9, KP728259, KP406475-6, KP406484, LN681339, KP739245, MG995027. Reference strains: AY426531, AB614423, KM881710.

4

EPIDEMIOLOGY OF ACUTE FLACCID MYELITIS IN CHILDREN IN THE NETHERLANDS, 2014 TO 2019

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EuroSurveillance 2022 Oct;27(42):2200157. doi:10.2807/1560-7917.ES.2022.27.42.2200157

ABSTRACT

Background

Acute flaccid myelitis (AFM) is a polio-like condition affecting mainly children and involving the central nervous system (CNS). AFM has been associated with different non-polio-enteroviruses (EVs), in particular EV-D68 and EV-A71. Reliable incidence rates in European countries are not available.

Aim

To report AFM incidence in children in the Netherlands and its occurrence relative to EV-D68 and EV-A71 detections.

Methods

In 10 Dutch hospitals, we reviewed electronic health records of patients diagnosed with a clinical syndrome including limb weakness and/or CNS infection and who were < 18 years old when symptoms started. After excluding those with a clear alternative diagnosis to AFM, those without weakness, and removing duplicate records, only patients diagnosed in January 2014–December 2019 were retained and further classified according to current diagnostic criteria. Incidence rates were based on definite and probable AFM cases. Cases' occurrences during the study period were co-examined with laboratory-surveillance detections of EV-D68 and EV-A71.

Results

Among 143 patients included, eight were classified as definite and three as probable AFM. AFM mean incidence rate was 0.06/100,000 children/year (95% CI: −0.03 to 0.14). All patient samples were negative for EV-A71. Of respiratory samples in seven patients, five were EV-D68 positive. AFM cases clustered in periods with increased EV-D68 and EV-A71 detections.

Conclusions

AFM is rare in children in the Netherlands. The temporal coincidence of EV-D68 circulation and AFM and the detection of this virus in several cases' samples support its association with AFM. Increased AFM awareness among clinicians, adequate diagnostics and case registration matter to monitor the incidence.

INTRODUCTION

Acute flaccid myelitis (AFM) is a polio-like condition, mainly occurring in children, and characterised by an acute onset of flaccid limb weakness, combined with abnormalities in the grey matter of the spinal cord on magnetic resonance imaging (MRI) and pleocytosis in cerebrospinal fluid (CSF). Weakness can be severe and persistent, leading to significant disability in affected patients [1]. AFM has been associated with different enteroviruses (EV), in particular EV-D68 and EV-A71, with increasing evidence for causality [2–5].

In North America, a biennial upsurge of AFM cases has been reported from 2014 onwards coinciding with an increased detection rate of EV-D68 [6–8]. Unlike in the United States (US), where surveillance targets the clinical picture of AFM, surveillance in Europe up to 2020 has mainly been centred on associated EVs [9]. There, an increased detection rate of EV-D68 occurred in 2014, 2016, 2018 and to a lesser extent in 2019, but the incidence of AFM in these years is not known [10–12]. During these times however, cases and case series of AFM were identified in particular in 2016, when a European working group composed of virologists and clinicians from 20 European countries described 29 EV-D68-positive AFM patients [13]. Also in 2016, an outbreak of EV-A71 occurred in Spain, during which 133 cases of severe neurological disorders were reported, including 12 presenting with a clinical picture compatible with AFM [14].

Despite more focus on EV in Europe, some publications from the United Kingdom have related outcomes from monitoring the clinical syndrome [15,16]. In 2018, coinciding with an increase of EV-D68 detections in this country, 40 cases of acute flaccid paralysis (AFP), defined as an acute onset of limb weakness and flaccidity, were reported. Sixteen cases were further classified as probable or confirmed AFM and for two of them EV-D68 was detected [16].

In North America, in the largest AFM cohort (n = 159) described so far [7], EVs were only detected in 20–45% of cases. This may be related to incomplete or inadequate testing or to testing which nevertheless resulted negative for the virus at the moment weakness occurred [6–8]. Because in AFM cases, EVs are often not detected or tested for, the mainly used ‘EV-focused’ approach in Europe has undoubtedly led to under-reporting of the number of AFM cases.

To be able to estimate the public health impact of AFM and to decide about the necessity and usefulness of introducing AFM surveillance, reliable incidence numbers are crucial. In

this study, we aimed to retrospectively identify cases of AFM in the Netherlands and examine the incidence in the context of circulation of EV-D68 and EV-A71.

METHODS

Identification of AFM cases

A stepwise approach was used to identify cases of AFM (Figure 1). First, electronic health records were searched, for children with disease onset before the age of 18 years and with specific diagnostic codes (International Classification of Diseases (ICD) and Dutch classification of diagnoses 'Diagnose Behandel Combinatie' (DBC)), including infectious diseases which affect the nervous system and/or disorders presenting with limb weakness (specific codes are listed in the Supplementary Files). This search was done in 10 Dutch hospitals (six university hospitals and four large community hospitals), covering all, but one, hospitals in the country, with a paediatric neurology department. This way, we presumed to be able to pick up any child presenting with acute weakness in the Netherlands, except for those in the referral area of one hospital. To be included in the search, a diagnostic code had to be registered from January 2014 through December 2019. Additionally, the paediatric neurology staff of every participating hospital was asked to list any cases of suspected AFM apart from the search and selection procedure. That was done to be able to include any suspected AFM cases who might have been missed by using the diagnostic codes, as there was no specific AFM-code in the study period.

Second, the files of patients included after the first step were screened. Patients without clinical weakness or with an obvious diagnosis other than AFM were excluded. In cases without weakness and an alternative diagnosis, the absence of weakness was noted as the reason for exclusion.

In case of uncertainty on inclusion, cases were discussed in the research group (minimally comprising JH, OFB, MtW, MdL) to decide on in- or exclusion.

Third, records were checked to exclude any that did not fulfil the January 2014–December 2019 inclusion-period criterion and a structured scoring list was applied to the remaining selected cases. This scoring list covered initial diagnosis, demographic features, clinical characteristics, disease course, and results of ancillary investigations including CSF analysis, MRI, electromyography and nerve conduction studies (EMG), serum analysis for

autoantibodies (myelin oligodendrocyte (MOG) and aquaporin 4 (AQP4)), and virological tests. The type and number of samples tested with PCR, as well as positivity for EV-D68, EV-A71 or any other EV in any of these samples was considered in the scoring.

Recently published diagnostic criteria for AFM, proposed by the international AFM working group, were applied to the obtained data, by JH, with two amendments [1]. First, the criterion of decreased muscle tone in at least one weak limb was omitted as this was not included in the scoring list. Second, the presence of demyelinating features on EMG was added as a factor compatible with an alternative diagnosis. Based on these criteria, cases were classified as: (i) definite AFM; (ii) probable AFM; (iii) possible AFM; (iv) uncertain; and (v) alternative diagnosis more likely.

Cases with uncertainty on classification were presented to two experienced clinicians (OFB and BCJ) for reassessment. Both were not aware of the initial classification. The final classification was determined by consensus between these clinicians.

Calculation of incidence rates

Mean AFM incidence rates over the 6-year period as well as yearly incidence rates, both with 95 per cent confidence intervals (95% CI), were calculated based on the number of cases classified as probable and definite AFM. Population numbers from Statistics the Netherlands (CBS) were used as a reference for the annual number of children under 18 years in the Netherlands. Since one university hospital did not participate in this study, the estimated number of children in their referral region, based on data from CBS, was subtracted from the total number of children in the Netherlands. The referral region as well as the area covered by the other university hospitals are shown in Supplementary Figure S1, where the former and latter are represented on a map of the country in different colours. The number of children in the referral region as well as the total number of children under 18 years of age in the Netherlands are shown in Supplementary Table S1.

Enterovirus surveillance

Data on the number of EV-D68 and EV-A71 detections (2014–2019) were obtained from two non-overlapping surveillance systems: (i) the general practitioner (GP)-based sentinel influenza like illness (ILI) and other acute respiratory infections (ARI) surveillance (Nivel and

RIVM) [17,18], and (ii) the national EV surveillance reported in EV-surveillance/VIRO-TypeNed [19–21]. The GP surveillance is performed year-round by ca 40 practices spread throughout the Netherlands, covering close to 1% of the general population with the percentage of the general population under the age of 20 years also close to 1% [22]. All combined nose and throat specimens collected from patients with ILI or ARI are subjected to RT-PCR for EV and all EV-positive specimens are typed by sequencing [17,18]. During the study period, among the tested patients, the percentage below the age of 18 years was per year on average 25% (range: 21–29%).

The national EV-surveillance comprises a year-round typing of samples from EV positive cases diagnosed in ca 30 medical microbiology laboratories conducting EV diagnostics in the Netherlands and predominantly covers patients attending hospitals with or without hospitalisation. The primary aim of this surveillance system is to exclude the circulation of poliovirus among EV positive cases, and secondary to characterise the circulation of non-polio EVs, such as EV-D68 and EV-A71. Denominator data for the national EV-surveillance system are not available.

RESULTS

Search and selection

In a first step, the diagnosis-based search in the electronic health records produced a total of 1,776 patients. Inquiry with paediatric neurology staff of the participating hospitals yielded two additional cases of suspected AFM, who had received a different DBC/ICD-code (DBC 0131 ‘peripheral nerve’ and DBC 0599 ‘other central nervous system conditions’). Screening of the 1,778 patient files in the second step resulted in the exclusion of 1,024 patients without weakness, and 584 with a clear alternative diagnosis, leaving 168 patients eligible for step 3 (Figure 1). From these 168 patients, eight were excluded because they had not been diagnosed in the inclusion period. Fifteen patients were counted twice, and one patient three times due to referrals between hospitals. After exclusion of these cases, 143 patients qualified for further analysis.

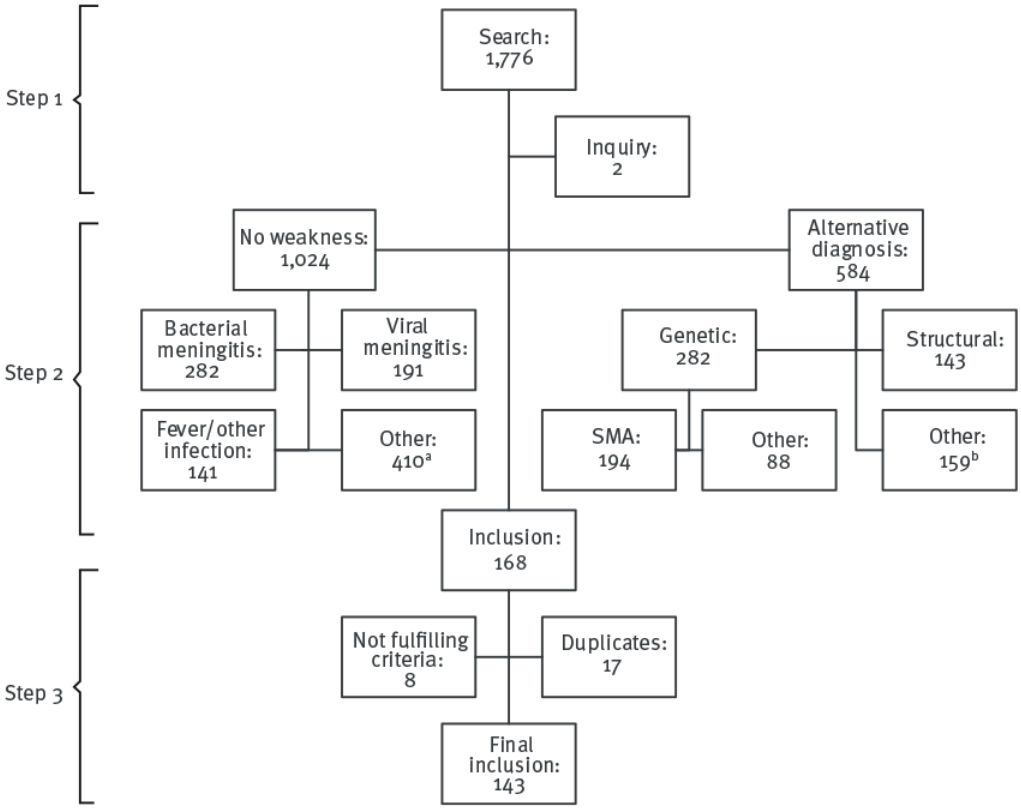

Figure 1: Results of the search and selection procedures, the Netherlands, January 2014–December 2019 (n = 1,778 patients screened)

CMV: cytomegalovirus; SMA: spinal muscular atrophy.

[a] Other diagnoses in patients without limb weakness included, among others, (suspected) autoimmune encephalitis (n = 57), congenital CMV infection (n = 39) and post-infectious ataxia (n = 29).

[b] Other diagnoses included, among others, neuroborreliosis (n = 19), slowly progressive spastic paraparesis (n = 12) and chronic inflammatory demyelinating polyneuropathy (n = 10).

In step 1, electronic healthcare systems were searched for diagnostic codes suggestive of disorders presenting with limb weakness and/or infectious diseases affecting the nervous system. Inquiry with the paediatric neurology staff of the participating hospitals yielded two

additional cases. In step 2, cases without weakness or with a clear alternative diagnosis were excluded. In step 3, cases diagnosed outside the inclusion period (not fulfilling inclusion criteria) and duplicates/triplicates were excluded.

Classification

The diagnostic criteria for AFM, as proposed by the international AFM working group, were applied to the included patients (n = 143), resulting in the classification as shown in Table 1. Fifteen cases were discussed with OFB and BCJ before final classification could be made. In two patients, insufficient information was available for classification (not included in Table 1). Furthermore, after discussion in the consensus meeting, one patient was classified as 'definite AFM', while not fulfilling all AFM criteria. This patient had clinical signs and symptoms compatible with AFM, with severe asymmetric flaccid limb weakness and minimal recovery over time. PCR in respiratory material was positive for EV-D68. EMG findings of absent or decreased motor responses in affected muscles, without signs of demyelination, were compatible with AFM. However, MRI of the spinal cord 1 day and 1 week after onset of weakness, both of which were reassessed during the classification process, did not show abnormalities.

Table 1: Characteristics of the different classification subgroups in the study, the Netherlands, January 2014–December 2019 (n = 141[a])

Characteristics	Definite[b] (total = 8)[c]	Probable[b] (total = 3)	Possible[b] (total = 3)	Uncertain[b] (total = 11)	Alternative diagnosis more likely[b] (total = 116)
Male: female[d] (percentage)	4:4 (NA[e])	2:1 (NA[e])	1:2 (NA[e])	8:3 (NA[e])	66:50 (57%)
Median age in years at diagnosis (IQR, full range)	5 (2.3–7.8; 1–11)	12 (NA[e]; 3–15)	2 (NA[e]; 0–15)	5 (3–15.5; 1–16)	6 (3–13; 0–17)
AFM criteria; proportions of patients					
Onset to nadir < 10 days	8/8	3/3	3/3	11/11	90/105
Prodrome	6/8	2/3	3/3	11/11	81/114
Hyporeflexia	8/8	3/3	0/3	11/11	91/114
MRI spinal cord abnormalities	7/8	3/3	3/3	0/1[c]	30/61
Predominant grey matter involvement	7/8	3/3	3/3	0/1	21/60
Pleocytosis	8/8	0/3	1/2	0/8	26/106
Factors suggestive of an alternative diagnosis; proportions of patients					
Encephalopathy	1/8	0/3	0/3	0/11	14/115
Sensory deficits	2/7	1/3	2/2	0/9	60/93
MRI brain abnormalities	2/6	0/3	0/3	0/4	16/65
Supratentorial white matter/cortex	0/6	0/3	0/3	0/4	15/64
AQP4 antibodies	0/6	0/1	0/2	0/0	0/32
MOG antibodies	1/6	0/1	0/3	0/1	5/26
Demyelination on EMG	0/2	0/1	0/0	0/1	33/54
Virology; proportions of patients					
Sample type investigated					
Respiratory sample	5/8	2/3	1/3	4/10	29/106
Faecal sample	4/6	1/3	0/3	1/10	22/104
CSF sample	8/8	3/3	1/3	5/10	56/105
All samples tested	3/6	1/3	0/3	1/10	10/105
Virus detected					
Enterovirus[f]	5/8	1/3	0/2	0/8	3/72

Characteristics	Definite[b] (total = 8)[c]	Probable[b] (total = 3)	Possible[b] (total = 3)	Uncertain[b] (total = 11)	Alternative diagnosis more likely[b] (total = 116)
EV-D68	4/8	1/3	0/2	0/8	0/72
EV-A71	0/8	0/3	0/2	0/8	0/72

AFM: acute flaccid myelitis; AQP4: aquaporin-4; EMG: electromyography including nerve conduction studies; EV-D68: enterovirus-D68; EV-A71: enterovirus-A71; IQR: interquartile range; MOG: myelin oligodendrocyte glycoprotein; MRI: magnetic resonance imaging; NA: not applicable.
[a] Of 143 patients included in the study, two do not figure in Table 1 because information for classification was insufficient.
[b] Information on several characteristics was not available for all patients. The number of patients without information are removed from the nominators and denominators of the fractions.
[c] Including one case without MRI abnormalities but with a compatible clinical picture and positive for EV-D68.
[d] Information on sex was collected as a binary variable.
[e] NA, as the numbers are low, so IQRs or percentages are not presented.
[f] Total number of enteroviruses detected, including EV-D68, EV-A71, other subtypes and untyped enteroviruses. The denominator indicates the number of patients in whom at least one sample was investigated.
Both 'positive' AFM criteria and factors that might suggest an alternative diagnosis are shown.

Definite AFM. In these eight patients, median age at onset was 5 years (interquartile range (IQR): 2.3–7.8; full range: 1–11). In five patients a respiratory sample was taken (day 2–5 after onset of weakness), four of whom were positive for EV, all subtyped as EV-D68. A faecal sample was taken in four patients (day 2–11 after onset of weakness), one of which was positive for EV, but could not be further subtyped. In the three patients for whom respiratory, faecal and CSF samples were tested, two respiratory samples were positive for EV-D68. In none of the samples, EV-A71 was detected. One patient tested positive for MOG antibodies but was still included in this group after careful consideration, because of lack of sensory abnormalities and significant proximal weakness at follow-up. Of the patients classified as definite AFM, four had also been initially diagnosed as AFM, and four as transverse myelitis.

Probable AFM. Of the three patients, one had been diagnosed as AFM and two as transverse myelitis. In two patients, respiratory specimen were tested (both taken at day 2 after onset of weakness), of which one tested positive for EV, subtyped as EV-D68. In the patient with a positive respiratory sample, the faecal sample (day 2 after onset of weakness) and CSF sample were negative.

Possible AFM. All three patients were initially diagnosed with transverse myelitis (Table 1). In one of these patients a respiratory and CSF sample were taken, in which no virus was isolated.

Uncertain diagnosis. In this group of 11 patients, one was initially diagnosed as AFM, with a compatible clinical picture. In this patient, IgM for EVs in blood was positive, but PCR testing of both respiratory material and faeces was negative. Both initial and repeated MRI of the spinal cord were of suboptimal quality but did not reveal clear abnormalities. All other patients in this group had initially been diagnosed with a Guillain–Barré syndrome (GBS) variant without sensory abnormalities (pure motor GBS).

Alternative diagnosis. This group of 116 patients included, among others, 75 patients who had been diagnosed as GBS, 20 as transverse myelitis and eight as acute disseminated encephalomyelitis (ADEM). In three patients an EV was isolated, two in respiratory material, with an EV which could not be further specified, and one in faeces, subtyped as EV CV-A9. The proportion of cases with respiratory or faecal samples in this group was 29/106 (27%) and 22/104 (21%), respectively, which is lower compared with the other groups. In the 10 patients (10%) in whom respiratory, faecal and CSF samples were tested, one respiratory sample was positive for an untyped EV. All CSF samples in both this group and other groups tested negative for EV and other viruses.

Incidence rate

All the patients classified as probable or definite AFM were seen in university hospitals, often after referral from a community hospital (Supplementary Figure S1). The incidence rate of AFM in the Netherlands, based on cases classified as probable or definite AFM together, was calculated as 0.06/100,000 children/year from January 2014 through December 2019 (95% CI: −0.03 to 0.14). There was variation over the different years with a minimal incidence rate of 0/100,000 children in 2015 and 2017 and a maximum incidence rate of 0.12/100,000 (95% CI: 0.00 to 0.24) children in 2016 (Supplementary Table S1; Yearly incidence rates of probable and definite acute AFM).

Enterovirus detection

Over the 6-year period (2014–2019), 220 EV-D68 positive cases were reported in the two surveillance systems (39 through GP surveillance and 181 through EV-surveillance/ VIRO-Typened). Respiratory samples were the main sample type found to be positive for EV-D68. Figure 2 shows that the virus was frequently detected in both surveillance systems in 2014, 2016, 2018, and 2019 as previously reported [12,17,18,23] with clearly high circulation in the autumn and winter months (September through November) in most years. The number of detections in 2019 was low and more spread out across the autumn and winter season, with most detections in December.

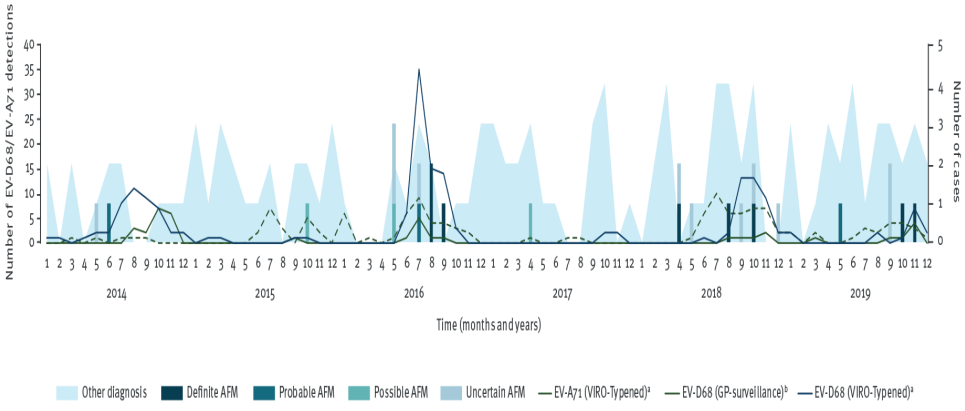

Figure 2: Temporal distribution of number of cases of AFM, according to their classification (bars) and cases with an alternative diagnosis (plane), and monthly number of EV-D68 and EV-A71 detections (lines), the Netherlands, January 2014–December 2019

AFM: acute flaccid myelitis; EV-A71: enterovirus A71; EV-D68: enterovirus D68; GP: general practitioner.

[a] EV-D68 and EV-A71 detections by enterovirus surveillance/VIRO-TypeNed.

[b] EV-D68 detections by the GP-surveillance.

EV-A71 was detected only twice in the GP-surveillance system and is therefore not shown.

EV-A71 was detected 130 times through EV-surveillance with similar circulation patterns to those seen in EV-D68 (Figure 2). Only two detections of EV-A71 were made by GP-surveillance during the study period.

A temporal relationship between AFM and EV-D68/A-71 can be suggested from Figure 2 with cases classified as definite or probable most commonly being seen in periods of increased EV-D68/EV-A71 circulation. AFM cases, including EV-D68 positive cases, are, however, also observed before onset of periods with increased EV circulation (Supplementary Table S2; Onset month of EV-positive cases according to definite, probable AFM or another likely diagnosis). The cases for whom an alternative diagnosis was considered more likely showed no clear seasonality or relation with EV-D68/EV-A71 circulation, as shown in Figure 2.

DISCUSSION

We provide a minimal estimate of the incidence of AFM of 0.06/100,000 children/year (95% CI: −0.03 to 0.14) in the Netherlands from January 2014 through December 2019. The number of cases whom we finally classified as probable or definite AFM is low, but in line with the reported incidence of AFM in the literature [24,25].

EV-D68 was detected in five of 11 cases, classified as probable and definite AFM, while EV-A71 was not identified in any of the included patients. We found indications for a temporal relationship between the number of AFM patients and the number of EV-D68 positive samples identified in two different surveillance systems during the study period, which supports the previously established association between AFM and EV-D68 infection [2,5].

While AFM cases in Europe have been described in several case reports and case series, in particular during years of increased EV-D68 and EV-A71 circulation, our study is, to our knowledge, the first to provide incidence rates of AFM in a European country [13,16,26]. Surveillance in Europe has particularly focused on the identification of EV-D68, in which no link can be made with AFM. In surveillance studies, a yearly variation similar to that observed in this study was reported [10–12].

In the US, AFM incidence rates have been reported, mainly based on passive surveillance, which is prone to under-reporting and underestimation of the real incidence rate. In the general US population, the incidence rates were calculated as 0.01–0.07/100,000 inhabitants/year based on data from the US Centers for Disease Control and Prevention

(CDC) from 2014 to 2020 with bi-annual peaks. Based on a short period of increased reporting in 2014, one study described an incidence of 0.32/100,000 population/year in individuals younger than 21 years [27]. From 2012 to 2015 incidence rates of 0.03–0.16/100.000 person-years in California in both children and adults were reported, with a clear temporal variation [25]. A retrospective cohort study in northern California reported higher incidence rates of 0.30–1.43/100,000 person-years in children between 1 and 18 years of age, with most cases reported in 2014 and 2016 [24]. Although all of these studies were performed before the introduction of the current AFM classification, diagnosis was also primarily based on the combination of acute flaccid limb weakness and spinal cord lesions largely restricted to the spinal cord grey matter. Similar to our findings, the temporal variation seemed to be connected to the circulation of different EVs, in particular EV-D68.

Our study has some limitations, including factors that may have led to an underestimation of the true incidence rate of AFM. First, patients with mild symptoms may not be referred to a paediatric neurologist, which may have led to a selection bias towards more severe cases. The full range of the clinical phenotype of AFM, possibly including milder cases, may only be revealed by large prospective cohort studies in both university and community hospitals.

Second, for the identification of patients we had to use diagnostic codes not specific for AFM, since the ICD-code for AFM has only been introduced in 2021. Some AFM cases are inevitably missed by this approach, as was confirmed by the identification of two suspected cases not included in the initial search.

Third, correct classification according to published AFM diagnostic criteria depends largely on additional investigations, in particular MRI. Cases with unrecognised or absent MRI abnormalities or in whom an MRI study was not performed are generally classified in a group with lower diagnostic certainty. On the other hand, cases of transverse myelitis may be unjustly classified as AFM as differentiation can be difficult based on current criteria. In particular, the clinical presentation of myelitis in the context of MOG-associated disease may be similar with AFP of the limbs and predominant grey matter abnormalities of the spinal cord on MRI [28].

Last, elucidating the temporal relationship between AFM cases and EV-D68/EV-A71 may be limited by suboptimal sensitivity of the surveillance systems at the beginning of an EV season. This might explain that AFM cases already occurred before increased EV-D68/EV-A71 detections were noted by the EV surveillance.

Despite its rarity and the lack of therapeutic options in the acute phase of the disease, the impact of AFM on affected children, which frequently results in severe residual deficits determines the urgency of monitoring this disease [29]. Insight in the epidemiology of AFM is important not only for the estimation of the burden of this disease, but also for better understanding of the causal relationship with viruses such as EV-D68 and EV-A71.

Increased awareness of AFM among clinicians will hopefully lead to its improved and early recognition, the relevance of which is shown in this study by the identification of several AFM cases (i.e. six of 11 in total) who were initially diagnosed with another neurological disorder, such as transverse myelitis. The relevance of awareness is illustrated by the identification of several cases in the Netherlands in the autumn of 2021. This is presumably related to an upsurge of EV-D68 across Europe that was identified to be higher than in previous years [30].

Appropriate viral diagnostics were not always performed in AFM cases in this study but are important to support the diagnosis and its relation with EV infections [31]. More intense collaboration between clinicians and virologists/microbiologists may help to ensure the performance of timely and adequate diagnostic tests, improving diagnostic accuracy as well as virus detection and identification. However, despite the importance of relating the incidence of AFM to EV epidemiology, surveillance solely based on EV infection would not be sufficient as an EV is not detected in all cases, both in our and prior studies.

Apart from adequate identification, registration of new AFM cases will be necessary to keep track of the incidence and determine the burden of disease and healthcare impact. Additionally, setting up national centres of expertise for spreading knowledge and information, for consultation and registration of AFM patients is recommended. The recently emerged European non-polio EV network (ENPEN) might provide a good structure to facilitate AFM case registration in Europe [9].

CONCLUSION

AFM is a rare disease, but with significant impact on individual patients worldwide. Our minimum estimate of 0.06/100,000 children/year from 2014 through 2019 in the Netherlands is in line with previously reported incidence values from other countries. Our findings support the association between EV-D68 infection and AFM and the importance of adequate and timely virological testing. Identification of new cases may be improved by stronger cooperation between clinicians and virologists/microbiologists, preferably based on specific guidelines.

Ethical statement

The 'Dutch Medical Research Involving Human Subjects Act' was considered not applicable for the current study. After consultation with the privacy unit of the legal department of the coordinating centre, a waiver of informed consent was obtained.

Local approval of the study protocol was obtained by the ethical committees of the participating hospitals according to their individual institutional research policy requirements. Data sharing agreements were concluded for each participating hospital, in line with the General Data Protection Regulation.

Funding statement

This study was funded by the Ministry of Health, Welfare and Sport of the Netherlands.

Acknowledgements

We wish to thank Nivel Primary Care Database – Sentinel Practices staff and participating general practitioners and patients. We also wish to thank all the virologists and laboratory staff of Laboratories participating in EV-surveillance/VIRO-TypeNed.

REFERENCES

1. Murphy OC, Messacar K, Benson L, Bove R, Carpenter JL, Crawford T, et al. Acute flaccid myelitis: cause, diagnosis, and management. Lancet (London, England). 2021 Jan;397(10271):334–46.
2. Messacar K, Asturias EJ, Hixon AM, Van Leer-Buter C, Niesters HGM, Tyler KL, et al. Enterovirus D68 and acute flaccid myelitis-evaluating the evidence for causality. Lancet Infect Dis [Internet]. 2018 Aug 1;18(8):e239–47. Available from: https://doi.org/10.1016/S1473-3099(18)30094-X
3. Schubert RD, Hawes IA, Ramachandran PS, Ramesh A, Crawford ED, Pak JE, et al. Pan-viral serology implicates enteroviruses in acute flaccid myelitis. Nat Med. 2019 Nov;25(11):1748–52.
4. Messacar K, Spence-Davizon E, Osborne C, Press C, Schreiner TL, Martin J, et al. Clinical characteristics of enterovirus A71 neurological disease during an outbreak in children in Colorado, USA, in 2018: an observational cohort study. Lancet Infect Dis. 2020 Feb;20(2):230–9.
5. Dyda A, Stelzer-Braid S, Adam D, Chughtai AA, MacIntyre CR. The association between acute flaccid myelitis (AFM) and Enterovirus D68 (EV-D68) - what is the evidence for causation? Euro Surveill. 2018 Jan;23(3):10.2807/1560-7917.ES.2018.23.3.17-00310.
6. Yea C, Bitnun A, Robinson J, Mineyko A, Barton M, Mah JK, et al. Longitudinal Outcomes in the 2014 Acute Flaccid Paralysis Cluster in Canada. J Child Neurol. 2017 Mar;32(3):301–7.
7. Messacar K, Schreiner TL, Haren K Van, Yang M, Glaser CA, Tyler KL, et al. Acute flaccid myelitis: A clinical review of US cases 2012-2015. Ann Neurol. 2016 Sep;80(3):326–38.
8. McKay SL, Lee AD, Lopez AS, Nix WA, Dooling KL, Keaton AA, et al. Increase in Acute Flaccid Myelitis - United States, 2018. MMWRMorbidity Mortal Wkly Rep. 2018 Nov;67(45):1273–5.
9. Harvala H, Benschop KSM, Berginc N, Midgley S, Wolthers K, Simmonds P, et al. European Non-Polio Enterovirus Network: Introduction of Hospital-Based Surveillance Network to Understand the True Disease Burden of Non-Polio Enterovirus and Parechovirus Infections in Europe. Microorganisms. 2021 Aug;9(9).
10. Bubba L, Broberg EK, Jasir A, Simmonds P, Harvala H. Circulation of non-polio enteroviruses in 24 EU and EEA countries between 2015 and 2017: a retrospective surveillance study. Lancet Infect Dis. 2020 Mar;20(3):350–61.
11. Poelman R, Schuffenecker I, Van Leer-Buter C, Josset L, Niesters HGM, Lina B. European surveillance for enterovirus D68 during the emerging North-American outbreak in 2014. J Clin Virol Off Publ Pan Am Soc Clin Virol. 2015 Oct;71:1–9.
12. Midgley SE, Benschop K, Dyrdak R, Mirand A, Bailly JL, Bierbaum S, et al. Co-circulation of multiple enterovirus D68 subclades, including a novel B3 cluster, across Europe in a season of expected low prevalence, 2019/20. Euro Surveill Bull Eur sur les Mal Transm = Eur Commun Dis Bull. 2020 Jan;25(2).
13. Knoester M, Helfferich J, Poelman R, Leer-Buter C Van, Brouwer OF, Niesters HGM, et al. Twenty-nine Cases of Enterovirus-D68-associated Acute Flaccid Myelitis in Europe 2016: A Case Series and Epidemiologic Overview. Pediatr Infect Dis J. 2019 Jan;38(1):16–21.
14. Gonzalez-Sanz R, Casas-Alba D, Launes C, Munoz-Almagro C, Ruiz-Garcia MM, Alonso M, et al. Molecular epidemiology of an enterovirus A71 outbreak associated with severe neurological disease, Spain, 2016. Euro Surveill Bull Eur sur les Mal Transm = Eur Commun Dis Bull. 2019 Feb;24(7).
15. Williams CJ, Thomas RH, Pickersgill TP, Lyons M, Lowe G, Stiff RE, et al. Cluster of atypical adult Guillain-Barre syndrome temporally associated with neurological illness due to EV-D68 in children, South Wales, United Kingdom, October 2015 to January 2016. Euro Surveill Bull Eur sur les Mal Transm = Eur Commun Dis Bull. 2016;21(4).

16. Force TUKAFPAT. An increase in reports of acute flaccid paralysis (AFP) in the United Kingdom, 1 January 2018-21 January 2019: early findings. Euro Surveill [Internet]. 2019 Feb 7;24(6):1900093. Available from: https://www.ncbi.nlm.nih.gov/pubmed/30755296

17. Meijer A, van der Sanden S, Snijders BEP, Jaramillo-Gutierrez G, Bont L, van der Ent CK, et al. Emergence and epidemic occurrence of enterovirus 68 respiratory infections in The Netherlands in 2010. Virology. 2012 Feb;423(1):49–57.

18. Meijer A, Benschop KS, Donker GA, van der Avoort HG. Continued seasonal circulation of enterovirus D68 in the Netherlands, 2011-2014. Euro Surveill Bull Eur sur les Mal Transm = Eur Commun Dis Bull. 2014 Oct;19(42).

19. Benschop KSM, Rahamat-Langendoen JC, van der Avoort HGAM, Claas ECJ, Pas SD, Schuurman R, et al. VIRO-TypeNed, systematic molecular surveillance of enteroviruses in the Netherlands between 2010 and 2014. Euro Surveill Bull Eur sur les Mal Transm = Eur Commun Dis Bull. 2016 Sep;21(39).

20. Niesters HG, Rossen JW, van der Avoort H, Baas D, Benschop K, Claas EC, et al. Laboratory-based surveillance in the molecular era: the TYPENED model, a joint data-sharing platform for clinical and public health laboratories. Euro Surveill Bull Eur sur les Mal Transm = Eur Commun Dis Bull. 2013 Jan;18(4):20387.

21. Donker G. Nivel Zorgregistraties Eerste Lijn - Peilstations 2018. 2019.

22. Donker GA. NIVEL Primary Care Database - Sentinel Practices. 2015.

23. van Dissel JT, Meijer A. [Enterovirus type D68 and acute flaccid paralysis: a new duo?]. Ned Tijdschr Geneeskd. 2017;161:D1825.

24. Kane MS, Sonne C, Zhu S, Malhotra A, Van Haren K, Messacar K, et al. Incidence, Risk Factors and Outcomes Among Children With Acute Flaccid Myelitis: A Population-based Cohort Study in a California Health Network Between 2011 and 2016. Pediatr Infect Dis J. 2019 Jul;38(7):667–72.

25. Haren K Van, Ayscue P, Waubant E, Clayton A, Sheriff H, Yagi S, et al. Acute Flaccid Myelitis of Unknown Etiology in California, 2012-2015. Jama. 2015 Dec;314(24):2663–71.

26. Antona D, Kossorotoff M, Schuffenecker I, Mirand A, Leruez-Ville M, Bassi C, et al. Severe paediatric conditions linked with EV-A71 and EV-D68, France, May to October 2016. Euro Surveill. 2016 Nov;21(46):10.2807/1560-7917.ES.2016.21.46.30402.

27. Sejvar JJ, Lopez AS, Cortese MM, Leshem E, Pastula DM, Miller L, et al. Acute Flaccid Myelitis in the United States, August-December 2014: Results of Nationwide Surveillance. Clin Infect Dis. 2016 Sep;63(6):737–45.

28. Bruijstens AL, Lechner C, Flet-Berliac L, Deiva K, Neuteboom RF, Hemingway C, et al. E.U. paediatric MOG consortium consensus: Part 1 - Classification of clinical phenotypes of paediatric myelin oligodendrocyte glycoprotein antibody-associated disorders. Eur J Paediatr Neurol. 2020 Nov;in press.

29. Martin JA, Messacar K, Yang ML, Maloney JA, Lindwall J, Carry T, et al. Outcomes of Colorado children with acute flaccid myelitis at 1 year. Neurology. 2017 Jul;89(2):129–37.

30. Benschop KS, Albert J, Anton A, Andrés C, Aranzamendi M, Armannsdóttir B, et al. Re-emergence of enterovirus D68 in Europe after easing the COVID-19 lockdown, September 2021. Euro Surveill Bull Eur sur les Mal Transm = Eur Commun Dis Bull. 2021 Nov;26(45).

31. Harvala H, Broberg E, Benschop K, Berginc N, Ladhani S, Susi P, et al. Recommendations for enterovirus diagnostics and characterisation within and beyond Europe. J Clin Virol. 2018 Apr;101:11–7.

SUPPLEMENTARY FILES

Supplementary Table S1: Yearly incidence rates based on the number of cases classified as probable or definite acute flaccid myelitis (AFM).

Year	Number of AFM cases (definite and probable	Total population under 18 years (x 10^6)[1]	Estimated population under 18 years in the area not covered (x 10^6)[2]	Population under 18 years in covered area (x 10^6)[3]	Incidence rate (/100.000 children)	Lower limit 95% CI	Upper limit 95% CI
2014	1	3.44	0.20	3.24	0.03	0.15	-0.02
2015	0	3.43	0.20	3.23	0	0	0
2016	4	3.44	0.20	3.24	0.12	0.24	0.00
2017	0	3.4	0.20	3.20	0	0	0
2018	3	3.38	0.19	3.19	0.09	0.29	0.02
2019	3	3.36	0.19	3.17	0.09	0.20	-0.01
Mean	1.8	3.40	0.20	3.21	0.06	0.17	-0.02

95% CI: 95 percent confidence interval.
[1]Total number of children in the Netherlands according to population numbers of Statistics Netherlands (CBS).
[2] Estimated number of children in the referral region of the university hospital not participating in this study (area indicated in supplementary figure 2)
[3] Number of children after subtraction of the estimate number of children in the referral region of the university hospital not participating in this study

Supplementary Table S2: Month of onset of the enterovirus positive cases in the different categories[1].

Year	Month	Definite AFM		Probable AFM		Other diagnosis more probable	
		EV[2]	EV-D68	EV[2]	EV-D68	EV[2]	EV-D68
2016	July	0	0	1	1	0	0
	August	2	1	0	0	0	0
2018	August	1	1	0	0	1	0
	October	1	0	0	0	0	0
2019	April	0	0	0	0	1	0
	May	0	0	0	0	1	0
	December	1	1	0	0	0	0

[1] The categories 'possible AFM' and 'uncertain' are not shown, because no enterovirus was found in these groups
[2] Total number of identified enteroviruses, including both EV-D68 and other subtypes.

Supplementary Figure S1: Map of the Netherlands, showing the estimated area covered by the participating university hospitals and the location of all participating hospitals in this study.

SECTION 2:

DIFFERENTIAL DIAGNOSIS

5

ACUTE FLACCID MYELITIS AND GUILLAIN-BARRÉ SYNDROME IN CHILDREN: A COMPARATIVE STUDY WITH EVALUATION OF DIAGNOSTIC CRITERIA

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European Journal of Neurology 2022 Feb;29(2):593-604. doi: 10.1111/ene.15170.

ABSTRACT

Background

Differentiation between acute flaccid myelitis (AFM) and Guillain–Barré syndrome (GBS) can be difficult, particularly in children. Our objective was to improve the diagnostic accuracy by giving recommendations based on a comparison of clinical features and diagnostic criteria in children with AFM or GBS.

Methods

A cohort of 26 children with AFM associated with enterovirus D68 was compared to a cohort of 156 children with GBS. The specificity of the Brighton criteria, used for GBS diagnosis, was evaluated in the AFM cohort and the specificity of the Centers for Disease Control and Prevention (CDC) AFM diagnostic criteria in the GBS cohort.

Results

Children with AFM compared to those with GBS had a shorter interval between onset of weakness and nadir (3 vs. 8 days, $p < 0.001$), more often had asymmetric limb weakness (58% vs. 0%, $p < 0.001$), and less frequently had sensory deficits (0% vs. 40%, $p < 0.001$). In AFM, cerebrospinal fluid leukocyte counts were higher, whereas protein concentrations were lower. Spinal cord lesions on magnetic resonance imaging were only found in AFM patients. No GBS case fulfilled CDC criteria for definite AFM. Of the AFM cases, 8% fulfilled the Brighton criteria for GBS, when omitting the criterion of excluding an alternate diagnosis.

Conclusions

Despite the overlap in clinical presentation, we found distinctive early clinical and diagnostic characteristics for differentiating AFM from GBS in children. Diagnostic criteria for AFM and GBS usually perform well, but some AFM cases may fulfill clinical diagnostic criteria for GBS. This underlines the need to perform diagnostic tests early to exclude AFM in children suspected of atypical GBS.

INTRODUCTION

Both acute flaccid myelitis (AFM) and Guillain–Barré syndrome (GBS) usually present with rapidly progressive limb weakness with low tendon reflexes, preceded by a prodromal illness. At onset of disease, it may be difficult to differentiate between these two conditions, especially in children. This is demonstrated by reported cases of AFM, which were initially diagnosed as atypical GBS[1]. Early differentiation is important, because there are considerable differences in diagnostic workup, treatment options, and prognosis.

AFM has been defined by the Centers for Disease Control and Prevention (CDC) as acute flaccid limb weakness, combined with a spinal cord lesion in the gray matter on magnetic resonance imaging (MRI)[2]. Other criteria for AFM have been proposed, with additionally required features regarding clinical course and outcome and results of cerebrospinal fluid (CSF) and nerve conduction studies (NCS) as well as positive diagnostic polymerase chain reaction (PCR) for enterovirus D68 (EV-D68) and EV-A71, which are among the viruses associated with AFM [3,4]. GBS has been defined by diagnostic criteria from the National Institute of Neurological Disorders and Stroke (NINDS) and more recently by diagnostic criteria from the Brighton Collaboration, in which clinical, CSF, and NCS parameters are used to classify the level of certainty of the diagnosis [5,6].

In this study, we compared two well-described cohorts of children diagnosed with AFM associated with EV-D68, or GBS with respect to clinical presentation and diagnostic features and compared the specificity of current diagnostic criteria for AFM and GBS. The results were used to provide additional recommendations for an early and accurate diagnosis of either AFM or GBS.

METHODS

Study cohorts

The AFM cohort consists of 26 children (<18 years old), who were selected from a previously described cohort of 29 European patients (adults and children) with AFM associated with EV-D68 [7]. This cohort included patients who were retrospectively identified by sending questionnaires to the European AFM Working Group. EV-D68-associated AFM was defined as acute onset focal limb weakness with MRI abnormalities and a positive

PCR for EV-D68 in either respiratory, fecal, blood, or CSF specimens. When MRI data were not available or MRI was described as normal, CSF pleocytosis was sufficient for a probable diagnosis of AFM, in concordance with the CDC case definition for AFM from 2018 [7,8].

The GBS cohort is composed of 156 children (<18 years old) from nine hospitals in the Netherlands. Most patients in the GBS cohort were included in previously published studies; 68 patients were collected in a retrospective study in one hospital[9]; and 14 patients were included in the International GBS Outcome Study, a prospective multicenter study[10]. The other 74 patients were retrospectively collected from nine Dutch hospitals. The NINDS diagnostic criteria from 1990 were used as guidelines for the diagnosis of GBS [6,9,11]. For defining the GBS electrophysiological subtypes, we used the Hadden classification [12]. From both cohorts, information was collected regarding preceding infection, first symptom, neurological deficits at admission and nadir, and results of additional tests (CSF, NCS, MRI of brain and spinal cord, and virology diagnostics), as well as treatment type and disease course. Severity of the disease at nadir was defined by the highest GBS disability score during the course of the disease. The GBS disability score includes 0 (normal), 1 (minor symptoms, capable of running), 2 (able to walk 10 m or more without assistance but unable to run), 3 (able to walk 10 m across an open space with help), 4 (bedridden or chair bound), 5 (requiring assisted ventilation for at least part of the day), and 6 (dead)[13]. Good clinical outcome was defined as reaching GBS disability score ≤ 2 at several time points during follow-up (1 month, 2 months, 3 months, 6 months, and 12 months after onset). CSF protein level was considered to be increased when >0.65 g/L for age 1–3 months, >0.37 g/L for 3–6 months, >0.35 g/L for 6–12 months, >0.31 g/L for 1–10 years, and >0.49 g/L for 10–18 years [14].

Comparison studies

A comparison between these two cohorts was made for (i) demographic characteristics, including age, sex, and month of onset; (ii) presence, type, and timing of preceding prodromal syndrome; (iii) clinical features at admission and nadir, including severity, localization, and symmetry of muscle weakness, cranial nerve involvement, sensory deficits, reflexes, respiratory failure, and autonomic dysfunction; (iv) results of additional investigations, including CSF, NCS, and MRI; and (v) clinical course and outcome.

Diagnostic criteria

The diagnostic criteria from the Brighton Collaboration, which were previously validated for GBS in children, were applied to both cohorts (excluding the criterion of absence of an alternative diagnosis in the AFM cohort) [5,9]. The Brighton criteria consist of the following items: (i) bilateral limb weakness, (ii) decreased or absent deep tendon reflexes in weak limbs, (iii) monophasic disease course, (iv) normal CSF cell count, (v) increased CSF protein level, and (vi) NCS findings consistent with GBS (Supplementary table 1).

The case definitions for AFM published by the CDC in 2018 and 2019 were applied to both cohorts. In the 2018 case definition, the combination of acute flaccid limb weakness and MRI abnormalities in the gray matter of the spinal cord was required for a definite diagnosis, whereas acute flaccid limb weakness combined with CSF pleocytosis fulfilled the criteria for a probable diagnosis of AFM. In the 2019 criteria, a definite diagnosis is described as a combination of acute flaccid weakness and MRI abnormalities predominantly in the gray matter, spanning one or more segments, with exclusion of malignancy, vascular disease, or anatomic abnormalities as an explanation for the spinal cord lesion. Criteria for a probable diagnosis are similar to those for a definite diagnosis, except that gray matter involvement of the spinal cord lesion has to be present but does not have to be predominant. A suspected case is defined as any case of acute flaccid limb weakness (Supplementary figure 1, Supplementary table 2) [2,8].

Statistics

For the statistical analysis, we used SPSS 25. Continuous data were presented as means and standard deviations if normally distributed, and otherwise as medians and interquartile ranges (IQRs). Categorical data were presented as proportions. Continuous data of the two cohorts were compared with *t*-test if normally distributed and with Mann–Whitney *U* test if not normally distributed. Proportions were compared using the chi-squared or Fisher exact test. The survival distribution of the two groups was calculated using the log-rank test. The Bonferroni correction was applied to correct for multiple comparisons. A two-sided p-value < 0.05 was considered significant.

Standard protocol approvals, registrations, and patient consents

Studies from which data were used were approved by the medical ethical review committee of the coordinating centers.

Data availability

The authors confirm that the data supporting the findings of this study are available within the article, within the limits of the General Data Protection Regulation privacy regulations.

RESULTS

Demographic characteristics

Included were 26 children diagnosed with AFM associated with EV-D68, and 156 children diagnosed with GBS. Median age of the AFM group was 3 years (IQR = 2–5, full range = 1–9) versus 7 years (IQR = 3–13, full range = 0–17) for the GBS cohort ($p < 0.001$;Table 1).

Whereas most children with AFM presented during summer and early autumn, children with GBS presented during the whole year, with the highest frequencies in June and December ($p = 0.038$).

Table 1: Demography and clinical presentation of AFM and GBS in children

	AFM (N=26)	**GBS** (N=156)	p-value
Demography			
Male: female (% M)	14:12 (54)	82:74 (53)	ns
Age, median (IQR, full range), years	3 (2-5, 8)	7 (3-13, 17)	<0.001
Antecedent events			
Time antecedent event-onset weakness, median (IQR, full range), days	7 (5-8, 10)	11 (7-15, 41)	ns
No antecedent event (%)	1/26 (4)	19/143 (13)	ns
Respiratory tract infection(%)	23/26 (89)	66/146 (45)	<0.001
Vomiting (%)	2/26 (8)	32/118 (27)	ns
Diarrhea (%)	5/26 (19)	47/145 (32)	ns
Fever (%)	22/24 (92)	51/140 (36)	<0.001
Vaccination (%)[a]	0/2 (0)	11/130 (9)	np
Time onset weakness-admission, median (IQR, full range), days[b]	0 (0, 5)	5 (3-8, 30)	<0.001
Time onset weakness-nadir, median (IQR, full range), days[c]	3 (2-5, 9)	8 (5-10, 38)	<0.001
Neurological symptoms at admission			
Sensory deficits (%)	0/26 (0)	41/102 (40)	<0.001
Pain (%)	8/24 (33)	92/130 (71)	<0.001
Limb weakness (%)	25/25 (100)	122/135 (90)	ns
Weakness arms (%)	19/25 (76)	90/131 (69)	ns
Weakness legs (%)	17/24 (71)	121/135 (90)	ns
Asymmetric weakness (%)	14/24 (58)	0/133 (0)	<0.001
Cranial nerve involvement (%)	6/24 (25)	51/141 (36)	ns
Autonomic dysfunction (%)	0/24 (0)	13/122 (11)	ns
Areflexia/hyporeflexia (%)	19/21 (91)	80/111 (72)	ns
Neurological symptoms at nadir			
Sensory deficits (%)	0/24 (0)	66/112 (59)	<0.001
Pain (%)	2/28 (7)	107/132 (81)	<0.001
Limb weakness (%)	25/25(58)	145/146 (99)[d]	ns
Weakness arms (%)	22/25 (88)	126/144 (88)	ns
Weakness legs (%)	20/24 (83)	142/144 (99)	ns
Asymmetric weakness (%)	11/20 (55)	0/142 (0)	<0.001
Cranial nerve involvement (%)	12/25 (48)	77/140 (55)	ns
Autonomic dysfunction (%)	3/25 (12)	64/136 (47)	ns
Areflexia/hyporeflexia (%)	21/23 (91)	120/129 (93)	ns

	AFM (N=26)	**GBS** (N=156)	p-value
Mechanical ventilation (%)	16 (64)	37 (24)	<0.001
Duration intubation, median (IQR, full range), days[e]	29 (15-365, 716)	20 (12-32, 134)	ns
Time onset weakness-respiratory failure, median (IQR, full range), days[f]	1 (1-4, 3)	6 (4-11, 61)	<0.001

Due to small patient numbers, not all items were compared (mentioned as np).
Abbreviations: AFM, acute flaccid myelitis; GBS, Guillain–Barré syndrome; IQR, interquartile range; np, not performed; ns, not significant.
[a] The information on vaccinations in the AFM cohort was missing for 24 patients.
[b] The median time between onset of weakness and admission was based on 18 AFM patients and 141 GBS patients.
[c] The median time between onset of weakness and nadir was based on 17 AFM patients and 129 GBS patients.
[d] One patient in the GBS cohort was diagnosed with sensory GBS and never developed (bilateral) limb weakness.
[e] The median time of intubation was based on six AFM patients; this information was missing for 10 patients. In the GBS cohort, this information was complete.
[f] The median time between onset of weakness and respiratory failure in the AFM cohort was based on five patients.

Clinical presentation and course

Most children with AFM or GBS had symptoms of a preceding infection, 96% and 87%, respectively.

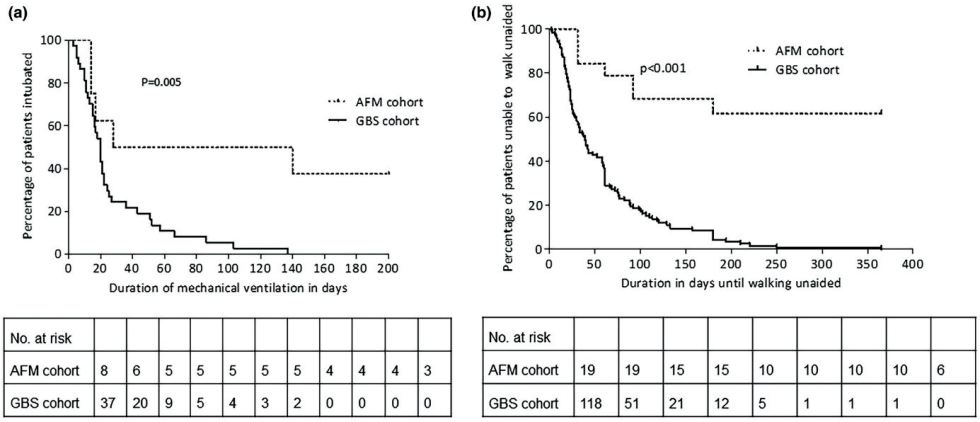
Time between onset of weakness and hospital admission was shorter for the children with AFM, with a median of 0 days (IQR = 0) versus 5 days (IQR = 3–8) in GBS patients (Table **1**).

At admission, patients with AFM more often had asymmetric weakness than children with GBS (58% vs. 0%, $p < 0.001$). None of the children with AFM had sensory deficits at onset, compared to 40% of children with GBS. At onset, in 33% of children with AFM pain was reported, compared to 71% of children with GBS (Table 1).

The time between onset of symptoms and time of nadir was shorter for AFM patients (median = 3 days, IQR = 2–5 vs. median = 8 days, IQR = 5–10). At nadir, 83% of AFM patients had bilateral weakness, compared to 99% of GBS patients. Only one patient with GBS did not have bilateral weakness but a purely sensory form.

More patients with AFM required mechanical ventilation, and respiratory failure developed earlier after onset of symptoms (Table 1). Duration of mechanical ventilation was longer in AFM patients, reflecting a more severe and prolonged clinical course and poorer

recovery (Figure 1A). The poor outcome of AFM compared to GBS is also reflected in the proportion of children able to walk unaided after 6 months (46% vs. 93%) and 12 months (50% vs. 99%;Figure 1B).



No. at risk											
AFM cohort	8	6	5	5	5	5	5	4	4	4	3
GBS cohort	37	20	9	5	4	3	2	0	0	0	0

No. at risk									
AFM cohort	19	19	15	15	10	10	10	10	6
GBS cohort	118	51	21	12	5	1	1	1	0

Figure 1

a. Duration of mechanical ventilation in the acute flaccid myelitis (AFM) and Guillain–Barré syndrome (GBS) cohorts. Three patients from the AFM cohort were still intubated at 200 days. The difference between the duration of intubation between the two groups was based on log-rank test. **b. Long-term prognosis, indicating time until the ability to walk unaided.** Included were the patients who were unable to walk unaided at nadir (GBS disability score >2). The long-term follow-up was available until 1 year after onset of weakness. The difference between the duration until independent walking between the two groups was based on log-rank test

Additional diagnostic tests

Lumbar puncture was performed in most patients with AFM and GBS, but the timing after onset of symptoms was earlier in patients with AFM compared to GBS (Table 2). In the patients with GBS, CSF protein level was more frequently elevated and higher than in the patients with AFM. GBS patients with normal CSF protein level had an earlier lumbar puncture after onset of symptoms than GBS patients with an elevated CSF protein level (median = 4 days, IQR = 3–5.75 vs. median = 7 days, IQR = 4–11, $p < 0.001$). The median number of leukocytes in CSF was higher for the AFM cohort (79 vs. 4/μL, $p < 0.001$), as was the proportion of patients with CSF pleocytosis (Table 2). A cytoalbuminologic dissociation was less often found in the AFM group.

Table 2: Additional diagnostic test results in children with AFM and GBS

	AFM (N=26)	**GBS** (N=156)	p-value
Lumbar puncture			
Lumbar puncture performed (%)	23/26 (89)	143/154 (93)	ns
Time onset weakness-LP, median (IQR, full range), days[a]	1 (1-2, 4)	6 (4-9, 32)	<0.001
Raised protein level (%)[b]	12/17 (71)	111/141 (79)	ns
Protein concentration in CSF, median (IQR, full range), (g/l)[c]	0.44 (0.30-0.59, 1.39)	0.76 (0.43-1.61, 7.57)	0.004
Leucocyte number in CSF, median (IQR, full range)[d]	79 (25-149, 414)	4 (1-9, 133)	<0.001
CSF leukocyte count ≤ 5 (%)	3/20 (15)	86/133 (65)	<0.001
CSF leukocyte count 5-50 (%)	5/18 (28)	40/133 (30)	ns
CSF leukocyte count ≥ 50 (%)	11/18 (61)	5/133 (4)	<0.001
Cytoalbuminologic dissociation (%)[e]	3/16 (19)	99/132 (75)	<0.001
MRI			
MRI performed (%)	24/24 (100)	16/113 (14)	<0.001
MRI laesions brainstem (%)	18/24 (75)	1/8 (13)	np
MRI laesion myelum (%)	19/23 (83)	0/8 (0)	np
MRI nerve root thickening (%)	5/21 (24)	3/7 (43)	np
Nerve conduction study			
Nerve conduction studies performed (%)	11/26 (42)	96/120 (80)	<0.001
Time onset weakness-NCS, median (IQR, full range), days[f]	6 (4-9, 14)	9 (5-14, 36)	Ns
Normal (%)	1/11 (10)	8/94 (9)	Np
Equivocal (%)	4/10 (40)	16/85 (19)	Np
Unresponsive (%)	0/10 (0)	1/85 (1)	Np
AMAN (%)	5/10 (50)	8/85 (9)	Np
AMSAN (%)	0/10 (0)	2/85 (2)	Np
AIDP(%)	0/10 (0)	50/85 (59)	Np

Where $p > 0.05$, this is mentioned as ns. Due to small patient numbers, not all items were compared (mentioned as np).
Abbreviations: AFM, acute flaccid myelitis; AIDP, acute inflammatory demyelinating polyradiculoneuropathy; AMAN, acute motor axonal neuropathy; AMSAN, Acute motor and sensory axonal neuropathy; CSF, cerebrospinal fluid; GBS, Guillain–Barré syndrome; IQR, interquartile range; LP, lumbar puncture; MRI, magnetic resonance imaging; NCS, nerve conduction studies; np, not performed; ns, not significant.

[a] The information on onset weakness and performing LP was available for 15 patients from the AFM cohort and for 117 patients from the GBS cohort.
[b] Raised protein was defined as a protein level of >0.65 g/L for the age of 1–3 months, >0.37 g/L for 3–6 months, >0.35 g/L for 6–12 months, >0.31 g/L for 1–10 years, and >0.49 g/L for 10–18 years.
[c] The information on protein concentration in CSF was available for 15 AFM patients and 138 GBS patients.
[d] The information on the number of leukocytes in CSF was available for 18 AFM patients and 133 GBS patients.
[e] Cytoalbuminologic dissociation: protein level > the age dependent reference values and leukocytes < 50.
[f] In 52 patients from the GBS cohort, the information for onset of weakness and NCS was missing.

MRI was performed in almost all AFM patients, but in only 14% of the GBS patients. Lesions of the spinal cord and brainstem were more often seen in AFM patients, whereas the presence of nerve root enhancement was found equally frequently (Table **2**).

NCSs were performed in 42% of patients with AFM and 80% of patients with GBS. These were normal in one AFM patient, examined on the first day of symptoms, but revealed abnormalities in most patients, most often consistent with axonal damage. In GBS patients, abnormalities were found in 91% of patients, most often compatible with a demyelinating polyneuropathy (Table 2).

Evaluation of clinical criteria

The Brighton criteria for GBS were evaluated in the AFM cohort (except for the criterion of excluding alternative diagnosis). Two (8%) of the patients with AFM fulfilled all the Brighton criteria for a diagnosis of GBS at Level 1 certainty, two (8%) reached Level 2, 12 (46%) reached Level 3, and 10 (39%) reached Level 4 (Figure 2, Supplementary table 1). For the 2018 CDC criteria for AFM, all of our AFM patients with sufficient data fulfilled the criteria for definite or probable AFM. Of a subset of 38 GBS patients with sufficient data, 32 fulfilled the criteria for probable AFM with a CSF pleocytosis (Supplementary figure 1, Supplementary table 2), but MRI was not performed in any of these patients. Of the patients with GBS, none fulfilled the 2019 CDC criteria for probable or definite AFM. From the AFM cohort, three patients (13%) also did not fulfill these criteria (Supplementary figure 1, Supplementary table 2).

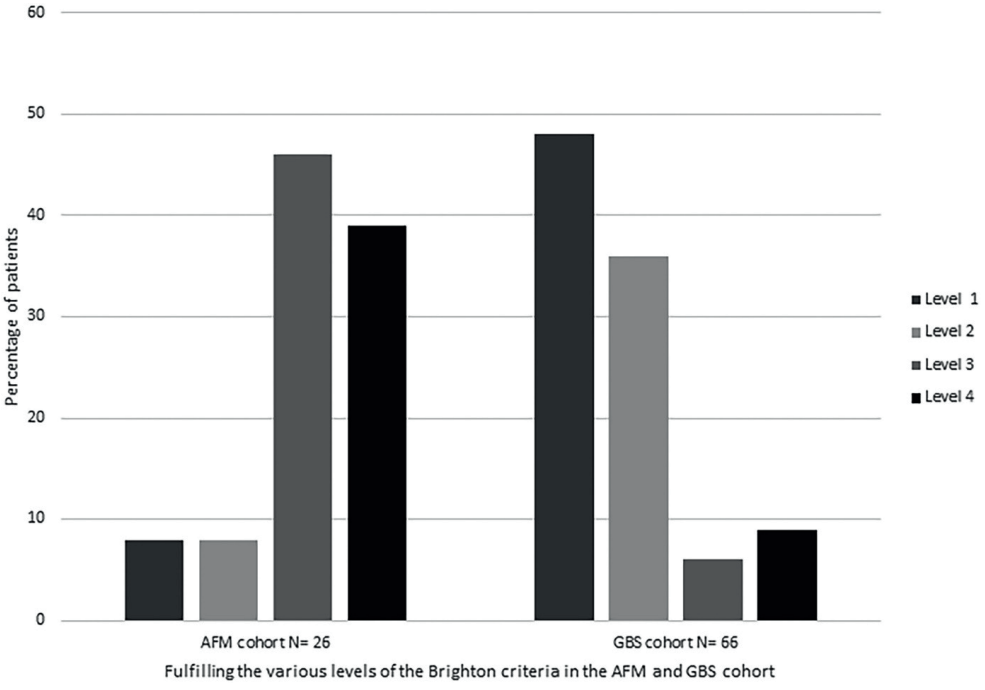


Figure 2: Performance of the Brighton diagnostic criteria for Guillain–Barré syndrome (GBS) in the acute flaccid myelitis (AFM) and GBS cohorts.

DISCUSSION

his comparative study in children shows that there is a considerable overlap in the clinical presentation of AFM and GBS, in accordance with the differential diagnosis in current practice. The majority of children with either AFM or GBS presented with a prodromal disease and progressive flaccid weakness of the limbs with reduced reflexes, and had a monophasic disease course. Some of the AFM patients even fulfilled the clinical diagnostic criteria for GBS, at least if involvement of spinal cord gray matter and viral infections related to AFM are not taken into account.

Nonetheless, our study also shows that there are important distinguishing early features, as illustrated in Figure 3. AFM compared to GBS in children is more rapidly progressive, and clinical nadir is usually reached within days and related to the presence of asymmetric limb weakness and absence of sensory deficits. The numbers for sensory deficits and pain in the AFM cohort may, however, be an underestimation, as the age in the AFM cohort is significantly lower and the assessment of pain and sensory deficits may be challenging in younger children.

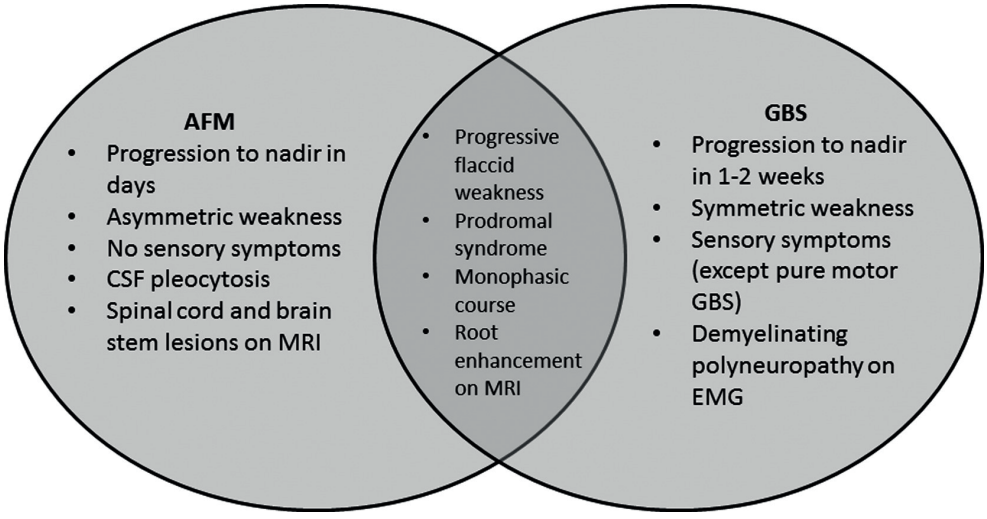


Figure 3: Venn diagram illustrating overlapping and differentiating features of acute flaccid myelitis (AFM) and Guillain–Barré syndrome (GBS). The indicated features are suggestive of either diagnosis, but they are not necessarily present or exclusive. CSF, cerebrospinal fluid; EMG, electromyography; MRI, magnetic resonance imaging

CSF pleocytosis (>50 cells/μL) is frequent in AFM but rare in GBS, but an increase in protein level or mildly elevated cell count (5–50/μL) does not differentiate between AFM and GBS. NCS performed after the acute stage of disease frequently show peripheral nerve involvement in both disorders, but usually demyelinating in GBS and always axonal in AFM. Later in the disease course, it becomes evident that a protracted course and persistent weakness are associated with AFM.

The demography, clinical presentation, diagnostic test results, and clinical course of the presented cohorts of children with AFM and GBS resemble other cohorts of patients with these conditions [15–20]. Therefore, these characteristics are likely representative for children with either AFM or GBS.

This is the first comparative study between GBS and AFM, both in children and adults. A recent study did compare a group with restrictively defined or "true" AFM with a group of patients with alternative diagnoses, matching the 2018 case definition of the CDC[3]. Several clinical factors, such as the asymmetry and the absence of sensory deficits, resemble the distinctive features found in our study. Further similarities are the CSF pleocytosis and MRI abnormalities that were more often found in the "true" AFM group[3].

The CDC criteria are highly selective in excluding GBS, as there is a focus on MRI findings to make a probable or definite diagnosis of AFM. Also in our cohort, none of the children with GBS fulfilled the 2019 CDC criteria. MRI may be normal in the early phase of AFM or can show only subtle abnormalities[15]. Therefore, a combination of clinical criteria and results from diagnostic tests may be more appropriate, as was suggested previously[3]. The content of different criteria does, however, depend on the goal for which these criteria are used; a broader case definition should be used for case detection, whereas a restricted definition is more suitable for research purposes. Recently, an international working group proposed new diagnostic criteria for AFM, which are broadly similar to most recent CDC criteria. CSF pleocytosis and the presence of sensory deficits were adequately suggested as markers for an alternate diagnosis[21].

The criteria developed by the Brighton Collaboration for the diagnosis GBS were developed as case definitions for vaccine safety studies but also reflect the diagnostic workup for GBS in current clinical practice[5]. The current study shows that children with AFM may fulfill the clinical diagnostic criteria for GBS of a rapidly progressive and bilateral weakness, reduced reflexes in affected limbs, and a monophasic disease course. In addition, patients with AFM may have the cytoalbuminologic dissociation in CSF and in half of the

cases have an axonal pattern in NCS that may be misclassified as the acute motor axonal neuropathy subtype of GBS. The implication of these findings for clinical practice is that when AFM is not excluded by conducting MRI and virology, these patients may be falsely diagnosed with GBS.

The Brighton classification requires the exclusion of other causes, but without specifying which other causes need to be excluded in which patients. Importantly, there is only a short time window early in the disease course when AFM can be accurately excluded by performing MRI and virological PCR testing to prove an infection with EV-D68 or EV-A71. These investigations can, however, be inconclusive and lose their diagnostic sensitivity later in the disease course[15,22]. Serological testing may indicate a previous enterovirus infection, but the subtype cannot be determined[22]. By the time of receiving the results of the NCS showing an axonal neuropathy or lack of recovery, it may be too late to exclude the diagnosis of AFM. Therefore, we recommend considering the diagnosis of AFM early in the disease course of patients suspected of GBS, especially if they present with a rapidly progressive or asymmetric limb weakness or lack of sensory deficits or a CSF pleocytosis. Prompt diagnostic studies should then be performed, including CSF investigations, MRI of the brain and spinal cord, and adequate virological testing, particularly on respiratory material.

Differentiation of AFM and GBS is important for several reasons. First, accurate diagnosis is important for informing patients and relatives about the expected prognosis and preparing patients for rehabilitation. The current study demonstrates the substantial difference in clinical course and outcome, which is much worse in AFM than GBS, in accordance with previous studies investigating the outcome either in AFM[23–25] or GBS.[18,19] Second, accurate diagnosis is important to start and develop targeted treatment of AFM and GBS. At present, proven effective treatments for GBS are intravenous immunoglobulins (IVIg) or plasma exchange, although these require further confirmatory studies. Although there is no proven effective treatment available for AFM yet, most patients are treated with IVIg, which might have a beneficial effect early in the disease course. Steroids were associated with a deterioration of motor symptoms in the mouse model of AFM [26–28]. Third, AFM and GBS may both occur during outbreaks, and to be able to monitor the background incidence rates accurate diagnosis is essential.

Our study has several strengths. First, we included two well-described cohorts of children with either AFM or GBS, which are compatible with reports in literature regarding

their clinical features, especially with respect to those features that were found to be discriminative between both conditions. Second, both cohorts are among the largest pediatric cohorts of AFM and GBS described in literature. Third, the AFM cohort included only EV-D68-positive cases, leading to a more certain diagnosis of AFM and improving the homogeneity of the AFM cohort.

On the other hand, the selection of EV-D68-positive cases could also be seen as a limitation, as it may lead to a selection bias. The phenotype of AFM with a proven EV-68 infection may be more severe than AFM associated with other viruses or AFM without a proven viral infection[16]. For example, patients with AFM associated with EV-A71 have an earlier onset of weakness after the prodromal syndrome, milder weakness, more rapid improvement, and a higher chance of full recovery, compared to patients with EV-D68-associated disease[29]. This could indicate that our results are not generalizable to AFM caused by other infectious agents. However, the similarities in clinical features and ancillary investigations between our cohort of AFM patients and larger cohorts described in literature suggest that the observed differences at onset of disease are also valid for the whole group of AFM patients. The poor prognosis of AFM in comparison with GBS in children observed in the current study, however, may be influenced by a selection bias toward more severe cases of AFM.

Further limitations include a selection bias by inclusion of cases matching current criteria for AFM and GBS, making some differences between the groups obvious. For example, as MRI abnormalities in the spinal cord are required for the diagnosis of AFM and only a limited number of children with GBS underwent MRI, it is not surprising that these abnormalities are more often found in the AFM group. However, we consider that this does not hinder the finding of differentiating features, which was the main purpose of this study. The identification of AFM patients by sending questionnaires to clinicians and microbiologists could lead to an overrepresentation of severe cases. Therefore, the outlined differences in outcome between AFM and GBS may be an overestimation of the true differences. Nonetheless, the available follow-up data on AFM show persistence of significant neurological deficits after 1 year, supporting the authenticity of the found differences[17,24,25]. The limited group size, predominantly in the AFM group, hinders multivariate analysis. The recommendations made are based on the differentiating features between AFM and GBS, and they are not specific for the differentiation between AFM and other conditions, such as transverse myelitis. Furthermore, the recommendations for

clinical differentiation and diagnostic studies are not externally validated. This will be necessary to confirm the validity of these recommendations.

CONCLUSIONS

A child with acute onset flaccid weakness may pose a diagnostic challenge for clinicians, with both AFM and GBS included in the differential diagnosis. We provide distinguishing features and recommendations, which may help clinicians in making the right diagnosis.

Diagnostic criteria for AFM and GBS usually perform well in children. However, in cases of atypical GBS, the diagnosis of AFM needs to be excluded early in the disease course, as AFM may fulfill the current clinical, CSF, and NCS diagnostic criteria for GBS.

Conflict of interest

M.C.d.W. has received honoraria paid to her institution by Novartis for serving on a steering committee and presenting at a conference, and has received research funding from the Epilepsiefonds (Dutch Epilepsy Foundation), Hersenstichting, and Sophia Foundation. B.C.J. has received funding for travel from Baxter International, and has received research funding from the Netherlands Organization for Health Research and Development, Erasmus MC, Prinses Beatrix Spierfonds, Stichting Spieren voor Spieren, CSL-Behring, Grifols, Annexon, Hansa Biopharma, and the GBS-CIDP Foundation International. None of the other authors has any conflict of interest to disclose.

Funding information

The Prinses Beatrix Spierfonds funded the PhD project of J.R. on GBS in children (project number: W.OR12-04)

REFERENCES

1. Williams CJ, Thomas RH, Pickersgill TP, Lyons M, Lowe G, Stiff RE, et al. Cluster of atypical adult Guillain-Barre syndrome temporally associated with neurological illness due to EV-D68 in children, South Wales, United Kingdom, October 2015 to January 2016. Euro Surveill Bull Eur sur les Mal Transm = Eur Commun Dis Bull. 2016;21(4).
2. CSTE. Revision to the Standardized Case Definition, Case Classification, and Public Health Reporting for Acute Flaccid Myelitis. 2019.
3. Elrick MJ, Gordon-Lipkin E, Crawford TO, Van Haren K, Messacar K, Thornton N, et al. Clinical Subpopulations in a Sample of North American Children Diagnosed With Acute Flaccid Myelitis, 2012-2016. JAMA Pediatr [Internet]. 2019 Feb 1;173(2):134–9. Available from: https://doi.org/10.1001/jamapediatrics.2018.4890
4. Kramer R, Lina B, Shetty J. Acute flaccid myelitis caused by enterovirus D68: Case definitions for use in clinical practice. Eur J Paediatr Neurol EJPN Off J Eur Paediatr Neurol Soc. 2019 Mar;23(2):235–9.
5. Sejvar JJ, Kohl KS, Gidudu J, Amato A, Bakshi N, Baxter R, et al. Guillain-Barré syndrome and Fisher syndrome: case definitions and guidelines for collection, analysis, and presentation of immunization safety data. Vaccine. 2011 Jan;29(3):599–612.
6. Asbury AK, Cornblath DR. Assessment of current diagnostic criteria for Guillain-Barre syndrome. Ann Neurol. 1990;27 Suppl:S21-4.
7. Knoester M, Helfferich J, Poelman R, Leer-Buter C Van, Brouwer OF, Niesters HGM, et al. Twenty-nine Cases of Enterovirus-D68-associated Acute Flaccid Myelitis in Europe 2016: A Case Series and Epidemiologic Overview. Pediatr Infect Dis J. 2019 Jan;38(1):16–21.
8. CSTE. Acute Flaccid Myelitis (AFM) 2018 Case Definition. 2018.
9. Roodbol J, de Wit MCY, van den Berg B, Kahlmann V, Drenthen J, Catsman-Berrevoets CE, et al. Diagnosis of Guillain-Barré syndrome in children and validation of the Brighton criteria. J Neurol. 2017 May;264(5):856–61.
10. Jacobs BC, van den Berg B, Verboon C, Chavada G, Cornblath DR, Gorson KC, et al. International Guillain-Barré Syndrome Outcome Study: protocol of a prospective observational cohort study on clinical and biological predictors of disease course and outcome in Guillain-Barré syndrome. J Peripher Nerv Syst. 2017 Jun;22(2):68–76.
11. Roodbol J, de Wit MCY, Walgaard C, de Hoog M, Catsman-Berrevoets CE, Jacobs BC. Recognizing Guillain-Barre syndrome in preschool children. Neurology. 2011 Mar;76(9):807–10.
12. Hadden RD, Cornblath DR, Hughes RA, Zielasek J, Hartung HP, Toyka K V, et al. Electrophysiological classification of Guillain-Barré syndrome: clinical associations and outcome. Plasma Exchange/Sandoglobulin Guillain-Barré Syndrome Trial Group. Ann Neurol. 1998 Nov;44(5):780–8.
13. Hughes RA, Newsom-Davis JM, Perkin GD, Pierce JM. Controlled trial prednisolone in acute polyneuropathy. Lancet (London, England). 1978 Oct;2(8093):750–3.
14. Kahlmann V, Roodbol J, van Leeuwen N, Ramakers CRB, van Pelt D, Neuteboom RF, et al. Validated age-specific reference values for CSF total protein levels in children. Eur J Paediatr Neurol EJPN Off J Eur Paediatr Neurol Soc. 2017 Jul;21(4):654–60.
15. Messacar K, Schreiner TL, Haren K Van, Yang M, Glaser CA, Tyler KL, et al. Acute flaccid myelitis: A clinical review of US cases 2012-2015. Ann Neurol. 2016 Sep;80(3):326–38.
16. Chong PF, Kira R, Mori H, Okumura A, Torisu H, Yasumoto S, et al. 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. Clin Infect Dis. 2017 Oct;

17. Yea C, Bitnun A, Robinson J, Mineyko A, Barton M, Mah JK, et al. Longitudinal Outcomes in the 2014 Acute Flaccid Paralysis Cluster in Canada. J Child Neurol. 2017 Mar;32(3):301–7.

18. Korinthenberg R, Schessl J, Kirschner J. Clinical presentation and course of childhood Guillain-Barre syndrome: a prospective multicentre study. Neuropediatrics. 2007 Feb;38(1):10–7.

19. Bradshaw DY, Jones HRJ. Guillain-Barre syndrome in children: clinical course, electrodiagnosis, and prognosis. Muscle Nerve. 1992 Apr;15(4):500–6.

20. Ye YQ, Wang KR, Sun L, Wang Z. Clinical and electrophysiologic features of childhood Guillain-Barre syndrome in Northeast China. J Formos Med Assoc. 2014 Sep;113(9):634–9.

21. Murphy OC, Messacar K, Benson L, Bove R, Carpenter JL, Crawford T, et al. Acute flaccid myelitis: cause, diagnosis, and management. Lancet (London, England). 2021 Jan;397(10271):334–46.

22. Schubert RD, Hawes IA, Ramachandran PS, Ramesh A, Crawford ED, Pak JE, et al. Pan-viral serology implicates enteroviruses in acute flaccid myelitis. Nat Med. 2019 Nov;25(11):1748–52.

23. Roodbol J, de Wit MCY, Aarsen FK, Catsman-Berrevoets CE, Jacobs BC. Long-term outcome of Guillain-Barré syndrome in children. J Peripher Nerv Syst. 2014 Jun;19(2):121–6.

24. Kirolos A, Mark K, Shetty J, Chinchankar N, Mcdougall C, Eunson P, et al. Outcome of paediatric acute flaccid myelitis associated with enterovirus D68: a case series. Dev Med Child Neurol. 2018 Nov;61(3):376–80.

25. Martin JA, Messacar K, Yang ML, Maloney JA, Lindwall J, Carry T, et al. Outcomes of Colorado children with acute flaccid myelitis at 1 year. Neurology. 2017 Jul;89(2):129–37.

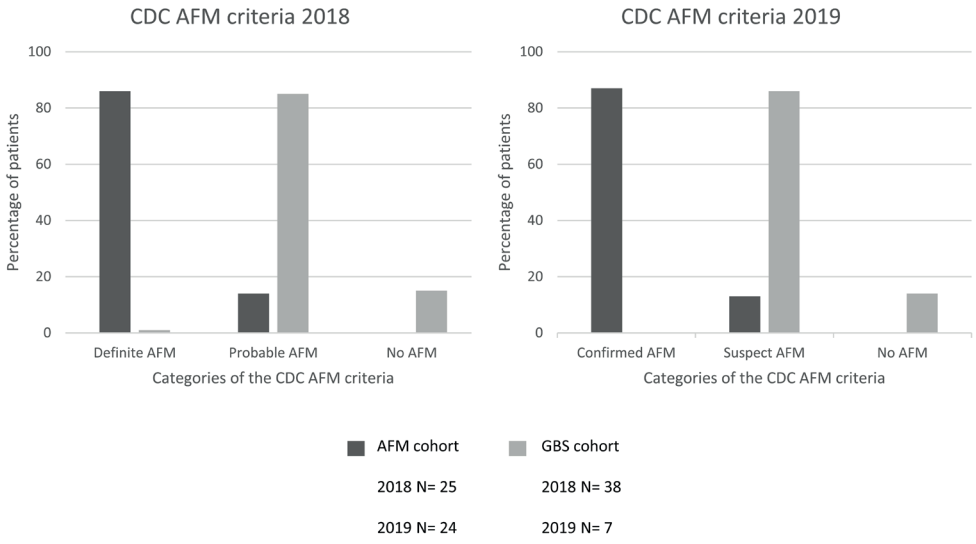
26. Hughes RAC, Swan A V, van Doorn PA. Intravenous immunoglobulin for Guillain-Barré syndrome. Cochrane database Syst Rev [Internet]. 2014 Sep 19;2014(9):CD002063–CD002063. Available from: https://pubmed.ncbi.nlm.nih.gov/25238327

27. Hixon AM, Clarke P, Tyler KL. Evaluating Treatment Efficacy in a Mouse Model of Enterovirus D68-Associated Paralytic Myelitis. J Infect Dis. 2017 Dec;216(10):1245–53.

28. Hopkins SE, Elrick MJ, Messacar K. Acute Flaccid Myelitis-Keys to Diagnosis, Questions About Treatment, and Future Directions. JAMA Pediatr. 2018 Nov;Published.

29. Messacar K, Spence-Davizon E, Osborne C, Press C, Schreiner TL, Martin J, et al. Clinical characteristics of enterovirus A71 neurological disease during an outbreak in children in Colorado, USA, in 2018: an observational cohort study. Lancet Infect Dis. 2020 Feb;20(2):230–9.

SUPPLEMENTARY FILES



Supplementary Figure 1: CDC AFM Criteria applied in both cohorts

Case definition CDC 2018:

Definite AFM: Acute flaccid limb weakness and MRI abnormalities in the spinal cord of the gray matter.

Probable AFM: Acute flaccid limb weakness and CSF pleocytosis.

Case definition CDC 2019:

Confirmed AFM: Acute flaccid weakness and MRI abnormalities predominantly in the gray matter, spanning one or more segments, with exclusion of malignancy, vascular disease or anatomic abnormalities as an explanation for the spinal cord lesion.

Suspect AFM: Any case of acute flaccid limb weakness.

Supplementary table 1: Brighton criteria in all patients.

Levels Brighton criteria all patients	**AFM cohort** (N=26)	**GBS cohort**[d] (N=66)
Level 1 (%)	**2 (8)**	**32 (48)**
AIDP, AMAN, AMSAN, unresponsive (%)	1 (4) (1)	25 (39)
Equivocal (%)	1 (4) (1)	7 (10%)
Level 2 (%)	**2 (8)**	**24 (36)**
Normal CSF protein level or normal NCS[a] (%)	-	8 (13)

Levels Brighton criteria all patients	**AFM cohort** (N=26)	**GBS cohort[d]** (N=66)
Normal CSF protein level and normal NCS[a] (%)	-	3 (5)
NCS or lumbar puncture not performed[a] (%)	1 (4)	6 (9)
Normal CSF protein level and no NCS performed[a] (%)	1 (4)	1 (2)
CSF or NCS missing[a] (%)	-	3 (5)
NCS and CSF missing [a] (%)	-	2 (3)
Level 3 (%)	**12 (46)**	**4 (6)**
NCS and lumbar puncture not performed[b] (%)	2 (8)	2 (3)
CSF cell count >50[b] (%)	8 (31)	1 (2)
Normal NCS and no lumbar puncture performed[b] (%)	-	1 (2)
NCS not performed, information CSF missing[b] (%)	2 (8)	-
Level 4 (%)	**10 (39)**	**6 (9)**
Information criteria missing[c] (%)	4 (15)	3 (5)
No monophasic disease[c] (%)	-	2 (3)
Course of disease unknown (%)	3 (12)	1 (2)
No bilateral weakness (%)	3 (12)	

To fulfill Level 1 to following criteria must be present:
-Presence of bilateral and flaccid weakness of limbs
-decreased or absent deep tendon reflexes in weak limbs
-a monophasic disease course and time between onset-nadir between 12 hours and 28 days
-CSF cell count < 50 /μl, and CSF protein concentration > normal value.
-NCS findings consistent with one of the subtypes of GBS (NCSs were interpreted as normal, equivocal or consistent with GBS, further subdivided in acute motor axonal neuropathy (AMAN), acute motor-sensory axonal neuropathy (AMSAN), acute inflammatory demyelinating polyneuropathy (AIDP), and unresponsive.
-no other alternative diagnosis.
Level 2: All items of level 1 except the CSF findings are not required.
Level 3: All items of level 2 except NCS findings are not required.
Level 4: No alternative diagnosis must be present, all other criteria are not required.
[a] Reasons why patients did not reach level 1
[b] Reasons why patients did not reach level 2
[c] Reasons why patients did not reach level 3
In level 3, in the group of patients with a CSF cell count >50: in 3 patients the NCS was not performed and 1 patient had a normal NCS.
[d] The validation of the Brighton criteria was performed in a previously published article[9] and was performed in 66 children with GBS.

Supplementary table 2: AFM criteria in both the AFM and GBS cohort.

	AFM cohort	GBS cohort
AFM CDC criteria 2018		
Definite AFM (%)	21/25 (84)[a]	0/38 (0)[b]
Probable AFM (%)	4/25 (16)[a]	32/38 (87)[b]
No MRI performed [c] (%)	1 (4)	32 (87)
Normal MRI spine [c] (%)	3 (12)	-
No AFM (%)	0/25 (0)[a]	6/38 (15)[b]
Normal MRI and no pleiocytosis [d](%)	-	5 (13)
No acute flaccid limb weakness [d](%)	-	1 (2)
AFM CDC criteria 2019		
Confirmed AFM (%)	21/24 (88)[e]	0/7 (0)
Suspect (%)	3/24 (13)[e]	6/7 (86)
Normal MRI[a](%)	3 (13)	6 (86)[f]
No AFM (%)	0/24 (0)[e]	1/7 (14)
No acute flaccid limb weakness and no MRI spine performed[b](%)	-	1 (14)

[a]1 patient missing because MRI and lumbar puncture were not performed
[b]In 98 patients no MRI was performed. In 51 patients the information on the MRI was missing.
[c] Reasons why patients did not reach Definite/confirmed AFM
[d] Reasons why patients did not reach Probable/suspect AFM
[e]2 patients missing. In one patient both MRI and lumbar puncture were not performed. In one patient no MRI was performed.

[f]In 149 patients no MRI was performed or the information was missing

6

COMPARISON OF ACUTE FLACCID MYELITIS AND TRANSVERSE MYELITIS IN CHILDREN AND EVALUATION OF DIAGNOSTIC CRITERIA

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European Journal of Neurology 2023 Aug;30(8):2430-2441. doi: 10.1111/ene.15861

ABSTRACT

Background

Acute flaccid myelitis (AFM) and transverse myelitis (TM) are serious conditions that may be difficult to differentiate, especially at onset of disease. In this study we compared clinical features of pediatric AFM and TM and evaluated current diagnostic criteria, aiming to improve early and accurate diagnosis.

Methods

Two cohorts of children with enterovirus D68-associated AFM and clinically diagnosed TM were compared regarding presenting clinical features, additional investigations and outcome. Current diagnostic criteria for AFM and TM were applied to evaluate their specificity.

Results

Children with AFM (n=21) compared to those with TM (n=36) were younger (median 3 vs. 10 years), more often had a prodromal illness (100% vs. 39%), predominant proximal weakness (69% vs. 17%) and hyporeflexia (100% vs. 44%), and less often had sensory deficits (0% vs. 81%), bowel and/or bladder dysfunction (12% vs. 69%) and hyperreflexia (0% vs. 44%). On MRI, brainstem involvement was more common in AFM (74% vs. 21%), while supratentorial abnormalities were only seen in TM (0% vs. 40%).

When omitting the criterion of a sensory level, 11/15 (73%) children with AFM fulfilled the diagnostic criteria for TM. Of children with TM, 4/33 (12%) fulfilled the diagnostic criteria for probable/definite AFM.

Conclusions

While there is considerable overlap between AFM and TM in children, we found important early differentiating clinical and diagnostic features. Meeting diagnostic criteria for AFM in children with TM and vice versa, underlines the importance of thorough clinical examination and early and accurate diagnostic studies.

INTRODUCTION

Both acute flaccid myelitis (AFM) and transverse myelitis (TM) are rare conditions, but with significant impact on individual patients. AFM is a polio-like disease characterized by acute flaccid limb weakness of presumed anterior horn origin, most commonly occurring in childhood.1,2 The pathophysiology is not completely clarified, but an association with specific viruses, in particular enterovirus D68 (EV-D68) and A71 (EV-A71) has been made.3–5 According to the diagnostic criteria for AFM, as proposed by the international AFM Working Group in 2021, a definite diagnosis can be made based on the combination of acute flaccid paralysis (AFP), spinal cord grey matter abnormalities on MRI and pleocytosis in cerebrospinal fluid (CSF), in the absence of factors suggesting an alternative diagnosis.1

TM is an immune-mediated condition of the spinal cord. The diagnosis is currently based on Transverse Myelitis Consortium Working Group (TMCWG) criteria.6 These include (1) the presence of sensory, motor, and/or autonomic dysfunction attributable to the spinal cord, (2) a clearly defined sensory level, and (3) signs of inflammation of the spinal cord, indicated by CSF pleocytosis, an elevated IgG index or gadolinium enhancement of the spinal cord on MRI. The presence of a sensory level is, however, often omitted in children.6–8 Evidence of an associated systemic or infectious disease, as well as the presence of optic neuritis or brain MRI abnormalities suggestive of multiple sclerosis (MS) would exclude the diagnosis of idiopathic TM, but may lead to a diagnosis of disease-associated TM according to the TMCWG criteria.

While early differentiation between AFM and TM is important, as there are significant differences with respect to treatment options and prognosis, this can be challenging in particular at onset of disease.6,8,9 This is especially true in children, for example because of the difficulty to assess sensory deficits on neurologic examination.9

In this study, we aimed to find early differentiating clinical and diagnostic features between AFM and TM in children, by comparing two well described cohorts of children with these conditions. Furthermore, we aimed to evaluate the specificity of diagnostic criteria for AFM and TM in these cohorts.

METHODS

Study cohorts

The AFM cohort comprised 21 pediatric cases (<18 years) from a previously described retrospectively collected cohort of 29 patients from Europe (adults and children) with AFM associated with EV-D68, diagnosed in 2015 or 2016.2 Part of this cohort was previously used for a comparison study between AFM and GBS.10 The diagnosis of AFM had been based on the presence of acute onset limb weakness, MRI abnormalities of the spinal cord and/or CSF pleocytosis, fulfilling the Centers of Disease Control and Prevention (CDC) case definition from 2018 for probable or definite AFM.11 Furthermore EV-D68 had to be detected by polymerase chain reaction in a sample of any origin.

The TM cohort consisted of 36 children (<18 years) with a clinical diagnosis of TM, selected from acquired demyelinating syndrome (ADS) patients included in the Dutch nationwide prospective multicenter PROUD-kids study (Predicting the Outcome of a Demyelinating event in childhood).12 Part of these patients with TM were described previously.13 Cases in which a diagnosis of AFM was considered by the treating physician were excluded. In line with the TMCWG criteria, children with TM in association with acute disseminated encephalomyelitis (ADEM), as well as those with optic neuritis or supratentorial MRI abnormalities suggestive of MS at onset of disease were excluded for the current study. Generally, these cases can easily be differentiated from idiopathic TM and they follow a different clinical course.13,14

Comparison studies

Both cohorts were compared with respect to demographic features, prodromal features, clinical characteristics at first presentation and time course. Furthermore, a comparison of MRI features in spinal cord and brain, CSF abnormalities and microbiology test results at onset of disease was made. Treatment type and outcome measures were compared. Outcome was reported in terms of ability to walk independently and recovery at final follow-up (full, partial or no recovery). Results of electrophysiologic studies for the AFM patients and serum aquaporin-4 (AQP4) and myelin oligodendrocyte protein (MOG) antibody results tested by cell-based assay for the TM patients were reported if available.

Diagnostic criteria

The TMCWG criteria (supplementary table 1) were applied to both cohorts. In the AFM group, the exclusion criterion of enterovirus associated pathology of the nervous system was discarded.6 Both the presence of a sensory level and criteria for proven inflammation are often omitted in children, as a sensory level may be difficult to assess and (repeated) CSF examinations are limitedly tolerated in children.7,8 Therefore, both the whole set of criteria was applied, as well as the criteria without a sensory level and/or without proven inflammation.

The AFM criteria (supplementary table 2), as proposed by the international AFM working group in 2021, were applied to both cohorts.15 The criterion of decreased muscle tone in at least one of the weak limbs was not included, as this had not been recorded in both cohorts. Both (1) diagnostic features of AFM and (2) factors suggestive for an alternative diagnosis were applied.

MRI examinations were reassessed if images were available; alternatively, the report of the radiologist was used. Terms such as 'mostly affecting the grey matter', 'presence of a central cord lesion' or 'anterior myelitis' were deemed consistent with gray matter involvement on MRI.

Statistics

SPSS 23 was used for statistical analysis. Continuous data were presented as means and standard deviations if normally distributed, and otherwise as medians and interquartile ranges (IQR). Categorical data were presented as proportions. Continuous data of the two cohorts were compared with t-test if normally distributed and with Mann-Whitney U test if not normally distributed. Proportions were compared using the Chi-square or Fisher exact test. The Bonferroni correction was applied to correct for multiple comparisons. A two-sided p-value <0.05 was considered significant.

Standard Protocol Approvals, Registrations, and Patient Consents

Studies from which data was used, were approved by the medical ethical review committee of the coordinating centers.

Data Availability

The authors confirm that the data supporting the findings of this study are available within the article, within the limits of the GDPR privacy regulations.

RESULTS

Demographic characteristics

The AFM cohort consisted of 21 children with AFM associated with EV-D68 and the TM cohort comprised 36 children with a clinical diagnosis of TM. Median age at onset of disease was three years (IQR 2-5, full range 8) for the AFM group versus ten years (IQR 5.5-15.6, full range 17) for the children with TM ($p<0.001$). (Table 1)

Table 1: Demography and clinical presentation of AFM and TM in children

	AFM (N=21)	**TM** (N=36)	p-value
Demography			
Male: female (% M)	11:10 (52)	16:20 (44)	NS
Age, median (IQR, full range), years	3 (2-5,1-9)	10 (6-16, 1-18)	<0.001
Prodromal illness			
Prodrome (%)	21/21 (100)	14/36 (39)	<0.001
Time prodrome-onset weakness, median (IQR, full range), days[a]	6 (4-7,2-12)	12 (5-16, 1-18)	NS
Respiratory symptoms(%)	20/21 (95)	7/36 (19)	<0.001
Gastrointestinal symptoms (%)	7/21 (33)	4/36 (11)	NS
Fever (%)	18/19 (95)	7/35 (20)	<0.001
Time onset weakness-nadir, median (IQR, full range), days[b]	3 (2-5, 1-10)	4 (2-5, 1-20)	NS
Neurological symptoms			
Limb weakness (%)	20/20 (100)	32/36 (89)	NS
Weakness arms (%)	18/20 (90)	12/36 (33)	<0.001
Weakness legs (%)	17/20 (85)	31/36 (86)	NS
Weakness arms only (%)	3/20 (15)	1/36 (3)	NS
Weakness legs only (%)	2/20 (10)	20/36 (56)	<0.001
Weakness in arms and legs (%)	15/20 (75)	11/36 (31)	0.002
Proximal>distal (%)	11/16 (69)	5/29 (17)	<0.001
Distal>proximal (%)	0/16 (0)	10/29 (34)	0.006
Asymmetric weakness (%)	11/18 (61)	17/34 (50)	NS
Bilateral symptoms (%)	17/20 (85)	34/35 (97)	NS
Sensory deficits (%)	0/19 (0)	29/36 (81)	<0.001
Sensory level (%)	0/19 (0)	15/30 (50)	<0.001
Areflexia/hyporeflexia at nadir (%)	20/20 (100)	16/36 (44)	<0.001
Hyperreflexia in affected limbs at nadir (%)	0/20 (0)	16/36 (44)	<0.001
Cranial nerve involvement (%)	10/20 (50)	5/36 (14)	0.005
Autonomic dysfunction (%)	4/20 (20)	26/36 (72)	<0.001
Bladder and/or bowel dysfunction (%)	2/17 (12)	24/35 (69)	<0.001
Pain (%)	6/19 (32)	19/36 (53)	NS
Encephalopathy (%)	2/19 (11)	1/36 (3)	NS
Mechanical ventilation (%)	13/21 (62)	1/29 (3)	<0.001
Follow-up			
Follow-up duration, median (IQR, full range), months	5 (2-12, 1-48)	30 (15-55, 3-123)	<0.001

	AFM (N=21)	**TM** (N=36)	p-value
Complete recovery (%)	1/18 (5)	7/36 (19)	NS
Walking independently (%)	9/21 (42)	27/28 (96)	<0.001

Data are presented as number (percentage) or median (IQR, full range). A p-value of > 0.05 was noted as non-significant (NS). Most children with AFM presented between July and October of 2016 (16/21, 76%) while the month of presentation was more evenly distributed over the year in the TM group, without the difference in seasonality reaching statistical significance.
aMedian time between prodromal illness and onset of weakness based on 18 AFM patients and 36 TM patients.
bMedian time between onset of weakness and nadir based on 17 AFM patients and 36 TM patients.

Most children with AFM presented between July and October of 2016 (16/21, 76%) while the month of presentation was more evenly distributed over the year in the TM group (no statistically significant difference). Of 13 TM-patients diagnosed after July 2016, when the first AFM case in the Netherlands was reported, three were diagnosed in a period of increased EV-D68-circulation. These patients had significant sensory deficits and recovered completely at final follow-up, which argues against a diagnosis of AFM. Also, all TM-patients were diagnosed in a tertiary center with expertise in the field of inflammatory conditions of the CNS, and most children in the TM cohort had a considerably long follow-up period, so we do assume that the diagnosis was made correctly.

Clinical presentation and course

A prodromal illness was more commonly seen in children with AFM, compared to children with TM (100% vs. 39%, p<0.001), and most often consisted of respiratory symptoms and/or fever in AFM patients. (Table 1)

Limb weakness was commonly asymmetric in both AFM and TM (61% and 50%). Weakness in the legs was equally observed in both groups, while the arms were more often affected in AFM (90% vs. 33%, p<0.001). Furthermore, a different pattern of weakness was observed in which the legs were less often solitarily involved in AFM (10% vs. 56%, p<0.001), whereas both arms and legs were more often involved in AFM (75% vs. 44%, p=0.002). Proximal weakness was often more prominent in AFM (69% vs. 17%, p=0.002), while predominant distal weakness was only seen in TM (0% vs. 34%, p=0.005). Sensory deficits were only observed in children with TM (0% vs. 81%, p<0.001) and a sensory level was observed in 15/30 TM patients in whom details regarding a sensory level were recorded.

Hyporeflexia at nadir was present in all patients with AFM and in almost half of TM patients (100% vs. 44%, p<0.001); hyperreflexia was not found in any patient with AFM, but in 44% of children with TM.

Autonomic dysfunction, most often presenting as bowel and/or bladder dysfunction, was noted in 20% of AFM patients and in 72% of TM patients (p<0.001). Cranial nerve dysfunction on the other hand was more commonly seen in AFM patients (50% vs. 14%, p=0.005).(Table 1)

Time course, reflected in the time period between prodromal symptoms and onset of weakness (median 6 vs. 12 days), and the time from onset of weakness to nadir (median 3 vs. 4 days), did not significantly differ between both groups.(Table 1) More children with AFM were treated with intravenous immunoglobulin (88% vs. 38%, p<0.001), whereas less were treated with steroids (61% vs. 89%, p=0.02). In both groups one patient died because of respiratory failure. Relapses occurred in none of the AFM patients, but in four patients in the TM group. Most patients had residual deficits at final follow-up in both groups (95% and 81%), but the proportion of children walking independently was smaller in the AFM group (42% vs. 96%, p<0.001). Follow-up duration was however also significantly longer in the TM group (median 30 months, IQR 3-123 vs. median 5 months, IQR 2-12).

Additional diagnostic tests

Spinal cord MRI showed spinal abnormalities in 88% of AFM and 97% of TM patients. These lesions affected the spinal cord grey matter in all ten AFM patients for which this data was obtained, but also in 23 of 29 of TM patients (79%) for which MRI assessment was possible. (Table 2)

Table 2: Additional diagnostic test results in children with AFM and TM

	AFM (N=21)	**TM** (N=36)	p-value
MRI			
Time onset weakness-MRI spinal cord, median (IQR, full range), days[a]	2 (2-2.3, 1-6)	4 (3-6, 1-50)	NS
Spinal cord lesion (%)	15/17 (88)	35/36 (97)	NS
Predominant gray matter involvement (%)	10/10 (100)	23/29 (79)	NS
Cervical involvement (%)	14/16 (88)	24/34 (71)	NS

	AFM (N=21)	**TM** (N=36)	p-value
Thoracic involvement (%)	8/16 (50)	25/34 (74)	NS
Lumbar involvement (%)	6/16 (38)	11/34 (32)	NS
Longitudinally extensive lesion (%)	14/16 (88)	26/33 (79)	NS
Spinal cord enhancement (%)	2/6 (33)	7/24 (29)	NS
Nerve root enhancement (%)	4/16 (25)	NR	np
Brainstem abnormalities (%)	14/19 (74)	6/29 (21)	<0.001
Supratentorial abnormalities (%)	0/19 (0)	13/32 (40)	<0.001
CSF			
LP performed (%)	18/21 (86)	34/36 (94)	NS
Time onset weakness-LP, median (IQR, full range), days[b]	1 (1-2, 0-4)	4 (3-7, 1-27)	<0.001
Leucocyte number in CSF, median (IQR, full range)[c]	81 (25-141, 3-175)	13 (3-43,0-239)	NS
CSF pleocytosis (%)	14/15 (93)	19/34 (56)	NS
Protein concentration in CSF, median (IQR, full range), (g/l)[d]	0.43 (0.31-0.58, 0.21-1.6)	0.29 (0.20-0.62, 0.17-1.17)	NS
Raised protein level (%)[e]	7/13 (54)	14/32 (44)	NS
IgG index abnormal (%)	NR	12/23 (52)	np
CSF oligoclonal bands (%)	NR	3/19 (16)	np
Antibodies			
MOG antibodies (%)	NR	3/23 (13)	np
AQP4 antibodies (%)	NR	1/26 (4)	np
Virology abnormalities			
CSF (%)	0/15 (0)	0/28 (0)	NS
Respiratory material (%)	18/19 (95)	1/6 (17)	np
Feces (%)	6/17 (35)	2/5 (40)	np

Data are presented as number (percentage) or median (IQR, full range). A p-value of > 0.05 was noted as non-significant (NS). Not all comparisons were performed, because of limited numbers; this is mentioned as np. Some items were not recorded in both patient groups, which is noted as NR. MOG=myelin oligodendrocyte glycoprotein; AQP4= aquaporin-4; MRI: magnetic resonance imaging CSF: cerebrospinal fluid; LP: lumbar puncture, IgG: Immunogloblin G.
aThe median time between onset of weakness and MRI was available in 15 patients from the AFM cohort and in 36 patients from the TM cohort.
bThe median time between onset of weakness and lumbar puncture was available in 15 patients from the AFM cohort and in 33 patients from the TM cohort.
cThe information on the number of leucocytes in CSF was available in 14 AFM patients and 33 TM patients.
dThe information on protein concentration in CSF was available in 12 AFM patients and 33 TM patients.
eRaised protein was defined as a protein level > 0.65 g/L for the age 1-3 months, >0.37 g/L for 3-6 months, >0.35 g/L for 6-12 months, >0.31 g/L for 1- 10 years and >0.49 g/L for 10-18 years.

Enhancement of the spinal cord was seen in a similar percentage in AFM and TM patients (33% vs. 29%). Nerve root enhancement was only recorded in AFM patients and was seen in 24% of these patients. Longitudinally extensive lesions were often reported in both groups (88% vs. 79%).

In AFM patients, MRI of the brain more often showed brain stem abnormalities (74% vs. 21%, $p<0.001$). Non-specific supratentorial abnormalities were not seen in AFM patients and in 13 of 32 TM patients (0% vs. 40% $p<0.001$). Of these 13, two had MOG antibodies, one AQP4 antibodies and one was diagnosed MS during follow-up, while nine had an idiopathic TM.

Presence of such serum MOG and APQ4 antibodies was only recorded in TM patients. Of all tested patients, one patient had AQP4 antibodies (4%) and three patients showed positivity for MOG antibodies (13%). (Table 2)

CSF investigations were performed in most AFM and TM patients (86% vs. 94%), but lumbar puncture was performed earlier after onset of weakness in AFM (1 vs. 4 days, $p<0.001$). (Table 2) Numbers of patients with CSF pleocytosis were not significantly different between AFM and TM patients (93% vs. 56%).

Electromyography was performed in nine AFM patients and revealed abnormalities suggestive for axonal damage in eight. Virology testing was commonly performed in CSF in TM patients, while respiratory (16%) and fecal (14%) samples were tested in a limited number of patients.

Evaluation of clinical criteria

The TMCWG criteria were applied to both cohorts. (Figure 1A, Supplementary Table 1) None of the AFM patients fulfilled the complete set of TMCWG criteria for TM, because of the absence of a sensory level. When omitting the criterion of the presence of a clearly defined sensory level, 73% (11/15) of AFM patients in whom sufficient information was available fulfilled the criteria. If inflammation of the spinal cord was also not required, 83% (15/18) patients fulfilled the criteria; in the remaining three patients, bilateral signs or symptoms attributable to spinal cord dysfunction, which is included in the TMWCG criteria, were not present.

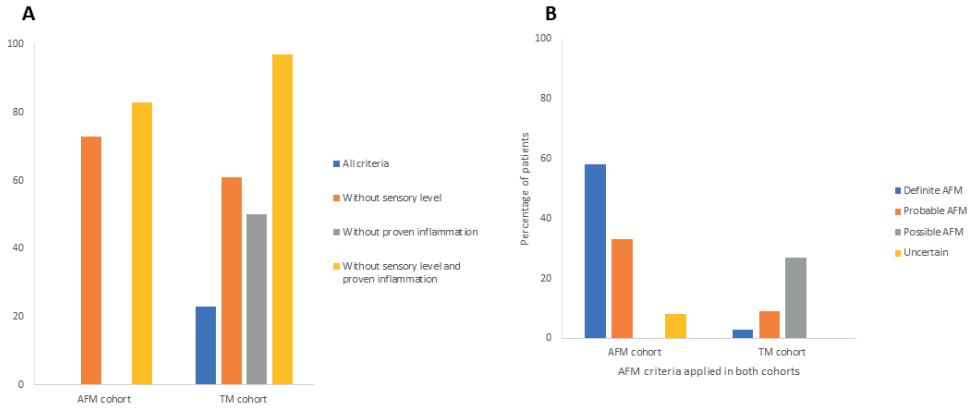

Figure 1

a. Percentage of patients fulfilling the complete set of TMCWG criteria for TM, and the criteria without the presence of a sensory border and/or proven inflammation, in both cohorts for patients from whom sufficient information was available. AFM: acute flaccid myelitis; TM: transverse myelitis.

b. Percentage of patients fulfilling the AFM criteria in both cohorts for the patients from whom sufficient information was available for classification. Nineteen patients with TM had clear signs suggestive of an alternative diagnosis (not shown). AFM: acute flaccid myelitis; TM: transverse myelitis.

Of 30 children with TM with sufficient information available, seven (23%) fulfilled all TCWMG criteria. In 15 of the remaining 23 patients not fulfilling all criteria, no sensory level was found. In the other 8 patients, no inflammation was demonstrated. When omitting the criterion of a sensory level, 61% (22/36) of TM patients fulfilled the criteria. In 13 of 14 patients not fulfilling the criteria, no inflammation was shown, while one patient did not have bilateral signs. (Supplementary table 1). When omitting the criterion of proven inflammation, 50% (15/30) of TM patients fulfilled the criteria. The 15 patients not fulfilling the criteria did not have a sensory level. When omitting both the presence of a sensory level and proven inflammation, only one TM patient without bilateral signs did not fulfill the criteria.

The AFM criteria, as proposed by the international AFM working group were applied to both cohorts. (Figure 1B, Supplementary Table 2) Of the 33 patients with TM with sufficient

information available, one (3%) fulfilled the criteria for definite, three (9%) for probable and nine (27%) for possible AFM. (Figure 1B)

Nineteen TM patients with a sensory level, with supratentorial MRI abnormalities and/or with MOG or AQP4 antibodies were excluded as these factors are noted to be suggestive for an alternative diagnosis in the AFM criteria.15 One patient with a time interval from onset to nadir of 20 days was also not included in the classification. (Supplementary Table 2). Of the remaining 12 AFM patients which could be classified, seven (58%) fulfilled the criteria for definite and four (33%) for probable AFM. One patient (8%) was classified as uncertain, because no MRI was performed. (Figure 1B)

DISCUSSION

This comparative study in two well-characterized pediatric cohorts of AFM and TM reveals both similarities and differentiating features between both disorders.

Both AFM and TM commonly present with acute onset limb weakness, which may be asymmetric, accompanied by pain and preceded by a prodromal illness. At onset of disease, hyporeflexia may be present in both conditions, including AFM and TM in the differential diagnosis of AFP. In both groups of patients, MRI of the spinal cord often reveals a longitudinally extensive lesion with significant grey matter involvement and CSF often shows pleocytosis. (Figure 2) The similarities between both conditions are further underlined by the fulfillment of many of the diagnostic criteria for TM by AFM patients.

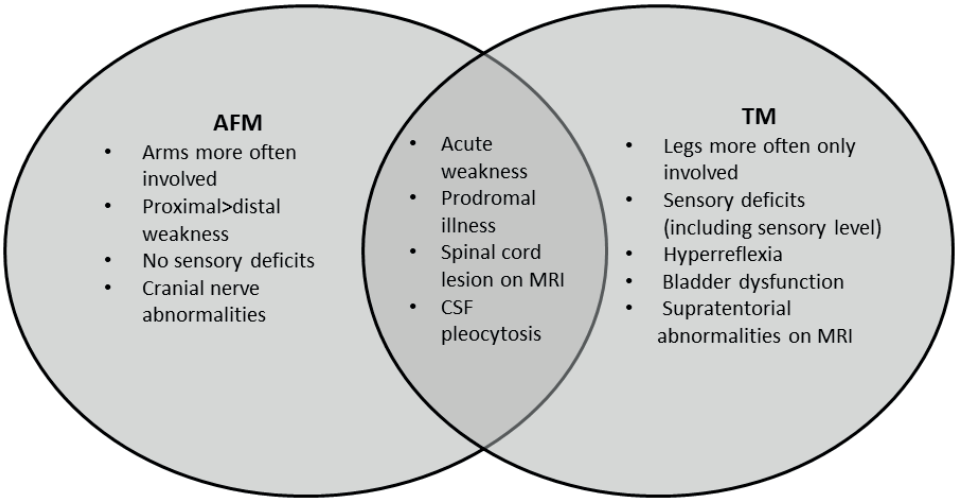

Figure 2: Venn diagram illustrating differentiating and overlapping features between AFM and TM in children.

Features mentioned under both conditions are suggestive for either diagnosis, but must be used in a clinical context, as they are neither exclusive nor always present. AFM: acute flaccid myelitis; TM: transverse myelitis; CSF: cerebrospinal fluid; MRI: magnetic resonance imaging.

Besides described similarities, this study showed that upper extremity weakness is typical for AFM, as are predominance of proximal weakness, cranial nerve involvement and brainstem abnormalities on MRI. On the other hand, the presence of a sensory level, bladder- and/or bowel dysfunction, as well as hyperreflexia, probably explained by more diffuse spinal cord involvement, are more typical for TM. Furthermore, supratentorial abnormalities on MRI were only seen in TM.

The clinical characteristics and findings on ancillary investigations described in this study are similar to previously described cohorts of AFM and pediatric TM, supporting the representativeness of our findings.16–20 Some studies have investigated the differences between AFM and TM. One prospective study, aiming to find differences in treatment outcome in pediatric myelitis patients, compared children with AFM and TM within their cohort.21 Similar to our study, weakness more commonly affected upper extremities and CSF leukocyte numbers were higher in the AFM group. Sensory deficits were noted in 24% of AFM patients, which is higher compared to our study and previous studies.19,21 More

detailed clinical information was not presented, impairing further comparison. Furthermore, diagnosis of AFM was based on the presence of flaccid weakness and grey matter abnormalities on MRI, regardless of enterovirus status, possibly leading to more heterogeneity within the AFM cohort.21 A comparison study between children with restrictively defined AFM and children fulfilling the AFM criteria of the CDC, but with a possible alternative diagnosis, including four with TM showed some findings similar to our study. This includes the differences in reflex pattern and sensory abnormalities.22

Differentiation between AFM and TM is important for several reasons. First, there are therapeutic consequences, since the first-line treatment in children with TM consists of high dose corticosteroids, while there are indications that steroid treatment may worsen outcome in AFM.8,23 Second, while residual deficits are common in both conditions, the motor outcome in TM is usually better than in AFM, which is also indicated by the number of children with TM that are ambulant at final follow-up in this study.8,24 An adequate diagnosis will help in counselling patients and their parents on the expected disease course. Third, especially for AFM, it is important to be aware of new clusters of cases to be able to relate these with outbreaks of associated enteroviruses for epidemiological purposes.25

Children with TM may fulfill the clinical criteria for definite AFM. This underlines the need for thorough investigation, both clinically and with further diagnostic procedures, to search for factors suggestive of TM in a suspected AFM case.9 These include the presence of sensory deficits and supratentorial abnormalities, mentioned in the diagnostic criteria and confirmed as differentiating factors in our study.1 Evaluation of MRI abnormalities may also provide valuable information, with the caveat that MRI abnormalities may be subtle or absent in AFM, especially at onset of disease. Finally, we believe that timely and adequate virologic tests are important, as the finding of associated enteroviruses will strongly support the diagnosis of AFM.

The TMCWG criteria were developed in 2002 to create a set of uniform diagnostic criteria for TM.6 These criteria were introduced before several important developments, in particular the discovery of AQP4 and MOG antibodies and a new classification strategy has been proposed.26 The TMCWG criteria are currently still in use and are believed to be applicable to children with suspected TM.8 Importantly, the presence of a sensory level as

a criterion, which would differentiate TM from AFM, is often omitted in childhood studies.7,8 The identification of a specific virus such as EV-D68 in suspected AFM cases would also lead to exclusion of idiopathic TM based on the criteria. However, as in many AFM cases no associated virus is isolated, possibly due to incomplete diagnostic testing or incongruence between the viral infection and onset of weakness, AFM cases may often fulfill the criteria for idiopathic TM. Conversely, the early identification of AQP4 or MOG antibodies would argue against an AFM diagnosis. Therefore, in children suspected of TM, a thorough clinical examination and complete diagnostic workup is important.

Our study has several limitations. First, the inclusion of patients was largely based on current diagnostic criteria, which may lead to circular reasoning. However, we do believe that this does not hinder the finding of differentiating features, which was the main purpose of this study. Second, sensory deficits are difficult to ascertain in young children. While we do believe that these are less common in AFM patients, the younger age of the AFM cohort may have led to underreporting of sensory symptoms and overestimation of the differences found.

Third, the selection of children with EV-D68 impedes generalizability of the distinguishing features for AFM in association with other viruses, such as EV-A71.5 This does however improve the homogeneity of this group and provides more certainty of the diagnosis.

Fourth, the retrospective questionnaire-based nature by which cases were identified in the AFM cohort and the selection of EV-D68 positive cases may both have led to a bias towards more severe cases. The similarity between the presented cohort and previous cohorts, however, supports the representativeness of the found differentiating features.

Fifth, certain tests may only be performed in selected patients in both cohorts, which may lead to confounding bias, inherent to the retrospective nature of this study. Finally, detailed information on the MRI results was not available from all children with AFM, so imaging characteristics could not be fully compared. This remains a topic for further research.

Patients were excluded from the TM cohort if, at onset of disease, signs of a disease-associated TM were present. As AQP4 and MOG antibody testing usually takes several weeks, and our aim was to find differentiating features at onset of TM presentation, we did not exclude the patients with a subsequent positive result for AQP4 or MOG antibodies, or the patients with MRI lesions suggestive of MS during follow-up. Therefore,

some patients of the TM cohort were finally diagnosed with MS (n=1), AQP4-positive neuromyelitis optica spectrum disorder (n=1) and MOG-antibody-associated disorder (n=3). Importantly, the differentiating features identified in this study and recommendations made in the discussion need validation in a prospective cohort.

In conclusion, we provide early distinguishing features between AFM and TM in childhood. Both disorders may, however present similarly and fulfill clinical criteria of the other condition. Therefore, a careful clinical evaluation with timely and adequate diagnostic tests is important to help differentiate between AFM and TM and guide decisions on treatment.

Conflicts of interest/disclosures

J.Helfferich, A.L. Bruijstens, M.Knoester and O.F. Brouwer have no conflicts of interest relevant to this study and no disclosures to report. Rinze F. Neuteboom participates in trials by Sanofi and Novartis, and received honorarium from Novartis and Zogenix.

Study funding

The Dutch MS Research Foundation funded the PROUD-kids study from which data was used in this study. This study was not industry sponsored.

Data access and responsibility: J. Helfferich had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

REFERENCES

1. Murphy OC, Messacar K, Benson L, Bove R, Carpenter JL, Crawford T, et al. Acute flaccid myelitis: cause, diagnosis, and management. Lancet [Internet]. 2020 Dec 28; Available from: https://doi.org/10.1016/S0140-6736(20)32723-9
2. Knoester M, Helfferich J, Poelman R, Leer-Buter C Van, Brouwer OF, Niesters HGM, et al. Twenty-nine Cases of Enterovirus-D68-associated Acute Flaccid Myelitis in Europe 2016: A Case Series and Epidemiologic Overview. Pediatr Infect Dis J. 2019 Jan;38(1):16–21.
3. Messacar K, Asturias EJ, Hixon AM, Leer-Buter C Van, Niesters HGM, Tyler KL, et al. Enterovirus D68 and acute flaccid myelitis-evaluating the evidence for causality. LancetInfectious Dis. 2018 Feb;
4. Dyda A, Stelzer-Braid S, Adam D, Chughtai AA, MacIntyre CR. The association between acute flaccid myelitis (AFM) and Enterovirus D68 (EV-D68) - what is the evidence for causation? Euro Surveill. 2018 Jan;23(3):10.2807/1560-7917.ES.2018.23.3.17-00310.
5. Messacar K, Spence-Davizon E, Osborne C, Press C, Schreiner TL, Martin J, et al. Clinical characteristics of enterovirus A71 neurological disease during an outbreak in children in Colorado, USA, in 2018: an observational cohort study. Lancet Infect Dis. 2020 Feb;20(2):230–9.
6. Transverse Myelitis Consortium Working Group. Proposed diagnostic criteria and nosology of acute transverse myelitis. Neurology. 2002;59(4):499–505.
7. Deiva K, Absoud M, Hemingway C, Hernandez Y, Hussson B, Maurey H, et al. Acute idiopathic transverse myelitis in children: Early predictors of relapse and disability. Neurology. 2015;84(4):341–9.
8. Absoud M, Greenberg BM, Lim M, Lotze T, Thomas T, Deiva K. Pediatric transverse myelitis. Neurology. 2016 Aug;87(9 Suppl 2):S46-52.
9. Theroux LM, Brenton JN. Acute Transverse and Flaccid Myelitis in Children. Curr Treat Options Neurol. 2019 Dec;21(12):64.
10. Helfferich J, Roodbol J, de Wit MC, Brouwer OF, Jacobs BC. Acute flaccid myelitis and Guillain-Barré syndrome in children: A comparative study with evaluation of diagnostic criteria. Eur J Neurol. 2022 Feb;29(2):593–604.
11. CDC. Revision to the Standardized Surveillance and Case Definition for Acute Flaccid Myelitis. 2017.
12. Ketelslegers IA, Catsman-Berrevoets CE, Neuteboom RF, Boon M, van Dijk KGJ, Eikelenboom MJ, et al. Incidence of acquired demyelinating syndromes of the CNS in Dutch children: a nationwide study. J Neurol. 2012 Sep;259(9):1929–35.
13. Helfferich J, Bruijstens AL, Wong YYM, van Pelt ED, Boon M, Neuteboom RF. Prognostic factors for relapse and outcome in pediatric acute transverse myelitis. Brain Dev. 2021;
14. Yiu EM, Kornberg AJ, Ryan MM, Coleman LT, Mackay MT. Acute transverse myelitis and acute disseminated encephalomyelitis in childhood: spectrum or separate entities? J Child Neurol. 2009;24(3):287–96.
15. Murphy OC, Messacar K, Benson L, Bove R, Carpenter JL, Crawford T, et al. Acute flaccid myelitis: cause, diagnosis, and management. Lancet (London, England). 2021 Jan;397(10271):334–46.
16. Meyer P, Leboucq N, Molinari N, Roubertie A, Carneiro M, Walther-Louvier U, et al. Partial acute transverse myelitis is a predictor of multiple sclerosis in children. Mult Scler J. 2014;20(11):1485–93.
17. Pidcock FS, Salorio CF, Trovato M, Krishnan C, Kerr DA, Crawford TO. Acute transverse myelitis in childhood: Center-based analysis of 47 cases. Neurology. 2007;68(18):1474–80.
18. De Goede CGEL, Holmes EM, Pike MG. Acquired transverse myelopathy in children in the United Kingdom - A 2 year prospective study. Eur J Paediatr Neurol [Internet]. 2010;14(6):479–87. Available from: http://dx.doi.org/10.1016/j.ejpn.2009.12.002

19. Messacar K, Schreiner TL, Haren K Van, Yang M, Glaser CA, Tyler KL, et al. Acute flaccid myelitis: A clinical review of US cases 2012-2015. Ann Neurol. 2016 Sep;80(3):326–38.

20. Chong PF, Kira R, Mori H, Okumura A, Torisu H, Yasumoto S, et al. 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. Clin Infect Dis. 2018 Feb;66(5):653–64.

21. Greenberg B, Plumb P, Cutter G, Dean J, Desena A, Hopkins S, et al. Acute flaccid myelitis: long-term outcomes recorded in the CAPTURE study compared with paediatric transverse myelitis. BMJ Neurol open. 2021;3(1):e000127.

22. Elrick MJ, Gordon-Lipkin E, Crawford TO, Van Haren K, Messacar K, Thornton N, et al. Clinical Subpopulations in a Sample of North American Children Diagnosed With Acute Flaccid Myelitis, 2012-2016. JAMA Pediatr [Internet]. 2019 Feb 1;173(2):134–9. Available from: https://doi.org/10.1001/jamapediatrics.2018.4890

23. Hixon AM, Clarke P, Tyler KL. Evaluating Treatment Efficacy in a Mouse Model of Enterovirus D68-Associated Paralytic Myelitis. J Infect Dis. 2017 Dec;216(10):1245–53.

24. Martin JA, Messacar K, Yang ML, Maloney JA, Lindwall J, Carry T, et al. Outcomes of Colorado children with acute flaccid myelitis at 1 year. Neurology. 2017 Jul;89(2):129–37.

25. Fischer TK, Simmonds P, Harvala H. The importance of enterovirus surveillance in a post-polio world. Lancet Infect Dis. 2022 Jan;22(1):e35–40.

26. Blackburn KM, Greenberg BM. Revisiting Transverse Myelitis: Moving Toward a New Nomenclature. Front Neurol. 2020;11:519468.

SUPPLEMENTARY FILES

Supplementary Table 1: TCMWG criteria applied in both cohorts.

	AFM cohort (N=21)	TM cohort (N=36)
Fulfilling all TCMWG criteria (%)	**0/19 (0)[1]**	**7/30 (23)[1]**
Reason(s) for not fulfilling criteria[2]	n=19	n=23
– No sensory level	– 19/19	– 15/23
– No proven inflammation	– 14/15	– 11/23
– No bilateral signs	– 3/19	– 1/22
TMCWG criteria without sensory level (%)	**11/15 (73)[3]**	**22/36 (61)**
Reason(s) for not fulfilling criteria[2]	n=4	n=14
– No proven inflammation	– 1/4	– 13/14[4]
– No bilateral signs	– 3/4	– 1/14
TMCWG criteria without proven inflammation (%)	**0/19 (0)**	**15/30 (50)**
Reason(s) for not fulfilling criteria[2]	n=19	n=15
– No sensory level	– 19/19	– 15/15
– No bilateral signs	– 3/19	– 0/15
TMCWG criteria without a sensory border and without proven inflammation (%)	**15/18 (83)**	**35/36 (97)**
Reason(s) for not fulfilling criteria[2]	n=3	n=1
– No bilateral signs	– 3/3	– 1/1

1No information on the presence of a sensory level was available in 2 AFM patients and 6 TM patients
2At least one of the reasons for not fulfilling the criteria listed below is present in these patients; multiple reasons may however be present within one patient.
3Insufficient information (no information on CSF examinations and MRI features) was available for 6 AFM patients.

4Absence of inflammation may be explained by the following: no lumbar puncture was performed in 3 patients. CSF examination was done early in the disease course (day 2 or earlier) in 3 patients. For 1 patient CSF leukocyte numbers were not available. For 1 patient CSF examination was done late in the disease course (day 25). For the remaining 6 patients, no clear explanation for the absence of inflammation can be given, besides the lack of gadolinium administration during MRI in one and the absence of an IgG index determination in another.

TCMWG criteria applied in both cohorts for patients in which sufficient information was available.

To fulfill TMCWG criteria patients must have:

1. Development of sensory motor, or autonomic dysfunction attributable to the spinal cord
2. Bilateral signs and/or symptoms (not necessarily symmetric)
3. Clearly defined sensory level
4. Inflammation of the spinal cord demonstrated by

a. CSF pleocytosis
b. Elevated IgG index
c. Gadolinium enhancement

5. Progression from onset to nadir between 4 hours and 21 days
6. Exclusion of

a. Compressive etiology or flow voids suggestive of a arterio-vascular malformation
b. Radiation to the spine within the last 10 years
c. Clear arterial distribution
d. Serologic or clinical evidence of a connective disease
e. CNS manifestation of an infectious disease (excluded in AFM patients in this cohort)
f. Brain MRI abnormalities suggestive of MS
g. History of/or clinically apparent optic neuritis

Supplementary Table 2: AFM criteria applied in all patients.

	AFM cohort (N=12)[1]	TM cohort (N=33)[1]
Definite AFM (%)	**7/12 (58)**	**1/33 (3)**
Probable AFM (%)	**4/12 (33)**	**3/33 (9)**
Reason(s) for not fulfilling criteria for definite AFM	n=4	n=3
– LP not performed	– 4/4	– 1/3
– No CSF pleocytosis		– 2/3
Possible AFM (%)	**0/12 (0)**	**9/33 (27)**
Reason(s) for not fulfilling criteria for probable AFM	n=0	n=9
– No CSF pleocytosis		– 9/9
Uncertain (%)	**1/12 (8)**	**0/33 (0)**
Reason(s) for not fulfilling criteria for possible AFM	n=1	n=0
– No MRI performed	– 1/1	
Factors suggestive of an alternative diagnosis (%)	**0/13 (0)**	**19/33**
	n=0	n=19
– Sensory level		– 15/19
– Supratentorial white matter abnormalities		– 13/18
– MOG antibodies		– 3/14
– AQP4 antibodies		– 1/15
– Time to nadir>10 days		– 1/19

AFM criteria applied in both cohorts for patients in which sufficient information was available. The criterium of decreased muscle tone in at least one weak limb was omitted, because this was not recorded.
1 AFM cases (n=9) and TM cases (n=3) were not classifiable because MRI was normal (2 AFM cases) or if MRI of if predominant gray matter involvement was not appreciable based on the provided description (7 AFM cases and 3 TM cases).
AFM: acute flaccid myelitis; TM: transverse myelitis; AQP4: aquaporin-4; CSF: cerebrospinal fluid; MOG: myelin oligodendrocyte protein; MRI: magnetic resonance imaging.

To fulfill the criteria for definite AFM patients must have:

1. Acute onset limb weakness (period from onset to nadir: hours to 10 days)
2. Weakness involving one or more limbs, neck, face or cranial nerves
3. Decreased muscle tone in at least one weak limb
4. Decreased or absent tendon reflexes in at least one weak limb
5. Spinal cord lesion with predominant grey matter involvement on MRI, with or without nerve root enhancement
6. Pleocytosis in CSF

- To fulfill the criteria for probable AFM patients must have all of the above mentioned features, but no pleocytosis in CSF, either because no lumbar puncture is performed or because no pleocytosis is found.
- To fulfill the criteria for possible AFM patients must have the features mentioned under 1,2 and 5. Decreased muscle tone and/or hyporeflexia are not required for a possible diagnosis of AFM.
- The diagnosis of AFM is classified as uncertain when no MRI is performed, when the items mentioned under 1-4 are present and there is a prodromal fever or illness.

Factors that might suggest an alternative diagnosis are:

1. Encephalopathy that cannot be explained by fever, illness, respiratory distress, metabolic abnormalities or medications
2. Presence of sensory deficits on examination, although there are no data describing the frequency of this feature in AFM
3. Presence of lesions in the supratentorial white matter or cortex
4. Absence of CSF pleocytosis
5. Positive serum aquaporin-4 would exclude AFM
6. Positive serum MOG-antibody, with would suggest MOG-antibody associated disease, although there are no data describing the frequency of this feature in AFM

SECTION 3:

DIAGNOSTIC CRITERIA

7

ACUTE FLACCID MYELITIS: CAUSE, DIAGNOSIS, AND MANAGEMENT

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Lancet. 2021 Jan 23;397(10271):334-346. doi: 10.1016/S0140-6736(20)32723-9.
Supplementary material available online with the published version of this manuscript

ABSTRACT

Acute flaccid myelitis (AFM) is a disabling, polio-like illness mainly affecting children. Outbreaks of AFM have occurred across multiple global regions since 2012, and the disease appears to be caused by non-polio enterovirus infection, posing a major public health challenge. The clinical presentation of flaccid and often profound muscle weakness (which can invoke respiratory failure and other critical complications) can mimic several other acute neurological illnesses. There is no single sensitive and specific test for AFM, and the diagnosis relies on identification of several important clinical, neuroimaging, and cerebrospinal fluid characteristics. Following the acute phase of AFM, patients typically have substantial residual disability and unique long-term rehabilitation needs. In this Review we describe the epidemiology, clinical features, course, and outcomes of AFM to help to guide diagnosis, management, and rehabilitation. Future research directions include further studies evaluating host and pathogen factors, including investigations into genetic, viral, and immunological features of affected patients, host-virus interactions, and investigations of targeted therapeutic approaches to improve the long-term outcomes in this population.

INTRODUCTION

Unusual clusters of a disabling, polio-like illness, now termed acute flaccid myelitis (AFM), were recognised in California in 2012, and Colorado in 2014 [1,2]. AFM is now recognised as a global disease, with hundreds of cases reported across Europe [3,4], Asia [5–7], Australia [8], Africa [9], North America [10,11], and South America [12,13]. Epidemic enteroviral infection is believed to be the main driver of AFM in recent years, particularly enterovirus D68 infection.[14] Cases have usually occurred in geographical clusters, with a distinct seasonal biennial pattern in temperate regions.[15] AFM most frequently affects young children, and is characterised by acute onset of flaccid weakness of one or more limbs, with MRI showing abnormalities of the spinal cord grey matter.[5] Trunk, neck, respiratory, bulbar, facial, and extraocular muscles can also be affected. The clinical presentation of AFM may mimic other causes of acute weakness such as Guillain-Barré syndrome, demyelinating myelitis, and other infectious myelitis. The diagnosis of AFM can be informed by interpretation of the clinical features alongside findings of laboratory, neuroimaging, and electrophysiological tests.

Acute management of AFM is largely supportive because there is an absence of therapeutic agents proven to alter outcomes. A substantial proportion of patients with AFM will become critically ill during the acute illness, requiring intubation due to respiratory failure or severe bulbar weakness [16,17]. Neurological recovery after AFM is usually incomplete, with many patients having substantial residual weakness and muscle atrophy. Over the long term, patients can be affected by a range of neurological, musculoskeletal, and psychological sequelae [18–20]. Appropriate rehabilitation can improve functional status and quality of life after AFM [19]. Additionally, surgical approaches including tendon or nerve transfer surgery have been used in individual cases to manage residual impairments [21,22]. In this Review we describe the epidemiology, clinical features, course, and outcomes of AFM to help to guide diagnosis, management, and rehabilitation.

Epidemiology and cause

Several features support a viral link to AFM cases. Most individuals affected by AFM report a febrile prodrome accompanied by respiratory symptoms in the days before the onset of weakness.[15,17] The primary sites of neurological involvement parallel

poliomyelitis, with lesions targeting the anterior horn cells of the spinal cord and motor nuclei of the brainstem. To date, the virus suspected to be the predominant driver of the seasonal, biennial outbreaks of AFM observed in many global regions is enterovirus D68, although other enteroviruses (particularly enterovirus A71) and some coxsackie virus strains have also been implicated. Evidence specifically supporting the causal association of AFM with enterovirus D68 includes: (1) temporal and geographical correlations between AFM cases and enterovirus D68 circulation [1,23]; (2) enterovirus D68 predominating amongst pathogens identified in biological specimens (typically respiratory samples) from individuals with AFM, across many geographical regions [3,15,17]; (3) recent emergence of strains of enterovirus D68 that could have acquired the ability to cause AFM [24–26]; (4) a higher frequency of enterovirus-specific antibodies in the cerebrospinal fluid (CSF) of patients with AFM than in controls (albeit without definitive evidence of intrathecal synthesis) [27,28]; and (5) mouse models in which recent enterovirus D68 strains cause AFM-like limb paralysis with virus isolated from and visualised in the spinal cord [25,29,30].

Search strategy and selection criteria

For this Review, we searched PubMed with the terms “acute flaccid myelitis”, “acute flaccid paralysis”, “polio-like”, “poliomyelitis”, and “enterovirus”, and sorted results into the following themes: epidemiology, clinical presentation, diagnosis, management, and outcomes. Search criteria were limited to publications in English from January, 2012, to July, 2020. In addition to identified primary research we included relevant materials such as published opinions and viewpoints, proposed case definitions, and other materials including conference abstracts, ongoing research work, and unpublished observations of AFM Working Group members based on their clinical experience.

The specific mechanism by which infection with enterovirus D68 leads to AFM is not fully understood and represents a key question for future research. Enterovirus D68 most commonly causes respiratory disease [31,32], but there is ample precedent amongst other enteroviruses (particularly poliovirus) for occasional spread to the grey matter of the spinal cord, supported by evidence from autopsies [33–35]. The pathogen, environmental, and host factors that can mediate progression to neurological disease are unknown. Mouse models and neuronal cell culture models suggest that recent strains of enterovirus D68 have evolved in terms of their capability of accessing the nervous system (neuroinvasion), their capacity to

infect neurons (neurotropism), their ability to cause nervous system disease (neurovirulence), or any combination thereof [25,29,36,37]. A lack of spinal cord or brain tissue specimens from affected patients has impeded direct confirmation of this possibility in humans. Also confounding characterisation of the recent AFM outbreaks is the infrequency of direct viral isolation or viral genome detection from the CSF at the time of clinical presentation, even with sensitive and unbiased pathogen discovery technologies [23,38]. This difficulty nonetheless parallels similar experience with other viruses manifesting occasional neurotropic spread (eg, wild type poliovirus, vaccine-derived poliovirus, and West Nile virus).

The potential of other alternative viral causes as major contributors to recent AFM outbreaks would appear to be diminished by clinical features, reported investigations, and epidemiological characteristics. The first of these, enterovirus A71 is generally associated with outbreaks of hand-foot-mouth disease but also shows similar occasional tropism for the nervous system manifesting a poliomyelitis-like paralysis, thus meeting the AFM criteria. In regions reporting the recent increases of AFM cases, however, identified cases associated with enterovirus A71 have been less frequent than those associated with enterovirus D68, and could differ in clinical phenotype [3,5,16,39]. Additionally, AFM cases associated with enterovirus A71 have been geographically restricted, with outbreaks mainly reported in the Asia-Pacific region (where the virus has been endemic since the 1990s) [34,40], and more recently on a smaller scale in the USA and Europe [39,41,42]. The other notable candidate viruses include the flaviviruses, whose members include the arboviruses, West Nile virus, and Japanese encephalitis virus, which can cause acute flaccid paralysis related to anterior horn cell involvement. Multiple epidemiological and clinical class characteristics of arboviral infections undermine the argument that they are a major cause of recent AFM outbreaks, including: (1) infections are vector-borne and occur seasonally in endemic regions, unlike the more ubiquitous recent worldwide distribution of AFM; (2) arboviral infections typically affect adults more commonly than they do children; (3) patients typically have characteristic systemic features such as rash or vomiting; and (4) when nervous system involvement is present it tends to include meningoencephalitis, with motor-neuron-limited presentations being less common [43]. Thus, diagnosis of a specific arboviral infection in a patient manifesting a clinical syndrome of AFM will trigger diagnostic and management protocols already in existence [44].

In reviewing the literature regarding AFM, some observational studies have been specifically restricted to patients with AFM associated with enterovirus D68 [3], whereas other studies have not applied this criterion [17]. For the purposes of clinical research, defining

the disease by the associated organism provides a study population with more uniform pathophysiology, which is unlikely to include clinical mimics. Indeed, AFM cases occurring in years with epidemic peaks (ie, with clear-cut outbreaks driven by a single virus) show much greater clinical and paraclinical homogeneity than do AFM cases occurring in non-peak years [15]. However, since enterovirus D68 may only be detectable in laboratory specimens in the early stage of the disease and other non-polio enteroviruses are likely to cause a small proportion of the AFM burden globally, defining the disease by the associated organism is not pragmatic for the purposes of clinical practice. Currently, the absence of sensitive confirmatory testing for specific non-polio enteroviruses (such as serological testing) represents a major barrier to aetiological confirmation, clinical assessment, and disease surveillance.

Clinical presentation

AFM is predominantly a childhood disease (median age 6·3 years) [16], with less than 15% of cases occurring in adults (more commonly in the immunocompromised), although AFM in adults could be under-recognised or under-reported [1,3–5,12,17,45]. A slight predilection for males has been suggested [4,5,15,17]. Most patients with AFM have a prodromal illness manifesting with fever and respiratory symptoms (cough, rhinorrhea, pharyngitis, or asthma-like illness). Gastrointestinal symptoms such as vomiting or diarrhoea are less frequent [15,17]. Household contacts with similar prodromal illnesses are common; however, there have been no reported occurrences in the USA of multiple cases of AFM occurring in one household or family. The epidemiological context can provide useful clues, because known outbreaks of enterovirus D68 or A71 (or other confirmed AFM cases) in an area might prompt clinicians to consider AFM in patients presenting with acute weakness. Onset of neurological symptoms typically occurs 1–10 days after onset of the infectious prodrome, with many patients reporting improvement in prodromal symptoms before onset of neurological symptoms [5,17].

The onset of neurological symptoms can be accompanied by headache, neck stiffness, or recurrence of fever (table 1). Meningism can be present in this early stage. In many patients, limb weakness is heralded by pain in the affected limb(s), neck, or lower back. Flaccid weakness is typically asymmetric and can affect one or more limbs, with predilection for the upper limbs and proximal muscle groups [17]. Unlike many other causes of acute weakness, AFM can present with severe weakness in affected upper limb(s) and normal

strength in the lower limbs, or marked asymmetry with a difference of more than 2 points on the medical research council (MRC) scale between right and left limbs [46]. Affected limbs become hyporeflexic or areflexic. Weakness can also affect the neck, trunk, diaphragm, or other respiratory muscles. In addition to limb weakness, approximately 30% of patients also have motor deficits localising to the cranial nerve motor nuclei of the brainstem, primarily consisting of bulbar and facial weakness, and, less commonly, extraocular muscle weakness [2]. Finally, although not meeting existing epidemiological criteria for AFM, in some patients weakness can be limited to the cranial nerve(s) or neck, in the absence of limb weakness [47]. Given that presentations limited to the cranial nerves have been excluded from most published case series of AFM (which have required at least one weak limb for study inclusion), the frequency of these cases is unknown. In our experience, such presentations can occur within the syndrome of AFM, but represent a minority of cases.

Table 1 Clinical presentation of acute flaccid myelitis

	Estimated frequency
Age <21 years	80–90%
Prodromal fever or viral illness	85–95%
Neurological onset to nadir <10 days	100%
Headache or neck stiffness at onset	12–60%
Asymmetric onset of weakness	65–95%
Limb weakness	85–95%
Upper limb weakness	60–85%
Flaccidity or hyporeflexia of affected limbs	95–100%
Neck, face, extraocular, or bulbar weakness	20–60%
Trunk weakness	30–70%
Requirement for mechanical ventilation	10–40%
Bladder or bowel dysfunction	5–40%
Non-specific sensory symptoms (eg, paresthesia)	10–20%
Cardiovascular autonomic dysfunction	<10%
CSF pleocytosis (with testing <5 days after onset)	85–95%
Grey-matter predominant spinal cord lesion (s) on MRI	95–100%
Brainstem lesion (s) on MRI	35–45%
Cerebral deep grey matter lesion (s) on MRI	<5%

CSF=cerebrospinal fluid.

The severity of weakness in an individual patient can range from mild to moderate weakness of one limb to complete paralysis of all limbs, and axial and bulbar muscles. About a third of patients admitted to hospital require intubation and ventilation [38], either due to respiratory muscle weakness or bulbar muscle weakness (with inability to protect the airway). Respiratory failure can be precipitated by procedural sedation. Dysphagia might necessitate supplemental hydration and nutrition. Bladder and bowel dysfunction are common in the acute phase [17], and autonomic manifestations such as labile blood pressure or irregular heart rate and breathing patterns can occur.18 Sensory symptoms or deficits other than neuropathic pain or paraesthesia are atypical [48]. Altered mental status is not common [5,17,48], and the contribution of factors such as metabolic or respiratory disturbances in cases of reported encephalopathy is uncertain. Especially notable in cases associated with enterovirus A71 infection, AFM can occur in conjunction with frank brainstem encephalitis, and common clinical features in this patient group are autonomic disturbance, myoclonus, ataxia, irritability, and drowsiness [41].

Some clinical features of AFM overlap with other causes of acute flaccid paralysis, including Guillain-Barré syndrome, spinal cord stroke, demyelinating myelitis (eg, aquaporin-4-IgG seropositive or seronegative neuromyelitis optica spectrum disorder, myelin oligodendrocyte glycoprotein [MOG]-antibody associated myelitis, multiple sclerosis, and acute disseminated encephalomyelitis), poliomyelitis (wild type poliovirus or vaccine-derived poliovirus), other infectious myelitis (eg, Japanese encephalitis, West Nile virus, tick-borne encephalitis virus, and varicella zoster virus myelitis), acute plexopathy, periodic paralysis, botulism, toxic synovitis, and orthopaedic conditions including nursemaid's elbow. Infections capable of causing acute weakness differ according to the endemic and epidemic organisms in each global region. Poliovirus remains an important consideration in areas where wild-type poliovirus has not been eradicated, or in areas where vaccine-derived poliovirus may circulate [49]. Certain vector-borne infections that can affect the anterior horn cells have clear regional distributions, with West Nile virus occurring in North America, Europe, Africa, and West Asia; Japanese encephalitis occurring in Asia and west pacific regions; and tick-borne encephalitis occurring in Europe, Russia, and some countries in Asia [50–52]. Important clinical clues for these infections include systemic features (eg, erythematous maculopapular rash in West Nile virus) and neurological features accompanying acute weakness (eg, seizures and prominent neuroimaging involvement of

the deep grey matter in Japanese encephalitis). Microbiological testing can be tailored according to these epidemiological and regional infectious considerations.

Guillain-Barré syndrome, particularly the acute motor axonal neuropathy subtype, can cause acute weakness in children (particularly in some regions) [53–55]; however, there are some clinical features that can help to distinguish AFM from Guillain-Barré syndrome (table 2). The weakness in AFM can be markedly asymmetrical, often completely sparing one or more limbs [17,46], and can appear in a descending pattern. Sensory symptoms are usually a prominent feature in Guillain-Barré syndrome (except in the acute motor axonal neuropathy subtype) [56], unlike AFM. Additionally, the clinical and radiological features of MOG-antibody associated myelitis can be strikingly similar to AFM [57,58]. A further overlapping characteristic of the two is the frequent triggering of MOG-antibody associated disease by a viral infection [58]. Concurrent optic neuritis, an encephalopathy-predominant clinical presentation, clinical evolution over more than 10 days, or a history of previous CNS inflammatory events suggests an alternative diagnosis such as multiple sclerosis, neuromyelitis optica spectrum disorder, or MOG-antibody associated disease, rather than AFM. Spontaneous spinal cord infarction often presents with acute flaccid weakness with a grey-matter predominant MRI lesion, and is under-recognised in children [59]; however, patients often report severe back or limb pain (eg, knife-like) at onset, progress to nadir within 4 h, and have symmetric weakness and a sensory level [60].

Table 2 Differentiating acute flaccid myelitis from clinical mimics

Acute flaccid myelitis	Guillain-Barré syndrome	Acute transverse myelitis (demyelinating or idiopathic)	Spontaneous spinal cord infarction	
Prodromal illness	+++	+++	+/−	−
Temporal evolution	Hours to days	Days to weeks	Days to weeks	Minutes to hours
Pattern of weakness	Asymmetric, arms>legs	Symmetric, ascending	Variable	Symmetric, severe
Facial/bulbar weakness	++	++	+/−	+/−
Respiratory failure	++	++	+/−	+/−
Numbness/ paraesthesia	+/−	+++ (except AMAN)	+++	+
Sensory level	−	−	++	++

Acute flaccid myelitis	Guillain-Barré syndrome	Acute transverse myelitis (demyelinating or idiopathic)	Spontaneous spinal cord infarction	
Encephalopathy	–	–	+/– (eg, ADEM)	–
Bowel/bladder dysfunction	+/–	+/–	++	+++
Possible associated symptoms or syndromes	Headache, neck pain/stiffness, neuropathic pain	Neuropathic pain	Optic neuritis, encephalitis, seizures	Severe back/ limb pain at onset
MRI spinal cord	Ill–defined grey–matter predominant lesion, +/– nerve root enhancement	Normal cord, +/– nerve root enhancement	Variable, but usually a well-defined enhancing white>grey matter lesion	Non-enhancing anterior cord or grey-matter lesion
CSF	Mild–moderate pleocytosis	Elevated protein	Mild–moderate pleocytosis	Sometimes elevated protein or mild pleocytosis
Microbiological tests	See panel 1	Stool sample: bacterial culture, viral RT-PCR panel; respiratory sample: viral RT-PCR panel; serum: *Campylobacter jejuni* and *Mycoplasma pneumoniae* IgM/IgG; other organisms according to region and season	If indicated based on clinical presentation	Not usually indicated
Other useful tests	+/– EMG/NCS	EMG/NCS; serum: anti-ganglioside antibodies	Serum: MOG-IgG, aquaporin-4-IgG; CSF: oligoclonal bands	Angiography

AMAN=acute motor axonal neuropathy subtype. CSF=cerebrospinal fluid. ADEM=acute disseminated encephalomyelitis. EMG/NCS=electromyography and nerve conduction studies. MOG=myelin oligodendrocyte glycoprotein.

Diagnosis

MRI of the spinal cord is the most useful diagnostic test in AFM. T2 hyperintensity of the spinal cord grey matter is the hallmark of AFM (figure 1). Lesions in the early acute phase (hours to days) are typically confluent and ill defined, and affect the entire grey matter of the spinal cord when viewed axially [46,61,62], with a varying degree of surrounding white matter involvement and oedema [46]. Spinal cord grey matter lesions are longitudinally extensive in most cases [61]. The cervical cord is the most commonly, and often most prominently, affected, with marked oedema in some cases [46]. T2 hyperintense lesions can also occur in the brainstem (most commonly in the dorsal pons) [46,62]. Spinal cord and brainstem lesions are usually non-enhancing or minimally enhancing. Swelling and hyperintensity of the brachial plexus in affected upper limbs has been identified in some patients in the acute stage using short tau inversion recovery MRI [63]. Supratentorial lesions in the cortex and white matter generally do not occur [59], although T2 hyperintensity of deep grey matter structures has been recognised in a few patients (unpublished observations). Between 1 and 4 weeks after clinical onset, oedema improves and residual spinal cord lesions (present in many cases) become more focal, localising to the anterior horn region of the grey matter, and nerve root or cranial nerve enhancement frequently emerges [61], which can persist for weeks to months. MRI abnormalities can be subtle early in the acute course, which might even be interpreted as normal in the clinical setting. However, detailed retrospective MRI analysis by neuroradiologists with experience in AFM suggests that subtle lesions are invariably present on the initial MRI [46,59,61,62,64].

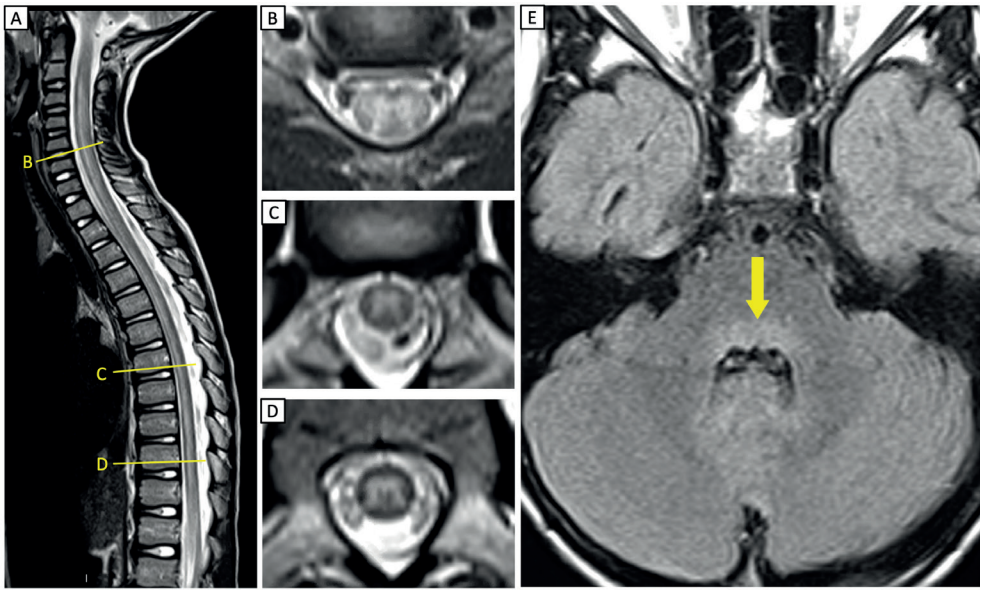

Figure 1: Typical MRI findings in the acute phase of AFM

Spinal MRIs are shown of an 8-year-old child with AFM, acquired 24 h after onset of neurological symptoms. (A) Sagittal T2 image showing an ill-defined longitudinally extensive central/anterior spinal cord lesion. (B) Axial T2 image from C5–C6 shows hyperintensity of the entire grey matter of the spinal cord, with associated oedema and some surrounding white matter hyperintensity. (C) Axial T2 image from T7 shows asymmetric hyperintensity of the grey matter (right more than left). (D) Axial T2 image from T10 shows hyperintensity of the entire grey matter. (E) Axial FLAIR image at the level of the middle cerebellar peduncle demonstrates hyperintensity of the dorsal pons (arrow). AFM=acute flaccid myelitis.

CSF pleocytosis is identified in almost all patients with AFM undergoing lumbar puncture in the acute phase, with a mild to moderate elevation in white blood cell count (usually <100 per μL with lymphocytic predominance), which appears to resolve over subsequent weeks [1,5,17,46,59]. How quickly pleocytosis evolves alongside the neurological syndrome is uncertain, and anecdotal reports suggest that if cell counts are normal very early in the course (within hours of neurological onset), pleocytosis can become apparent with repeat testing. A few patients with a clinical syndrome and imaging otherwise suggestive of AFM do not develop any CSF pleocytosis. The reason for this discrepancy is unclear but it can make differentiation of AFM from some clinical mimics more challenging. CSF protein can be mildly or moderately raised (usually <100 mg/dL), with occasional reports of values of almost 1000 mg/dL [5,17,38,46]. CSF analysis can be helpful during the acute phase in

differentiating AFM from other causes of flaccid paralysis less likely to produce pleocytosis (such as spinal cord infarction or Guillain-Barré syndrome).

Investigations outside the CNS or CSF are necessary to search for causes of AFM and its mimics (table 2). Respiratory (nasopharyngeal and oropharyngeal) and stool or rectal swab samples can show the presence of enterovirus D68, enterovirus A71, or other enterovirus RNA by reverse transcription polymerase chain reaction (RT-PCR), with detection most likely early in the clinical course. The highest yield for viral identification is in respiratory samples for enterovirus D68 [38], and in rectal or stool samples for enterovirus A71 [41].Additionally, stool viral culture for poliovirus (with RT-PCR of isolated virus to differentiate between wild-type and vaccine-derived virus) is indicated in some regions. Although not standard practice across all regions, the routine inclusion of enterovirus RT-PCR in viral respiratory and stool panels (as opposed to combined detection of enterovirus or rhinovirus species) would improve detection of these viruses in patients with AFM, and facilitate improved disease surveillance. Detection of enterovirus D68 or A71 in the CSF by RT-PCR is extremely rare [17]. Serum testing for MOG-IgG (cell-based assay only) and aquaporin-4 (AQP4-IgG, cell-based assay preferable since low titre false-positive results can occur with ELISA testing) can identify these important treatable clinical mimics. Positive AQP4-IgG detected by cell-based assay is highly specific for a diagnosis of neuromyelitis optica spectrum disorder [65,66]. The positive predictive value of MOG-IgG for a diagnosis of MOG-antibody associated disease is high (with high titres showing higher specificity and reproducibility than borderline or low-positive titres) [67,68]. Identification of anti-ganglioside antibodies in the serum can support an alternative diagnosis of Guillain-Barré syndrome, although specificity is incomplete, and positive anti-ganglioside antibodies have been reported with other neuropathies (eg, diabetic neuropathy) and in some patients with AFM [5,69]. Thus, detection of one or more anti-ganglioside antibodies (particularly at a low titer) does not exclude a diagnosis of AFM. Collection of serum samples for serological testing before intravenous immunoglobulin is administered will provide the most reliable results (given that intravenous immunoglobulin can alter sensitivity and specificity of auto-antibody tests) [70].

Electromyography or nerve conduction studies are often not required to make a diagnosis of AFM (in fact characteristic findings may only emerge 1 week after neurological onset); however, these studies can be a useful early investigation when differential diagnoses of Guillain-Barré syndrome or other acute neuromuscular disorders are being considered. Electromyography or nerve conduction studies can also have a role in the diagnosis of AFM

in regions where MRI is not readily available, for patients for whom there is diagnostic uncertainty (eg, with equivocal MRI findings), or for patients with a delayed presentation (or initial misdiagnosis) in whom electrophysiologic changes are likely to be established by the time of assessment. Electrophysiological changes of AFM emerge over several weeks. Diminished or absent compound motor action potentials (CMAP) are an early finding, and can occur as soon as several days from symptom onset [1,71]. By 2 weeks after onset, CMAP abnormalities tend to be evident, and lower CMAP amplitude appears to correlate with more severe injury (unpublished data). Decreased or absent F waves can also be detected [5,71]. Sensory nerve conduction studies are normal [20,59,71]. If electromyography or nerve conduction studies are completed early in AFM, reduced or absent recruitment of voluntary motor potentials might be the sole finding on needle electromyography. Fibrillations and positive sharp waves can develop as early as 1 week after symptom onset, followed by progressively increasing motor unit potential amplitude and duration consistent with denervation or reinnervation occurring over weeks to months or longer [1,3,59,71]. Electromyography findings indicative of denervation can be seen even in limbs with apparently normal strength [20]. Collectively, these findings are indicative of motor neuronopathy or axonal motor neuropathy, and may closely mimic the electrophysiologic changes seen in the acute motor axonal neuropathy subtype of Guillain-Barré syndrome [72]. The symmetry and the relative length-dependence of the findings can be helpful clues in such cases; abnormalities that are asymmetrical and proximal>distal are characteristic of AFM. Alternatively, conduction block with early reversal of findings on serial studies is suggestive of acute motor axonal neuropathy [73,74].

The poor availability of MRI or CSF analysis, or both, in resource-limited health-care settings is a particular challenge in the diagnosis of AFM. A typical prodromal illness and characteristic clinical presentation is suggestive of AFM even in the absence of advanced diagnostic testing, and electromyography or nerve conduction studies can be a useful adjunctive diagnostic tool when available. Epidemiological context can also be a useful clinical clue, because surveillance for enteroviral infections and AFM cases by regional public health systems may highlight seasonal periods and geographical locations associated with increased AFM risk. Although identification of a non-polio enterovirus species in respiratory or stool samples is not required to make a diagnosis of AFM, it can help to increase diagnostic certainty when MRI is not available. Serological testing for neuroimmune diseases with relapsing potential (specifically MOG IgG and AQP4 IgG) is not widely available

in some resource-limited regions, and the onset of a second neurological event is an important flag for these treatable disorders. Clinical evolution that is atypical for AFM might also be a clue to these disorders—eg, a robust clinical response to any empirical steroid treatment if used, or development of upper motor neuron signs (spasticity and hyper-reflexia) during clinical recovery.

Acute management

Patients with AFM progress from neurological onset to nadir of weakness within hours to days [17]. In 2018, 96% of identified AFM cases in the USA were admitted to hospital, and 58% to an intensive-care unit [16]. Supportive treatment with careful monitoring focused upon potential emerging vital complications is the mainstay of early management. Although there is no specific evidence for optimal management of AFM, acute supportive management is similar to other causes of acute neuromuscular weakness. Supportive management includes optimising cardiorespiratory status including securing the airway and providing ventilatory support for respiratory failure when needed; treating bladder, bowel, or other autonomic dysfunction; managing pain; preventing complications of acute immobility (such as pressure ulcers and venous thromboembolism); and commencing early rehabilitation. AFM is a notifiable illness in many regions, requiring notification of the relevant public health authorities according to local protocols.

Since the pathophysiology of AFM is not fully understood, with the disease occurring in association with identified enterovirus infection in some patients or absent isolated virus in others, which biological process(es) should be targeted in acute disease to modify clinical outcomes is uncertain [75]. There have been no prospective, controlled trials of specific medical therapies in AFM. Given that most experts believe neuroinvasive viral infection to be the primary cause of neurological disease in AFM, intravenous immunoglobulin (which has been shown to include neutralising antibodies against contemporary strains of enterovirus D68) [76] is frequently used for its possible antiviral and immunomodulatory effects, along with a favourable adverse-effect profile. On the basis of the postulated mechanism of action, theoretical potential benefits of intravenous immunoglobulin treatment could be considered greatest when administered early in the course of the illness. Intravenous steroids and plasma exchange are sometimes used for their potential immunomodulatory effect, but the potential for therapeutic benefit versus harm remains

controversial. Some physicians have used steroids in cases manifesting critical spinal cord oedema with secondary cord compression, although individual benefit in such cases is uncertain. In mouse models of enterovirus D68 nervous system infection, early administration of intravenous immunoglobulin reduced paralysis whereas steroid treatment resulted in increased viral titre in the spinal cord and worse outcomes [77]. The applicability of these murine studies to a human disease of incompletely understood cause and pathogenesis such as AFM is uncertain and represents a key area of future research. In low-resource settings where insufficient advanced diagnostic testing precludes confirmation of AFM, and immune-mediated myelitis (ie, demyelinating or idiopathic myelitis) remains in the differential diagnosis, clinicians might need to consider a trial of treatment with high-dose steroids in individual cases. Antienteroviral and neuroprotective activity of fluoxetine has been shown in vitro [78], but not in a small retrospective uncontrolled cohort study in patients with AFM [79], or in the murine model [77]. Small molecule antivirals and monoclonal antibodies against non-polio enteroviruses are being investigated as potential therapies [80–82].

Recovery, rehabilitation, and long-term sequelae

AFM seems to be a monophasic disorder with high potential for residual impairment. Prognostication is challenging, but electromyography or nerve conduction studies and MRI could both have potential utility [20,61,64]. Denervated muscles with severe neurogenic changes on electromyography or nerve conduction studies in the weeks to months after AFM onset are likely to experience residual weakness [5,20,71]. Quantitative measures of grey matter MRI involvement during the acute phase of the illness show promise in predicting motor outcomes, based on findings from a small case series [64]. Evolution of MRI abnormalities occurs in the weeks to months after onset of AFM, and the location of residual MRI lesions in the anterior horns, could correlate with the distribution of residual limb weakness [61]. Localising these characteristic residual lesions could help to map areas of more severe injury in affected individuals. Future research to elucidate which combination of clinical or paraclinical factors (or both) best predicts long-term clinical outcomes in AFM is needed.

The extent of recovery in AFM is highly variable, although few patients (<10%) recover completely [3,5,18,20,47]. After neurological nadir (which may last days to weeks), most

patients show some improvement in motor strength, with recovery being most rapid in the first few months after onset. Cranial nerve dysfunction is more likely to improve and resolve than is limb weakness [20,47]. In the limbs, early recovery appears to occur in a distal to proximal pattern [19]. Profoundly affected muscle groups (particularly MRC grade 0 of 5) at neurological nadir are the least likely to recover, and thus recovery can be markedly asymmetrical [20,46]. Respiratory muscle weakness can persist, although only a small proportion of patients remain ventilator dependent at approximately 1 year follow-up [19,46,47,83]. Reports of death have been rare and are limited to immunocompromised adults, and children with early or late complications of respiratory failure [3,17].

Nerve transfer surgery has been undertaken in some patients with poor clinical recovery of affected areas. Case series have shown generally positive outcomes from nerve transfers for restoration of elbow function in appropriately selected patients, with less positive outcomes for restoration of shoulder function [21,22,84]. Muscle or tendon transfer, or both, has been reported in a few cases, with generally positive outcomes for restoration of elbow or hand function [21,84]. Anecdotal reports indicate that lower limb nerve transfers, nerve transfer to the phrenic nerve, and diaphragmatic pacing have been undertaken in individual cases [85]; however, there are few published data regarding outcomes in these cases.

Data for medium-term to long-term neurological and functional outcomes of patients with AFM since 2012 are limited to small cohorts followed up for 2 years or less. Some data suggest that severity and prognosis vary according to viral pathogen detected in relation to AFM, given that patients with AFM associated with enterovirus A71 can have milder muscle weakness and better recovery than patients with AFM associated with enterovirus D68 [41]. Patients engaged in multimodal rehabilitation can achieve functional improvements for years even after recovery of motor strength plateaus [19]. However, many patients have substantial residual weakness, muscle atrophy, and functional impairment, with potential for a secondary broader range of developmental sequelae. Medium-term to long-term complications described to date in AFM include neurological sequelae (neuropathic pain, chronic constipation, chronic ventilator dependence, and dependence on artificial nutrition and hydration), musculoskeletal sequelae (joint subluxation and dislocation, particularly proximal joints with profound muscle weakness, limitation in range of joint motion, scoliosis, limb-length discrepancies, and chest wall abnormalities), and psychological sequelae (such as anxiety and depression) [18–20,48]. Given shared mechanisms, the known consequences of similar

neurological disorders can provide some clues to potential complications even later in life, such as accelerated degenerative joint disease, reduced bone mineral density in affected limbs, cardiometabolic syndrome (including obesity, insulin resistance, hypertension, and dyslipidaemia), restrictive respiratory insufficiency, sleep disordered breathing, and nocturnal hypoventilation [86–91]. Entrapment neuropathies can arise from the use of walking aids or wheelchairs [92,93], while scoliosis can predispose to later compressive myelopathy or radiculopathy [93]. Finally, in other disorders causing substantial neurological disability in early life, such as poliomyelitis, some patients have reported deterioration in strength or function with ageing [94]. Whether patients with AFM could have a similar decline later in life, or whether continued rehabilitation may mitigate this decline, is unclear.

Implications of current evidence: diagnostic criteria and clinical care

Literature to date focused on AFM is limited by no uniform diagnostic criteria, which is a barrier to advances in knowledge about treatment and outcomes in patients with AFM. Additionally, management approaches have been variable and centre based. On the basis of best evidence from published knowledge from multiple cohorts, we provide pathogen-agnostic diagnostic criteria (figure 2), and an approach to the clinical assessment (panel 1), management (panel 2), and rehabilitation (panel 3) of patients with suspected AFM. The pathogen-agnostic diagnostic criteria for AFM include elements of clinical history, examination, neuroimaging, and CSF analysis. The core clinical syndrome of AFM is defined by acute onset of limb weakness, with lower motor neuron findings evident on neurological examination. Prodromal fever or illness is supportive but not essential to diagnose AFM, because not all patients report prodromal symptoms, including those for whom enterovirus D68 is identified [5]. The diagnosis of AFM can be considered definite when characteristic MRI findings and CSF pleocytosis are present in addition to the previously mentioned core clinical features. The diagnosis of AFM can be considered probable when the core clinical features and characteristic MRI findings are present, but CSF pleocytosis is absent (or not checked). The diagnosis of AFM can be considered possible in cases with a limited or milder clinical syndrome, with characteristic MRI findings; and uncertain when the core clinical features are present, but without adequate MRI studies to evaluate. Additionally, factors that suggest an alternative diagnosis are: (1) encephalopathy that cannot be explained by fever, illness, respiratory distress, metabolic abnormalities, or medications; (2) presence of

sensory deficits on examination; (3) presence of lesions in supratentorial white matter or cortex, which should prompt consideration of ADEM, MOG-antibody associated disease, neuromyelitis optica spectrum disorder, encephalomyelitis, and others; (4) absence of CSF pleocytosis, which should prompt consideration of Guillain-Barré syndrome, botulism, ischaemic cord lesions, and others; (5) positive serum aquaporin-4 (AQP-4) antibody, which would exclude AFM; and (6) positive serum MOG antibody, which would suggest MOG-antibody associated disease.

Diagnostic Items	Level of Diagnostic Certainty			
	Definite	**Probable**	**Possible**	**Uncertain**
H1: Acute onset of limb(s) weakness (Period from onset to nadir: Hours to ten days)	P	P	P[a]	P
H2: Prodromal fever or illness[b]	P/A	P/A	P/A	P
E1: Weakness involving one or more limbs, neck, face, and/or cranial nerves	P	P	P[a]	P
E2: Decreased muscle tone in at least one weak limb	P	P	P/A	P
E3: Decreased or absent deep tendon reflexes in at least one weak limb[c]	P	P	P/A	P
MRI: Spinal cord lesion with predominant gray matter involvement, with or without nerve root enhancement[d]	P	P	P	ND
CSF: Pleocytosis (white cell count > 5 cell/L)[e]	P	A or ND	P/A or ND	P/A or ND
Abbreviations: **H:** History, **E:** Examination, **CSF:** cerebrospinal fluid **P:** Diagnostic element is present, **A:** Diagnostic item is absent **P/A:** Presence of this diagnostic element is supportive but not required, **ND:** Test was not done				
Factors that may suggest an alternative diagnosis				
1: Encephalopathy that cannot be explained by fever, illness, respiratory distress, metabolic abnormalities, or medications.				
2: Presence of sensory deficits on exam.[f]				
3: Lesions in supratentorial white matter or cortex should prompt consideration of ADEM, MOG-antibody associated disease, neuromyelitis optica spectrum disorder, encephalomyelitis, and others.				
4: Lack of CSF pleocytosis should prompt consideration of Guillain-Barré syndrome, botulism, ischemic cord lesions, and others.				
5: Positive serum aquaporin-4 (AQP-4) antibody will exclude AFM.				
6: Positive serum myelin oligodendrocyte glycoprotein (MOG) antibody suggests MOG-antibody associated disease.[f]				

Figure 2: Diagnostic criteria for AFM

These criteria apply to the acute stage of the disease. AFM=acute flaccid myelitis. H=history. E=examination. CSF=cerebrospinal fluid. P=diagnostic element is present. A=diagnostic item is absent. P/A=presence of this diagnostic element is supportive but not required. ND=test was not done. ADEM=acute disseminated encephalomyelitis. MOG=myelin oligodendrocyte glycoprotein. *Subjective (H1) or objective (E1) weakness must be present in any of: limb(s), neck, or cranial nerves. † Prodromal illness can include respiratory, gastrointestinal, or other symptoms of viral illness. ‡ Normal or increased reflexes can be found in other limbs. § If MRI obtained very early (within hours of neurological onset) appears normal, repeat MRI after clinical evolution might show diagnostic findings. MRI obtained at late stages (≥4 weeks) might be normal. ¶ CSF may be normal at very early (hours) or late (≥4 weeks) stages of AFM. ||At present, there are no data describing the frequency of these features in patients with AFM.

The frequency of each element of the diagnostic criteria as reported in the existing literature is outlined in the appendix (p 12), although these studies are notable for substantial heterogeneity of inclusion criteria (eg, inclusion or exclusion according to age or enterovirus detection). These diagnostic criteria are specific to the acute phase of the illness, and allow classification of the level of certainty of an AFM diagnosis, and to distinguish AFM from other causes of acute flaccid paralysis. The diagnostic criteria outlined do not replace epidemiological case definitions for acute flaccid paralysis or AFM that public health organisations (such as WHO or US Centers for Disease Control and Prevention) use for surveillance purposes. Furthermore, a clinical diagnosis of AFM to guide management of an individual patient remains nuanced and must take into account the particular characteristics of each case. These diagnostic criteria classify AFM cases using typical features, although clinicians might encounter patients with atypical features outside of the outlined criteria.

CONCLUSIONS AND FUTURE DIRECTIONS

The increasing incidence, since 2012, of a likely enterovirus-driven severe paralytic disease with lifelong sequelae identifies AFM as a major global public health concern of high priority. Its relative rarity, widely disparate distribution, and resemblance to other causes of acute weakness argues for widespread education of clinicians and health-care providers on the characteristics necessary to appropriate diagnosis, acute management, and chronic rehabilitation. Whether the pattern of seasonal, biennial outbreaks of AFM will continue is uncertain, but preparedness for potential future increases in AFM cases is essential. Understanding of factors driving the seasonal, cyclic circulation of non-polio enteroviruses could be key to predicting and preparing for future AFM outbreaks. The mechanism by which common exposures, such as enterovirus infections, lead to severe neurological disease in the few affected by AFM remains unknown. Potential host genetic and immunological factors, as well as virological or environmental determinants, need to be elucidated. To determine whether anti-infective therapies (ie, antivirals, monoclonal antibodies), immunomodulatory therapies (ie, intravenous immunoglobulin, steroids, plasma exchange), or a combined therapeutic approach may be most effective, there is a need to understand the pathophysiological role of direct viral infection, immune activation, and inflammation on neuronal damage. Ultimately, if cases of this disabling paralytic disease

continue or increase, a preventative approach, including development of vaccine candidates against the leading suspected viral causes, might be necessary. All the above advances are dependent upon increased awareness of the presenting clinical features of AFM, allowing accurate case ascertainment to understand epidemiology and burden of disease, early recognition to allow prompt specimen collection and causal diagnosis, and early initiation of potential therapies.

Panel 1

Clinical and paraclinical evaluation of patients with suspected AFM

Initial clinical assessment

- Consider AFM in patients presenting with rapid-onset weakness, particularly when occurring during or shortly following a suspected viral illness.
- Complete neurological examination should include specific tests for proximal muscle weakness (such as standing up from a seated position on the floor), axial weakness (neck and trunk flexion and extension), and cranial nerve abnormalities.
- Clinical features atypical for AFM include encephalopathy unrelated to metabolic disturbance, seizures, extensive sensory abnormalities, or evolution to nadir over more than 10 days.
- Neurology and infectious disease specialists should be consulted (where available) to help with diagnosis, evaluation, and treatment.
- Admission to intensive care unit should be considered when indicated, and close monitoring for respiratory or autonomic deterioration, or both, is essential.

Radiological evaluation

- MRI whole spine and brain should be prioritised, including T2 and T1 pre-contrast and post-contrast sequences in both axial and sagittal planes.
- The characteristic MRI abnormality is grey-matter predominant T2 hyperintensity of the spinal cord with associated spinal cord oedema; lesion(s) are usually longitudinally extensive and non-enhancing. Nerve root enhancement might be present.
- Repeat MRI can be considered after further clinical evolution in patients with a suggestive clinical presentation but in whom early MRI of the spinal cord is apparently normal.

Laboratory evaluation

- Obtain specimens as soon as possible (ie, within hours of clinical presentation).
- Respiratory samples (both nasopharyngeal and oropharyngeal): respiratory viral RT-PCR testing (to include enterovirus RT-PCR). When possible, a positive enterovirus RT-PCR result should be subtyped (to include enterovirus D68, enterovirus A71, and other common subtypes).
- Stool samples or rectal swab: enterovirus RT-PCR, viral culture for poliovirus when epidemiologically relevant (with RT-PCR of isolated virus to differentiate between wild-type and vaccine-derived virus).
- Blood sample: microbiological tests (enterovirus RT-PCR and other epidemiologically appropriate micro-organism tests—eg, West Nile virus serology), and testing for specific alternative myelopathy diagnoses to include MOG IgG and aquaporin-4 IgG.
- CSF sample: cell counts, protein, glucose, oligoclonal bands, enterovirus RT-PCR (although yield is very low), and other epidemiologically appropriate micro-organism tests.
- When RT-PCR is not readily available, samples can still be acquired and frozen for future analysis or transfer to public health authorities.
- Respiratory, stool, serum, and CSF samples should also be sent to the relevant public health authorities, according to local protocols.

Low-resource settings

- When MRI is not possible, rapid completion of available laboratory testing should be prioritised (CSF analysis, microbiological sampling), and EMG/NCS can be incorporated in the initial evaluation when available.

AFM=acute flaccid myelitis. MOG=myelin oligodendrocyte glycoprotein. CSF=cerebrospinal fluid. EMG/NCS=electromyography or nerve conduction studies.

Panel 2

Acute management of patients with suspected AFM

Respiratory status

• Patients with respiratory muscle weakness are at risk of hypoventilatory respiratory failure. Bulbar muscle weakness can lead to inability to protect the airway.

• Poor head control, drooling, proximal upper limb weakness, neck weakness, or altered voice quality suggest a risk of respiratory failure. Settings without intensive care facilities should consider transfer of patients with risk of evolving respiratory failure to higher level of care institutions.

• Respiratory function should be assessed every 4 h until clinical stabilisation. Monitoring may include testing of negative inspiratory force, vital capacity, oxygen saturation levels, and blood gas analysis (to detect evolving hypercarbia).

• The possibility of concomitant respiratory tract infection should be considered and treated as appropriate.

• Typical thresholds for non-invasive ventilation or intubation in patients with neuromuscular weakness or bulbar weakness should be applied.

• Tracheostomy can be considered in patients requiring prolonged intubation.

Sedation

• Sedation for procedures (eg, MRI) carries a risk of respiratory decompensation; patients should be closely monitored and short-acting agents used when possible.

• Where intubation is required, medications with the least effect on respiration should be used—eg, dexmedetomidine.

Pain and autonomic dysfunction

• Neuropathic pain is frequent and should be treated. In sedated patients or young children, pain might be recognised by irritability, tachycardia, and refusal to move.

• Bladder function should be assessed with post-void residual volumes. Urinary catheterisation might be required.

• Constipation is common and should be treated appropriately.

• Autonomic involvement may manifest with hypertension, labile blood pressure, diaphoresis, and even cardiac arrhythmia, requiring close monitoring and treatment.

Immunomodulatory therapies or antiviral therapies

• With little evidence regarding potential benefit or harm of therapies in humans, no standardised pharmacological treatment can be universally recommended.

• A common, but unproven, approach is to provide intravenous immunoglobulin during the acute phase, which might provide anti-enteroviral neutralising antibodies, with minimal potential harm.

• In low-resource settings where differentiating between AFM and immune-mediated myelitis can be challenging (eg, because of no MRI and a clinical presentation that is not wholly typical), a trial of steroids can be considered on an individualised basis.

Early rehabilitation

• Physical, occupational, and speech therapy should be commenced early.

• Consider early initiation of electrical stimulation therapy to minimise disuse muscle atrophy.

• Psychological support should be provided to assist the child and family with coping and adjustment.

• In settings with limited rehabilitation resources, early mobilisation and activity-based therapy should still be encouraged.

AFM=acute flaccid myelitis.

Panel 3

Rehabilitation and long-term clinical care of patients with AFM

Inpatient rehabilitation

- After the acute phase of AFM, medically stable children with significant residual neurological deficits should transfer to an inpatient rehabilitation programme with a multidisciplinary team.
- Although specific evidence regarding rehabilitation in AFM is minimal, the approach can draw on methods used in other monophasic neurological injuries (eg, spinal cord injury) and in other motor neuronopathies (eg, poliomyelitis, Guillain-Barré syndrome).
- Intensive rehabilitation should include short-term goals to facilitate developmentally appropriate functional independence and use of compensatory devices, while simultaneously working towards long-term goals for recovery of function and avoidance of musculoskeletal complications.
- Intensive activity-based therapy can include weight loading of limbs, massed practice, and task-specific practice.
- Locomotor gait training or functional electrical stimulation, or both, can be used when available, although data supporting the specific effect of these approaches on AFM outcomes are scarce.
- Consider orthotic devices, mobility equipment, assistive technology, identification of home care needs, a plan for school and community re-entry, psychosocial support, and education for the child and family.
- In low-resource settings with little access to skilled therapy, education of patients and care-givers regarding home-based activities is essential.

Nerve and tendon transfer surgery

- Patients with poor recovery in an affected muscle group 3 months or longer after onset of AFM should be considered for potential nerve or tendon transfer surgery, or both, by a centre experienced in the relevant procedures (where available).
- Experience with nerve and tendon transfers in the upper extremity has shown promising results in appropriately selected patients with AFM.
- Phrenic nerve transfer, lower extremity nerve transfers, or pacing of the phrenic nerve or diaphragm can also be considered in selected patients, although data on outcomes are scarce.
- The appropriate timing for nerve transfer surgery is uncertain, but a delay in consideration could result in a missed window of opportunity (because muscle viability wanes with extended periods of denervation). Tendon-transfer surgery is not time sensitive and can be completed months or years after the initial injury.
- EMG/NCS can aid in the planning of nerve transfer surgery, and should include evaluation of the donor nerve and acceptor muscle.

Medium-term to long-term rehabilitation

- After home discharge, continued rehabilitation with periodic skilled therapy should be provided to achieve acquisition of developmentally appropriate milestones and functional independence.
- Educational and developmental transitions, age-appropriate self-advocacy skills, increasing independence in self-care, and responsibility for medical management will aid successful transition to adulthood.

Long-term medical management

- Patients should continue vaccination protocols according to national guidelines (including delayed live vaccinations if intravenous immunoglobulin has been administered).
- Long-term follow-up should be provided by neurology and physiatry or rehabilitation medicine services where available, alongside primary care.
- Specialist input might be required to manage complications such as joint contracture, scoliosis, shoulder or hip subluxation, limb length difference, and loss of bone mineral density.
- Children requiring long-term ventilatory support or artificial nutrition will require additional specialist input.

AFM=acute flaccid myelitis. EMG/NCS=electromyography or nerve conduction studies.

Declaration of interests

CAP, LB, BG, KM, and CLS are unpaid advisors to the AFM Task Force of the US Centers for Disease Control and Prevention (CDC). ML serves (unpaid) on the UK AFP Task Force and is the lead for the Clinical Working Group. SEH, CO, and GYG receive salary support from the CDC for AFM surveillance. LB reports research support from Biogen outside the submitted work, and is serving as a consultant to the national vaccine injury compensation programme. RB reports grants from Akili interactive; and personal fees from EMD Serono, Roche Genentech, Novartis, Alexion, Sanofi Genzyme, and Biogen, outside the submitted work. JDes reports receiving funds from EFGLA, UCB, Novartis, Ovid, Aquestive, and Neurelis, and for serving on advisory boards as medico-legal expert. BG reports grants from National Institutes of Health, National MS Society, Siegel Rare Neuroimmune Association, Guthy Jackson Charitable Foundation, and CLENE Nanomedicine; personal fees from Novartis, Genentech, EMD Serono, and Alexion, outside the submitted work; and serves as board member to Siegel Rare Neuroimmune Association. RK reports grants from Ministry of Health, Labour and Welfare of Japan for AFM study, and personal fees from Eisai, Otsuka Pharmaceutical, and UCB Japan, outside the submitted work. ML receives research grants from Action Medical Research, the Dancing Eyes Syndrome society, Great Ormond Street Hospital Children's (GOSH) charity, National Institute for Health Research, MS Society, and Sparks charity; receives research support grants from the London Clinical Research Network and Evelina Appeal; has received consultation fees from CSL Behring, Novartis, and Octapharma; has received travel grants from Merck Serono; and was awarded educational grants to organise meetings by Novartis, Biogen Idec, Merck Serono, and Bayer. KM reports grants from the National Institute of Allergy and Infectious Diseases, outside the submitted work. TLS reports to have received a stipend from the American Academy of Neurology for lecturing on AFM. KTT reports grants by the National Institute of Health. EAY reports grants from the Canadian Network of MS Clinics, during the conduct of the study; grants from Biogen, National MS society, Consortium of Multiple Sclerosis Centers, MS Society of Canada/MS Foundation, Ontario Institute for Regenerative Medicine, Stem Cell Network, Sickkids Foundation, Centre for Brain and Mental Health, TEVA Pharmaceuticals, and Guthy Jackson Foundation; and personal fees from ACI Clinical, US Food and Drug Administration, and Juno, outside the submitted work. CAP reports grants from the National Institute of Health and Bart McLean Fund for Neuroimmunology Research, serves as co-investigator in

the Natural History Study of Acute Flaccid Myelitis, and serves as a member of the National Institute of Health Scientific Board of the Siegel Rare Neuroimmune Association. All other authors declare no competing interests.

Acknowledgments

We are grateful for the support provided by the Siegel Rare Neuroimmune Association (SRNA) for facilitating administrative support to the AFM Working Group, and the Bart McLean Fund for Neuroimmunology Research for AFM research. We thank Janell A Routh and Sarah E Kidd at the Centers for Disease Control and Prevention for sharing their expertise. OCM was funded through the SRNA James T Lubin Fellowship. CAP is supported by National Institutes of Health (R01 NS108358) and the Bart McLean Fund for Neuroimmunology Research. KM is supported by National Institutes of Health (K23 AI128069). KTT is supported by National Institutes of Health (1K23NS105935).

REFERENCES

1. Haren K Van, Ayscue P, Waubant E, Clayton A, Sheriff H, Yagi S, et al. Acute Flaccid Myelitis of Unknown Etiology in California, 2012-2015. Jama. 2015 Dec;314(24):2663–71.
2. Messacar K, Schreiner TL, Maloney JA, Wallace A, Ludke J, Oberste MS, 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 (London, England). 2015 Apr;385(9978):1662–71.
3. Knoester M, Helfferich J, Poelman R, Van Leer-Buter C, Brouwer OF, Niesters HGM. Twenty-nine Cases of Enterovirus-D68-associated Acute Flaccid Myelitis in Europe 2016: A Case Series and Epidemiologic Overview. Pediatr Infect Dis J. 2019;38(1).
4. Force TUKAFPAT. An increase in reports of acute flaccid paralysis (AFP) in the United Kingdom, 1 January 2018-21 January 2019: early findings. Euro Surveill [Internet]. 2019 Feb 7;24(6):1900093. Available from: https://www.ncbi.nlm.nih.gov/pubmed/30755296
5. Chong PF, Kira R, Mori H, Okumura A, Torisu H, Yasumoto S, et al. 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. Clin Infect Dis. 2018 Feb;66(5):653–64.
6. Sarmast SN, Gowda VK, Ahmed M, Basvaraja G V, Saini J, Benakappa A. Acute flaccid myelitis-Clustering of polio-like illness in the tertiary care centre in Southern India. J Trop Pediatr. 2018 Aug;fmy052.
7. Chen IJ, Hu SC, Hung KL, Lo CW. Acute flaccid myelitis associated with enterovirus D68 infection: A case report. Medicine (Baltimore). 2018 Sep;97(36):e11831.
8. Andersen EW, Kornberg AJ, Freeman JL, Leventer RJ, Ryan MM. Acute flaccid myelitis in childhood: a retrospective cohort study. Eur J Neurol. 2017 Aug;24(8):1077–83.
9. Fall A, Ndiaye N, Jallow MM, Barry MA, Touré CSB, Kebe O, et al. Enterovirus D68 Subclade B3 Circulation in Senegal, 2016: Detection from Influenza-like Illness and Acute Flaccid Paralysis Surveillance. Sci Rep. 2019 Sep;9(1):13881.
10. Crone M, Tellier R, Wei XC, Kuhn S, Vanderkooi OG, Kim J, et al. Polio-Like Illness Associated With Outbreak of Upper Respiratory Tract Infection in Children. J Child Neurol. 2016 Mar;31(4):409–14.
11. Centers for Disease Control and Prevention. AFM cases in the United States. 2020.
12. Ruggieri V, Paz MI, Peretti MG, Rugilo C, Bologna R, Freire C, et al. Enterovirus D68 infection in a cluster of children with acute flaccid myelitis, Buenos Aires, Argentina, 2016. Eur J Paediatr Neurol. 2017 Jul;21(6):884–90.
13. Carballo CM, Erro MG, Sordelli N, Vazquez G, Mistchenko AS, Cejas C, et al. Acute Flaccid Myelitis Associated with Enterovirus D68 in Children, Argentina, 2016. Emerg Infect Dis. 2019 Mar;25(3):573–6.
14. Messacar K, Asturias EJ, Hixon AM, Leer-Buter C Van, Niesters HGM, Tyler KL, et al. Enterovirus D68 and acute flaccid myelitis-evaluating the evidence for causality. LancetInfectious Dis. 2018 Feb;
15. McLaren N, Lopez A, Kidd S, Zhang JX, Nix WA, Link-Gelles R, et al. Characteristics of Patients with Acute Flaccid Myelitis, United States, 2015-2018. Emerg Infect Dis. 2020 Feb;26(2):212–9.
16. Counselors B of scientific. National overview of acute flaccid myelitis —United States, 2014–2018 [Internet]. [cited 2020 Aug 18]. Available from: https://www.cdc.gov/ddid/bsc/afm-overview-2018.html
17. Messacar K, Schreiner TL, Haren K Van, Yang M, Glaser CA, Tyler KL, et al. Acute flaccid myelitis: A clinical review of US cases 2012-2015. Ann Neurol. 2016 Sep;80(3):326–38.
18. Bove R, Rowles W, Carleton M, Olivera E, Sheehan M, Werdal HP, et al. Unmet Needs in the Evaluation, Treatment, and Recovery for 167 Children Affected by Acute Flaccid Myelitis Reported by Parents Through Social Media. Pediatr Neurol. 2020 Jan;102:20–7.

19. Melicosta ME, Dean J, Hagen K, Oppenheimer K, Porter C, Rybczynski S, et al. Acute flaccid myelitis: Rehabilitation challenges and outcomes in a pediatric cohort. J Pediatr Rehabil Med. 2019;12(3):245–53.

20. Martin JA, Messacar K, Yang ML, Maloney JA, Lindwall J, Carry T, et al. Outcomes of Colorado children with acute flaccid myelitis at 1 year. Neurology. 2017 Jul;89(2):129–37.

21. Saltzman EB, Rancy SK, Sneag DB, Md JHF, Lange DJ, Wolfe SW. Nerve Transfers for Enterovirus D68-Associated Acute Flaccid Myelitis: A Case Series. Pediatr Neurol. 2018 Nov;88:25–30.

22. Pino PA, Intravia J, Kozin SH, Zlotolow DA. Early results of nerve transfers for restoring function in severe cases of acute flaccid myelitis. Ann Neurol. 2019 Oct;86(4):607–15.

23. Messacar K, Robinson CC, Pretty K, Yuan J, Dominguez SR. Surveillance for enterovirus D68 in colorado children reveals continued circulation. J Clin Virol. 2017 Jul;92:39–41.

24. Greninger AL, Naccache SN, Messacar K, Clayton A, Yu G, Somasekar S, et al. A novel outbreak enterovirus D68 strain associated with acute flaccid myelitis cases in the USA (2012-14): a retrospective cohort study. LancetInfectious Dis. 2015 Jun;15(6):671–82.

25. Brown DM, Hixon AM, Oldfield LM, Zhang Y, Novotny M, Wang W, et al. Contemporary Circulating Enterovirus D68 Strains Have Acquired the Capacity for Viral Entry and Replication in Human Neuronal Cells. MBio. 2018 Oct;9(5):10.1128/mBio.01954-18.

26. Uprety P, Curtis D, Elkan M, Fink J, Rajagopalan R, Zhao C, et al. Association of Enterovirus D68 with Acute Flaccid Myelitis, Philadelphia, Pennsylvania, USA, 2009-2018. Emerg Infect Dis. 2019 Sep;25(9):1676–82.

27. Mishra N, Ng TFF, Marine RL, Jain K, Ng J, Thakkar R, et al. Antibodies to Enteroviruses in Cerebrospinal Fluid of Patients with Acute Flaccid Myelitis. MBio. 2019 Aug;10(4).

28. Schubert RD, Hawes IA, Ramachandran PS, Ramesh A, Crawford ED, Pak JE, et al. Pan-viral serology implicates enteroviruses in acute flaccid myelitis. Nat Med. 2019 Nov;25(11):1748–52.

29. Hixon AM, Yu G, Leser JS, Yagi S, Clarke P, Chiu CY, et al. A mouse model of paralytic myelitis caused by enterovirus D68. PLoS Pathog. 2017 Feb;13(2):e1006199.

30. Sun S, Bian L, Gao F, Du R, Hu Y, Fu Y, et al. A neonatal mouse model of Enterovirus D68 infection induces both interstitial pneumonia and acute flaccid myelitis. Antiviral Res. 2019 Jan;161:108–15.

31. Midgley CM, Watson JT, Nix WA, Curns AT, Rogers SL, Brown BA, et al. Severe respiratory illness associated with a nationwide outbreak of enterovirus D68 in the USA (2014): a descriptive epidemiological investigation. LancetRespiratory Med. 2015 Nov;3(11):879–87.

32. Kramer R, Sabatier M, Wirth T, Pichon M, Lina B, Schuffenecker I, et al. Molecular diversity and biennial circulation of enterovirus D68: a systematic screening study in Lyon, France, 2010 to 2016. Euro Surveill. 2018 Sep;23(37):10.2807/1560-7917.ES.2018.23.37.1700711.

33. Menant J, Gandevia S. Poliomyelitis. In: Day B, Lord S, editors. Handbook of clinical neurology. Elsevier Health Sciences; 2018. p. 337–44.

34. Chang LY, Lin HY, Gau SSF, Lu CY, Hsia SH, Huang YC, et al. Enterovirus A71 neurologic complications and long-term sequelae. J Biomed Sci. 2019 Aug;26(1):57.

35. BODIAN D. Histopathologic basis of clinical findings in poliomyelitis. Am J Med. 1949 May;6(5):563–78.

36. Hixon AM, Clarke P, Tyler KL. Contemporary circulating enterovirus D68 strains infect and undergo retrograde axonal transport in spinal motor neurons independent of sialic acid. J Virol. 2019 Jun;

37. Rosenfeld AB, Warren AL, Racaniello VR. Neurotropism of Enterovirus D68 Isolates Is Independent of Sialic Acid and Is Not a Recently Acquired Phenotype. MBio. 2019 Oct;10(5).

38. Ayers T, Lopez A, Lee A, Kambhampati A, Nix WA, Henderson E, et al. Acute Flaccid Myelitis in the United States: 2015-2017. Pediatrics. 2019 Nov;144(5).

39. Messacar K, Burakoff A, Nix WA, Rogers S, Oberste MS, Gerber SI, et al. Notes from the Field: Enterovirus A71 Neurologic Disease in Children - Colorado, 2018. MMWR Morb Mortal Wkly Rep. 2018 Sep;67(36):1017–8.

40. Puenpa J, Wanlapakorn N, Vongpunsawad S, Poovorawan Y. The History of Enterovirus A71 Outbreaks and Molecular Epidemiology in the Asia-Pacific Region. J Biomed Sci. 2019 Oct;26(1):75.

41. Messacar K, Spence-Davizon E, Osborne C, Press C, Schreiner TL, Martin J, et al. Clinical characteristics of enterovirus A71 neurological disease during an outbreak in children in Colorado, USA, in 2018: an observational cohort study. Lancet Infect Dis. 2020 Feb;20(2):230–9.

42. Casas-Alba D, de Sevilla MF, Valero-Rello A, Fortuny C, García-García JJ, Ortez C, et al. Outbreak of brainstem encephalitis associated with enterovirus-A71 in Catalonia, Spain (2016): a clinical observational study in a children's reference centre in Catalonia. Clin Microbiol Infect Off Publ Eur Soc Clin Microbiol Infect Dis. 2017 Nov;23(11):874–81.

43. Sejvar JJ, Bode A V, Marfin AA, Campbell GL, Ewing D, Mazowiecki M, et al. West Nile virus-associated flaccid paralysis. Emerg Infect Dis. 2005 Jul;11(7):1021–7.

44. Centers for Disease Control and Prevention. West Nile virus [Internet]. [cited 2020 Aug 18]. Available from: https://www.cdc.gov/westnile/healthcareproviders/healthCareProviders-Diagnostic.html

45. Giombini E, Rueca M, Barberi W, Iori AP, Inghilleri M, Castilletti C, et al. Enterovirus D68-Associated Acute Flaccid Myelitis in Immunocompromised Woman, Italy. Emerg Infect Dis. 2017 Oct;23(10):1690–3.

46. Gordon-Lipkin E, Munoz LS, Klein JL, Dean J, Izbudak I, Pardo CA. Comparative quantitative clinical, neuroimaging, and functional profiles in children with acute flaccid myelitis at acute and convalescent stages of disease. Dev Med Child Neurol. 2018 Sep;61(3):366–75.

47. Nelson GR, Bonkowsky JL, Doll E, Green M, Hedlund GL, Moore KR, et al. Recognition and Management of Acute Flaccid Myelitis in Children. Pediatr Neurol. 2016 Feb;55:17–21.

48. Yea C, Bitnun A, Robinson J, Mineyko A, Barton M, Mah JK, et al. Longitudinal Outcomes in the 2014 Acute Flaccid Paralysis Cluster in Canada. J Child Neurol. 2017 Mar;32(3):301–7.

49. Organization WH. Global polio eradication initiative [Internet]. [cited 2020 Aug 18]. Available from: http://polioeradication.org/polio-today/polio-now/this-week/

50. Turtle L, Solomon T. Japanese encephalitis - the prospects for new treatments. Nat Rev Neurol. 2018 Apr;14(5):298–313.

51. Sejvar JJ. Clinical manifestations and outcomes of West Nile virus infection. Viruses. 2014 Feb;6(2):606–23.

52. Lindquist L, Vapalahti O. Tick-borne encephalitis. Lancet (London, England). 2008 May;371(9627):1861–71.

53. Islam Z, Jacobs BC, Islam MB, Mohammad QD, Diorditsa S, Endtz HP. High incidence of Guillain-Barre syndrome in children, Bangladesh. Vol. 17, Emerging infectious diseases. United States; 2011. p. 1317–8.

54. Ho TW, Mishu B, Li CY, Gao CY, Cornblath DR, Griffin JW, et al. Guillain-Barré syndrome in northern China. Relationship to Campylobacter jejuni infection and anti-glycolipid antibodies. Brain. 1995 Jun;118 (Pt 3):597–605.

55. Doets AY, Verboon C, van den Berg B, Harbo T, Cornblath DR, Willison HJ, et al. Regional variation of Guillain-Barré syndrome. Brain. 2018 Oct;141(10):2866–77.

56. Willison HJ, Jacobs BC, van Doorn PA. Guillain-Barre syndrome. Lancet (London, England). 2016 Aug;388(10045):717–27.

57. Wang C, Narayan R, Greenberg B. Anti-Myelin Oligodendrocyte Glycoprotein Antibody Associated With Gray Matter Predominant Transverse Myelitis Mimicking Acute Flaccid Myelitis: A Presentation of Two Cases. Pediatr Neurol. 2018 Sep;86:42–5.

58. Dubey D, Pittock SJ, Krecke KN, Morris PP, Sechi E, Zalewski NL, et al. Clinical, Radiologic, and Prognostic Features of Myelitis Associated With Myelin Oligodendrocyte Glycoprotein Autoantibody. JAMA Neurol. 2019 Mar;76(3):301–9.

59. Elrick MJ, Gordon-Lipkin E, Crawford TO, Van Haren K, Messacar K, Thornton N, et al. Clinical Subpopulations in a Sample of North American Children Diagnosed With Acute Flaccid Myelitis, 2012-2016. JAMA Pediatr [Internet]. 2019 Feb 1;173(2):134–9. Available from: https://doi.org/10.1001/jamapediatrics.2018.4890

60. Zalewski NL, Rabinstein AA, Krecke KN, Brown RDJ, Wijdicks EFM, Weinshenker BG, et al. Characteristics of Spontaneous Spinal Cord Infarction and Proposed Diagnostic Criteria. JAMA Neurol. 2019 Jan;76(1):56–63.

61. Okumura A, Mori H, Fee Chong P, Kira R, Torisu H, Yasumoto S, et al. Serial MRI findings of acute flaccid myelitis during an outbreak of enterovirus D68 infection in Japan. Brain Dev. 2019 May;41(5):443–51.

62. Maloney JA, Mirsky DM, Messacar K, Dominguez SR, Schreiner T, Stence N V. MRI Findings in Children with Acute Flaccid Paralysis and Cranial Nerve Dysfunction Occurring during the 2014 Enterovirus D68 Outbreak. Am J Neuroradiol [Internet]. 2014 Nov 20;36(2):245–50. Available from: http://www.ajnr.org/content/early/2014/11/20/ajnr.A4188.abstract

63. Chong PF, Yoshida T, Yuasa S, Mori H, Tanaka-Taya K, Kira R. Acute Flaccid Myelitis With Neuroradiological Finding of Brachial Plexus Swelling. Pediatr Neurol. 2020 Aug;109:85–8.

64. McCoy DB, Talbott JF, Wilson M, Mamlouk MD, Cohen-Adad J, Wilson M, et al. MRI Atlas-Based Measurement of Spinal Cord Injury Predicts Outcome in Acute Flaccid Myelitis. AJNRAmerican J Neuroradiol. 2017 Feb;38(2):410–7.

65. Waters PJ, McKeon A, Leite MI, Rajasekharan S, Lennon VA, Villalobos A, et al. Serologic diagnosis of NMO: a multicenter comparison of aquaporin-4-IgG assays. Neurology. 2012 Feb;78(9):665–71; discussion 669.

66. McKeon A, Fryer JP, Apiwattanakul M, Lennon VA, Hinson SR, Kryzer TJ, et al. Diagnosis of neuromyelitis spectrum disorders: comparative sensitivities and specificities of immunohistochemical and immunoprecipitation assays. Arch Neurol. 2009 Sep;66(9):1134–8.

67. Reindl M, Waters P. Myelin oligodendrocyte glycoprotein antibodies in neurological disease. Nat Rev Neurol. 2019 Feb;15(2):89–102.

68. Waters PJ, Komorowski L, Woodhall M, Lederer S, Majed M, Fryer J, et al. A multicenter comparison of MOG-IgG cell-based assays. Neurology. 2019 Mar;92(11):e1250–5.

69. Matà S, Betti E, Masotti G, Pinto F, Lolli F. Motor nerve damage is associated with anti-ganglioside antibodies in diabetes. J Peripher Nerv Syst. 2004 Sep;9(3):138–43.

70. Grüter T, Ott A, Meyer W, Jarius S, Kinner M, Motte J, et al. Effects of IVIg treatment on autoantibody testing in neurological patients: marked reduction in sensitivity but reliable specificity. J Neurol. 2020 Mar;267(3):715–20.

71. Hovden IA, Pfeiffer HC. Electrodiagnostic findings in acute flaccid myelitis related to enterovirus D68. Muscle Nerve. 2015 Nov;52(5):909–10.

72. Kuwabara S, Yuki N. Axonal Guillain-Barré syndrome: concepts and controversies. Lancet Neurol. 2013 Dec;12(12):1180–8.

73. Kokubun N, Nishibayashi M, Uncini A, Odaka M, Hirata K, Yuki N. Conduction block in acute motor axonal neuropathy. Brain. 2010 Oct;133(10):2897–908.

74. Peediackal S, Jose J, Gafoor VA, Smitha B. Reversible conduction failure in acute motor axonal neuropathy. Ann Indian Acad Neurol. 2014 Jan;17(1):142–3.

75. Centers for Disease Control and Prevention. Acute flaccid myelitis: clinical management [Internet]. [cited 2020 Aug 18]. Available from: https://www.cdc.gov/acute-flaccid-myelitis/hcp/clinical-management.html

76. Zhang Y, Moore DD, Nix WA, Oberste MS, Weldon WC. Neutralization of Enterovirus D68 isolated from the 2014 US outbreak by commercial intravenous immune globulin products. J Clin Virol Off Publ Pan Am Soc Clin Virol. 2015 Aug;69:172–5.

77. Hixon AM, Clarke P, Tyler KL. Evaluating Treatment Efficacy in a Mouse Model of Enterovirus D68-Associated Paralytic Myelitis. J Infect Dis. 2017 Dec;216(10):1245–53.

78. Rhoden E, Zhang M, Nix WA, Oberste MS. In Vitro Efficacy of Antiviral Compounds against Enterovirus D68. Antimicrob Agents Chemother. 2015 Dec;59(12):7779–81.

79. Messacar K, Sillau S, Hopkins SE, Otten C, Wilson-Murphy M, Wong B, et al. Safety, tolerability, and efficacy of fluoxetine as an antiviral for acute flaccid myelitis. Neurology. 2018 Nov;92(18):e2118–26.

80. Vogt MR, Fu J, Kose N, Williamson LE, Bombardi R, Setliff I, et al. Human antibodies neutralize enterovirus D68 and protect against infection and paralytic disease. Sci Immunol. 2020 Jul;5(49).

81. Musharrafieh R, Ma C, Zhang J, Hu Y, Diesing JM, Marty MT, et al. Validating Enterovirus D68-2A(pro) as an Antiviral Drug Target and the Discovery of Telaprevir as a Potent D68-2A(pro) Inhibitor. J Virol. 2019 Apr;93(7).

82. Musharrafieh R, Zhang J, Tuohy P, Kitamura N, Bellampalli SS, Hu Y, et al. Discovery of Quinoline Analogues as Potent Antivirals against Enterovirus D68 (EV-D68). J Med Chem. 2019 Apr;62(8):4074–90.

83. Kirolos A, Mark K, Shetty J, Chinchankar N, Mcdougall C, Eunson P, et al. Outcome of paediatric acute flaccid myelitis associated with enterovirus D68: a case series. Dev Med Child Neurol. 2018 Nov;61(3):376–80.

84. Doi K, Sem SH, Hattori Y, Sakamoto S, Hayashi K, De la Red-Gallego MA. Surgical Reconstruction for Upper-Extremity Paralysis Following Acute Flaccid Myelitis. JB JS open access. 2019;4(4):e0030.

85. Edmiston TL, Elrick MJ, Kovler ML, Jelin EB, Onders RP, Sadowsky CL. Early use of an implantable diaphragm pacing stimulator for a child with severe acute flaccid myelitis-a case report. Vol. 5, Spinal cord series and cases. England; 2019. p. 67.

86. Dahan V, Kimoff RJ, Petrof BJ, Benedetti A, Diorio D, Trojan DA. Sleep-disordered breathing in fatigued postpoliomyelitis clinic patients. Arch Phys Med Rehabil. 2006 Oct;87(10):1352–6.

87. Kay L, Nielsen NM, Wanscher B, Jennum P. Neurological Symptoms in Danes with a History of Poliomyelitis: Lifelong Follow-Up of Late Symptoms, their Association with Initial Symptoms of Polio, and Presence of Postpolio Syndrome. Eur Neurol. 2018;80(5–6):295–303.

88. Darras BT. Spinal muscular atrophies. Pediatr Clin North Am. 2015 Jun;62(3):743–66.

89. Haziza M, Kremer R, Benedetti A, Trojan DA. Osteoporosis in a postpolio clinic population. Arch Phys Med Rehabil. 2007 Aug;88(8):1030–5.

90. Nash MS, Bilzon JLJ. Guideline Approaches for Cardioendocrine Disease Surveillance and Treatment Following Spinal Cord Injury. Curr Phys Med Rehabil reports. 2018;6(4):264–76.

91. Arens R, Muzumdar H. Sleep, sleep disordered breathing, and nocturnal hypoventilation in children with neuromuscular diseases. Paediatr Respir Rev. 2010 Mar;11(1):24–30.

92. Tsai HC, Hung TH, Chen CC, Lieu FK, Cho H, Tung TH, et al. Prevalence and risk factors for upper extremity entrapment neuropathies in polio survivors. J Rehabil Med. 2009 Jan;41(1):26–31.

93. Kidd D, Howard RS, Williams AJ, Heatley FW, Panayiotopoulos CP, Spencer GT. Late functional deterioration following paralytic poliomyelitis. QJM. 1997 Mar;90(3):189–96.

94. Dalakas MC, Elder G, Hallett M, Ravits J, Baker M, Papadopoulos N, et al. A long-term follow-up study of patients with post-poliomyelitis neuromuscular symptoms. N Engl J Med. 1986 Apr;314(15):959–63.

8

PEDIATRIC ACUTE FLACCID MYELITIS: EVALUATION OF DIAGNOSTIC CRITERIA AND DIFFERENTIATION FROM OTHER CAUSES OF ACUTE FLACCID PARALYSIS

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Eur J Paediatr Neurol 2023 May;44:28-36. doi: 10.1016/j.ejpn.2023.03.002

ABSTRACT

Background

Acute flaccid paralysis (AFP) is characterized by rapidly progressive limb weakness with low muscle tone. It has a broad differential diagnosis, which includes acute flaccid myelitis (AFM), a rare polio-like condition that mainly affects young children. Differentiation between AFM and other causes of AFP may be difficult, particularly at onset of disease. Here, we evaluate the diagnostic criteria for AFM and compare AFM to other causes of acute weakness in children, aiming to identify differentiating clinical and diagnostic features.

Methods

The diagnostic criteria for AFM were applied to a cohort of children with acute onset of limb weakness. An initial classification based on positive diagnostic criteria was compared to the final classification, based on application of features suggestive for an alternative diagnosis and discussion with expert neurologists. Cases classified as definite, probable, or possible AFM or uncertain, were compared to cases with an alternative diagnosis.

Results

Of 141 patients, seven out of nine patients initially classified as definite AFM, retained this label after further classification. For probable AFM, this was 3/11, for possible AFM 3/14 and for uncertain 11/43. Patients initially classified as probable or possible AFM were most commonly diagnosed with transverse myelitis (16/25). If the initial classification was uncertain, Guillain-Barré syndrome was the most common diagnosis (31/43). Clinical and diagnostic features not included in the diagnostic criteria, were often used for the final classification.

Conclusion

The current diagnostic criteria for AFM usually perform well, but additional features are sometimes required to distinguish AFM from other conditions.

INTRODUCTION

Acute flaccid myelitis (AFM) is characterized by rapidly progressive flaccid weakness of the limbs, caused by damage of anterior horn cells in the spinal cord. Young children are mostly affected [1–3]. According to current diagnostic criteria, MRI abnormalities in the grey matter of the spinal cord and pleocytosis in cerebrospinal fluid (CSF) are required to make a definite diagnosis of AFM [4].

In typical cases, limb weakness develops over several days and is preceded by a respiratory illness. While limb weakness is required for the diagnosis, respiratory and cranial muscles are also frequently involved [1,5]. Recovery is often incomplete with severe residual deficits being common in affected patients [6].

Different viruses may cause AFM, probably by invasion of the anterior horn cells [2,3]. Poliomyelitis, caused by poliovirus, may fulfill the clinical criteria for AFM and was the most common cause before the implementation of effective vaccination strategies.[7] However, since 2014 cases have frequently been associated with non-polio enteroviruses, in particular enterovirus D68 (EV-D68) and A71 (EV-A71) [5,8–10],

AFM is included in the broad differential diagnosis of acute flaccid paralysis (AFP), which covers other disorders of peripheral motor neurons and innervated muscles, including Guillain-Barré syndrome (GBS), toxic neuropathy or myopathy and botulism. However, central motor neuron disorders may also present with flaccid limb weakness, especially in the acute phase. These include transverse myelitis (TM), acute disseminated encephalomyelitis (ADEM), spinal cord ischemia and acute spinal cord compression [11]. Similarly to AFM, GBS as well as TM and ADEM may be preceded by a prodromal illness [12]. Also, in both TM and ADEM longitudinally extensive lesions of the spinal cord on MRI and CSF pleocytosis are commonly found [13,14]. Because of this clinical and diagnostic overlap between AFM and GBS, TM and ADEM, differentiation of these disorders may be particularly difficult, especially early in the disease course [12,15–17].

In a child with AFP, it is important to consider AFM early in the disease course. This enables the performance of early and proper investigations, which are required to confirm the diagnosis of AFM. Also, associated viruses are best identified early in the disease course, if appropriate sampling is performed [4]. Furthermore, patients with AFM may show rapid clinical deterioration, urging clinical monitoring [17,18]. Lastly, in the mouse model of AFM, early administration of immunoglobulin improved outcome, and administration of

monoclonal antibodies against specific strains of EV-D68 was effective in inhibiting progression of muscle weakness even several days after onset [19,20]. Also, in this mouse model, treatment with steroids was associated with deterioration of weakness [20]. While these findings need confirmation, these studies suggest that early treatment with immunoglobulin may be beneficial, whereas steroids may have negative effects. An early diagnosis will be required to investigate the effects of treatment in children with AFM.

To test the clinical usefulness of the present diagnostic criteria for AFM, we evaluated their application in a real-world cohort of children with acute onset limb weakness. By doing this, we aimed to identify both clinical and diagnostic features suggestive for AFM, or indicative for an alternative diagnosis.

METHODS

Study population

The study population consists of a cohort of children with acute onset weakness, diagnosed between January 2014 and December 2019, previously used to estimate the incidence of AFM in children (<18 years) in the Netherlands [21]. These children had been identified by searching electronic health care data systems of ten hospitals in The Netherlands for specific diagnostic codes (ICD and DBC), related to acute weakness and/or infection. Children without weakness or with a clear diagnosis other than AFM, such as a genetic disease (e.g., spinal muscular atrophy) or structural abnormalities (e.g., traumatic spinal cord injury, malignancy, or congenital abnormalities) had been excluded [21]. Only children of whom sufficient data was available to apply the current AFM classification, were included in this study.

Application of diagnostic criteria

The current diagnostic criteria for AFM had been used in the previously described study to classify cases [4,21](Table 1). In this study we further describe and analyze the previously performed classification process and the dilemmas encountered during this exercise.

Table 1: Diagnostic criteria for AFM (adapted from Murphy et al.[4]**)**

Diagnostic criteria for AFM classification				
Diagnostic items	**Definite AFM**	**Probable AFM**	**Possible AFM**	**Uncertain**
Acute onset of limb weakness (period from onset to nadir: hours to 10 days)	P	P	P	P
Prodromal fever or illness	P/A	P/A	P/A	P
Weakness involving one or more limbs, neck, face, or cranial nerves	P	P	P	P
Decreased muscle tone in at least one weak limb	P	P	P/A	P
Decreased or absent deep tendon reflexes in at least one weak limb	P	P	P/A	P
MRI: spinal cord lesion with predominant grey matter involvement, with or without nerve root enhancement	P	P	P	NP
CSF pleocytosis (white cell count> 5 cells/L)	P	A or NP	P/A or NP	P/A or NP
Factors that might suggest an alternative diagnosis:				
Encephalopathy that cannot be explained by fever, illness, respiratory distress, metabolic abnormalities or medications				
Presence of sensory deficits on examination				
Presence of lesions in the supratentorial white matter or cortex				
Absence of CSF pleocytosis				
Positive serum aquaporin-4 would exclude AFM				
Positive serum MOG-antibody, which would suggest MOG-antibody associated disease[6]				

Permission for reproduction was obtained. AFM: acute flaccid myelitis; P: diagnostic item is **present**; P/A: presence of this diagnostic item is supportive but not required; A: diagnostic item is **absent**; NP test was **not performed**; MRI: magnetic resonance imaging; CSF: cerebrospinal fluid; MOG: myelin oligodendrocyte glycoprotein.

First, an initial classification was made by merely applying the 'positive criteria' - acute flaccid limb weakness, abnormalities of the spinal cord grey matter on MRI, and pleocytosis in CSF - leading to a subdivision of five categories: (1) definite AFM, (2) probable AFM, (3) possible AFM, (4) uncertain or (5) no AFM (figure 1 step 1).

Hereafter, features mentioned in the diagnostic criteria as suggestive for an alternative diagnosis, shown in Table 1, were applied to consider exclusion of applicable patients [4] (Figure 1 step 2),

Third, cases in which there was a dilemma on the final classification, were discussed with two expert clinical neurologists (BCJ, OFB).

These three steps led to the final classification, in which patients could either retain or loose the label from the initial classification (definite AFM, probable AFM etc.). If the label from the initial classification was lost, the original diagnosis, as made by the treating clinician, was used for comparison.

The clinical features and diagnostic test results leading to the final classification were described. It was also described if clinical data was not available, or if tests were not performed and their results might have led to a different final classification.

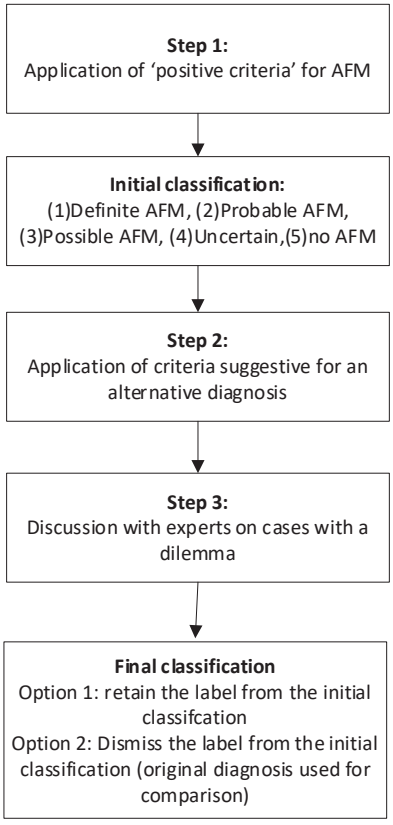

Figure 1: Flow chart showing the different steps which were taken to arrive at the initial and final classification.

Differentiating features

To further identify features that differentiate between AFM and other causes of AFP beyond the criteria suggestive for an alternative diagnosis, these cases, finally classified as definite, probable, or possible AFM and those classified as 'uncertain', were compared to cases with an alternative diagnosis.

For this comparison, we used demographic and clinical features and results of ancillary investigations including MRI of the spinal cord and brain, CSF, nerve conduction studies (NCS), auto-antibodies and virological tests.

Statistics

Relative numbers are given for dichotomous or ordinal variables. Median, interquartile range and full range are used for continuous variables.

RESULTS

Classification and dilemmas

A total of 141 patients younger than 18 years with rapidly progressive weakness, diagnosed in 2014-2019 in ten hospitals in The Netherlands, and with sufficient data available for classification were included [21]. The 'positive' criteria were applied, resulting in the subgroups as shown in Figure 2. Features suggestive of an alternative diagnosis were present in 7/10 cases fulfilling the positive diagnostic criteria for definite AFM, 11/11 for probable AFM (9/11 when excluding absence of CSF pleocytosis), 12/14 for possible AFM and 43/43 for 'uncertain' (21/43 when excluding absence of CSF pleocytosis). After discussing cases in which it was difficult to make a final classification, cases were subdivided into diagnostic groups. (Figure 2) Nineteen cases eventually retained the label from the initial classification, even if features suggestive of an alternative diagnosis were present (including 13 patients with absence of CSF pleocytosis). In twelve cases additional features were required for classification, including the presence of demyelinating features on NCS in nine of the cases, initially classified as uncertain. In fifteen cases further discussion with experts was necessary, as further described below.

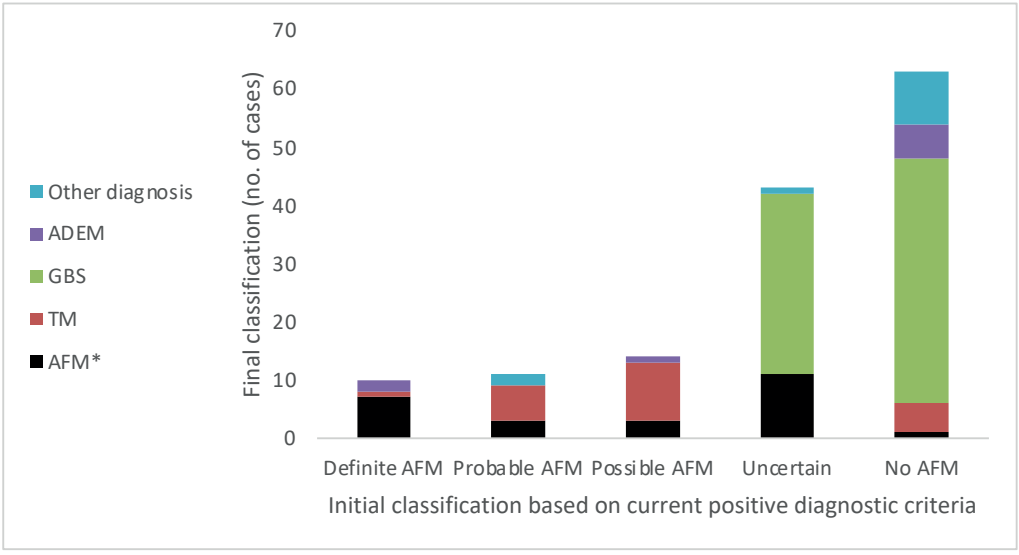

Figure 2: The initial classification based on the 'positive diagnostic criteria' for AFM[4] **is compared to the final classification.**

*AFM includes all cases which retained the label from the initial classification, except for the 'no AFM'-category that contained one case finally classified as AFM. AFM: acute flaccid myelitis, TM: transverse myelitis, GBS: Guillain-Barré syndrome, ADEM: acute disseminated encephalomyelitis.

'Definite' AFM'

According to recently published criteria, a definite diagnosis of AFM requires a combination of acute flaccid limb weakness with hyporeflexia and hypotonia in the affected limb, a spinal cord lesion with predominant grey matter involvement on MRI, and pleocytosis in CSF (Table 1)[4]. In our cohort, ten children fulfilled these criteria. Seven of them were finally classified as definite AFM. (Supplementary Table 1)

These seven patients all had a prodromal illness, followed by limb weakness, which was asymmetric in five. A respiratory sample was taken in four of seven (day 1-3 after onset), three of which were positive for EV-D68. In one of these seven patients, MOG-antibodies were found in serum. (Box 1) In two cases, sensory deficits were found at onset of disease. The presence of predominant proximal and asymmetric weakness at onset, the persistence of flaccid weakness and the absence of a sensory level, led to a final classification of definite AFM. One patient with definite AFM had encephalopathy at onset. The presence of predominant proximal asymmetric weakness in the arms, the absence of supratentorial

white matter abnormalities on MRI, and NCS abnormalities compatible with motor axonal damage led to the final diagnosis of AFM.

Three of the ten patients fulfilling the 'positive criteria' for AFM were finally classified as 'no AFM'. In one of them, a nasopharyngeal and fecal sample were tested and found negative at the first day after onset of weakness. In two patients, a diagnosis of ADEM was made, with encephalopathy in one patient and supratentorial white matter abnormalities in both patients. In the other patient a diagnosis of TM was made, based on the combination of bilateral sensory and autonomic abnormalities, symmetric weakness of only the legs and hyperreflexia at follow-up, combined with the presence of MOG-antibodies.

Box 1: Illustrative case

An 11-year-old patient presented in 2016 with asymmetric flaccid weakness of the limbs, three days after a prodromal illness with head- and neck pain. The arms were more severely affected than the legs and weakness was predominantly proximal. There were no sensory deficits. Because of respiratory failure, mechanical ventilation was required for 15 days. CSF showed a mononuclear pleocytosis and the MRI showed mostly centrally located myelopathy of the entire spinal cord. MOG-antibodies tested in serum at day 3 after onset were positive with a low titer. Only CSF was tested for viruses, showing negative results. The presence of MOG-antibodies led to an initial diagnosis of MOG-associated disease (MOGAD). The persistence of asymmetric, flaccid weakness predominantly of the arms four years after onset, as well as the absence of sensory deficits and bladder- or bowel dysfunction made us decide to finally classify this case as definite AFM.

'Probable' AFM

For a probable diagnosis of AFM, a combination of acute flaccid limb paralysis and a spinal cord lesion on MRI, predominantly affecting the grey matter, is required (Table 1)[4]. Eleven patients fulfilled these criteria, three of whom were finally classified as probable AFM. (Supplementary Table 2)

In these three patients CSF investigations were performed early (one day before until two days after onset of weakness). In two patients with a prodromal illness, a respiratory sample was investigated (day 2-3 after onset), one of which was positive for EV-D68. In one patient sensory deficits were present on examination, without a sensory level. The MRI of this patient showed predominant central conus involvement, but serum MOG-antibodies were negative.

Eight of eleven patients fulfilling the criteria for probable AFM were finally classified as 'no AFM'. In only one of these eight a respiratory sample was tested (day 3 after onset) and

was found negative. In six, a diagnosis of TM was made, five of them with a sensory level on examination. CSF investigations were performed early in the disease course in these patients (two days before until one day after onset of weakness). In the patient diagnosed with TM, but without a sensory level, the presence of sensory deficits and the development of spasticity at follow-up was deemed more compatible with TM than with AFM. In another patient, diagnosed with TM, MOG antibodies were found. This patient also had two separate spinal cord lesions on MRI. In two of the eight patients finally classified as 'no AFM', the clinical diagnosis was uncertain, but spinal cord ischemia was considered. One of these patients had a sensory level; the other patient did have sensory deficits and developed spasticity at follow-up, again making spinal cord ischemia more probable than AFM.

Box 2: Illustrative case

A 16-year-old patient with a final diagnosis of TM, had predominantly proximal flaccid weakness of the right arm. On examination a cervical sensory level was found. MRI showed a longitudinally extensive central myelopathy at the cervical level and subtle supratentorial abnormalities in the cerebral white matter. CSF showed no pleocytosis. At follow-up after 18 months there was persistent proximal weakness of the right arm with slight atrophy of the shoulder muscles and sensory abnormalities of the right leg. While the pattern of weakness is suggestive for AFM, the presence of a sensory level and the supratentorial abnormalities were deemed more compatible with TM.

'Possible' AFM

For a possible diagnosis of AFM, a combination of acute onset limb weakness and MRI spinal cord lesions predominantly affecting the grey matter is required. Hyporeflexia does not necessarily have to be present (Table 1)[4]. Fourteen patients fulfilled these criteria, of whom three were finally classified as possible AFM. (Supplementary Table 3)

In these three cases, a central longitudinally extensive myelopathy was seen on MRI. In one of the two patients in which CSF investigations were performed, pleocytosis was found. Virological testing of feces and a nasopharyngeal aspirate (day 3 after onset) was performed in one of the three, showing parechovirus, adenovirus and rhinovirus. One of these patients had asymmetric proximal weakness, more dominant in the arms with persistent proximal arm weakness over time. Two of these three patients had symmetric diffuse weakness of the legs and sensory deficits, without a sensory level. In one of these two patients there was also bladder involvement.

Of the fourteen patients fulfilling the positive criteria for possible AFM, eleven were finally classified as 'no AFM'. Ten of these eleven were diagnosed with TM and one with ADEM. In five of these patients virological testing was performed on a respiratory sample, all of which were negative.

Of the ten patients with a final diagnosis of TM, five had a sensory level on examination. Supratentorial abnormalities on MRI were present in five patients, including both patients with MOG-antibodies. In five of eight patients in whom CSF investigations were done, pleocytosis was found. Two patients who were diagnosed as TM did not have a sensory level nor supratentorial MRI abnormalities. One had asymmetric weakness, predominantly distal in the arms and proximal in the legs, while the other had symmetric leg weakness. At follow-up both patients had persistent hyperreflexia; one patient also had extensor plantar responses and spasticity. Two children had isolated involvement of the central conus on MRI. In these patients the presence of sensory deficits as well as the prominent bladder and/or bowel dysfunction were considered more consistent with a diagnosis of TM than with AFM.

The patient with a final diagnosis of ADEM had encephalopathy at onset. Furthermore, the MRI of the brain showed abnormalities in the supratentorial white matter.

Box 3: Illustrative case

An eight-month-old child had global symmetric weakness of the legs and bladder dysfunction. On examination there were hyperreflexia and sensory deficits, but a sensory level could not be found. MRI showed a longitudinally extensive central lesion of the cervical and thoracic cord, CSF showed a mononuclear pleocytosis. PCR of feces and respiratory material was positive for parechovirus, adenovirus and rhinovirus. After three years there was persistent leg weakness. This case was classified as possible AFM, since a sensory level was not identified. However, the symmetric leg weakness, the sensory deficits and bladder dysfunction suggest a diagnosis of TM.

Uncertain diagnosis

Cases with acute flaccid limb weakness and a prodromal illness or fever would be classified as uncertain if no MRI is performed or reliable assessment of the MRI is not possible, and if CSF analysis is normal or has not been performed (Table 1)[4]. In our cohort, 43 patients fulfilled these criteria. Of those, eleven were finally classified as uncertain. (Supplementary Table 4)

Of these eleven patients classified as uncertain, one patient had an MRI of the spinal cord twice, both of which could not reliably be assessed because of movement artifacts. This patient, who was diagnosed in a period of increased EV-D68 circulation (July 2016), had asymmetric predominantly proximal weakness. CSF showed a mononuclear pleocytosis and abnormalities compatible with motor axonal damage were seen on NCS. No respiratory or fecal samples were taken; serology did reveal positivity for enterovirus IgM, providing a possible clue for an enterovirus infection.

The other ten patients had symmetric weakness which was diffuse or predominantly distal in nine. None of these ten patients had sensory deficits and a significantly raised protein in CSF was found in eight. Virological testing on a respiratory sample was done in four (day 3-9), showing Haemophilus Influenzae in one patient. Five patients were completely recovered at final follow-up. Two of these ten patients had NCS compatible with acute motor axonal injury. (Box 4) They were initially diagnosed with acute motor axonal neuropathy, both in a period of increased EV-D68 circulation. Although in these ten patients a diagnosis of an axonal variant of GBS may be considered, especially in the patients with complete recovery, these patients were classified as uncertain, as no MRI was performed.

Of the 43 patients fulfilling the criteria for an uncertain AFM diagnosis, 32 were finally classified as 'no AFM'. Virological tests on a respiratory sample were performed in eight, one of which was positive for an adenovirus. Of these, 31 were diagnosed with GBS. One patient who recovered spontaneously several days after onset of weakness was diagnosed with probable functional limb weakness.

Of the 31 patients diagnosed with GBS, 20 had sensory abnormalities. CSF was performed in 29 patients and showed a raised protein without pleocytosis in 25. NCS was performed in 29 patients, showing features of a demyelinating neuropathy in twelve and of an axonal neuropathy in two.

Box 4: Illustrative case

A 15-year-old patient had symmetric distally predominant flaccid weakness of the limbs, five days after a gastrointestinal infection. The legs were more severely affected than the arms. There were no sensory deficits. CSF showed a slightly raised protein and no pleocytosis. NCS at 11 days after onset of weakness showed a motor axonal neuropathy, without sensory abnormalities. No MRI was performed. At follow-up after two months there was persistent distal weakness of the legs.

This patient was classified as uncertain as no MRI was performed, but clinically a motor axonal variant of GBS seems to be the most likely diagnosis.

No AFM

A total of 63 patients did not fulfill the criteria for AFM. These included 42 patients with GBS, five patients with TM, six patients with ADEM, nine patients with another diagnosis and one patient with AFM, further described in box 5. A substantial number of patients had acute onset flaccid limb weakness, but none had MRI abnormalities in the spinal cord grey matter.

Box 5: Illustrative case

A two-year-old patient had asymmetric mostly proximal flaccid weakness of all limbs with the legs being more severely affected. One day before onset there was a respiratory infection. CSF showed a mononuclear pleocytosis. NCS showed absent motor responses in the legs. In a respiratory sample EV-D68 was isolated. Repeated MRI scans of the brain and spinal cord at the first and eighth day after onset of weakness showed no abnormalities, even after careful reassessment.

While the clinical presentation and the identification of EV-D68 are compatible with a diagnosis of AFM, this patient was initially included in the 'no-AFM group' because of the absence of abnormalities on MRI of the spinal cord.

DISCUSSION

In this qualitative study, the current criteria for AFM were evaluated by applying them to a cohort of children with acute onset weakness. It may be difficult to make a correct diagnosis in children presenting with AFP and to differentiate AFM from other conditions, in particular at onset of disease. While the diagnostic criteria for AFM mostly performed well, in some cases additional features were required for proper classification. Furthermore, in many cases investigations required to make the diagnosis more or less likely were not adequately or timely performed.

Despite limited evidence for treatment in the acute phase of AFM, arguments for an early diagnosis of AFM include the need for clinical monitoring, and improved counselling to patients and parents [18]. Furthermore, early consideration of AFM would lead to early and adequate investigations, which is necessary to confirm the diagnosis, as CSF and MRI abnormalities may disappear and associated viruses may be undetectable later in the disease course [1,22].

We will discuss clinical and diagnostic features suggestive for AFM or for an alternative diagnosis, both from this study and previous studies, and evaluate the items included in the diagnostic criteria [4]. The additional features that were used for a final classification are summarized in Table 2.

Table 2: Summary of features, additional to those included in the diagnostic criteria, supportive for the diagnosis of AFM or for an alternative diagnosis.

Supportive features for the diagnosis AFM	Features supportive for an alternative diagnosis	Supportive for alternative diagnosis
Predominantly proximal weakness	Predominantly distal weakness	GBS
Asymmetric weakness	Strictly symmetric weakness	GBS
Arms more severely affected than legs	Only involvement of the legs	TM, SCI
Time course from prodrome till onset of < 5 days	Sensory level	TM, SCI
Features suggestive of axonal damage on NCS	Hyperreflexia in affected limbs	TM, SCI
PCR positive for EV-D68 or another associated virus in any material	Development of spasticity over time	TM, SCI
	Demyelinating features on NCS	GBS
	Significantly raised CSF protein level, especially in absence of pleocytosis	GBS
	Isolated conus involvement on MRI	TM/MOGAD

AFM: acute flaccid myelitis; CSF: cerebrospinal fluid; EV-D68: enterovirus D68; GBS: Guillain-Barré syndrome; MOGAD: Myelin oligodendrocyte glycoprotein antibody associated disease; MRI: magnetic resonance imaging; NCS: Nerve conduction studies; PCR: polymerase chain reaction; SCI: spinal cord ischemia; TM: transverse myelitis.

Clinical features

For definite or probable AFM, as well as for an uncertain diagnosis, the presence of acute flaccid limb weakness with hyporeflexia in at least one affected limb is required [4]. The presence of flaccid weakness at onset often does not differentiate between AFM and other causes of AFP, such as TM and GBS. However, the pattern of weakness may provide distinguishing features [12,15]. Both this study and previous studies indicate that asymmetry of weakness, predominance of proximal weakness, and involvement of arms more than legs, are supportive for AFM [1,15]. In addition, strictly symmetric and predominantly distal weakness are more compatible with GBS [12,23]. In TM, symmetric involvement of only the

legs is a commonly observed pattern, but other patterns such as asymmetric predominant proximal weakness may be seen. While differentiation between AFM and TM may be difficult at onset, in most TM cases, spasticity with hyperreflexia, often accompanied by extensor plantar responses, will develop over time.

Cases of acute weakness with normo- or hyperreflexia may fulfill the criteria for possible AFM. In many of these cases a diagnosis of TM or another cause of spinal cord injury with central pyramidal involvement is more probable, which can be further supported by additional features such as the pattern of weakness and the presence of sensory deficits. These sensory deficits are included in the diagnostic criteria as a feature suggestive for an alternative diagnosis [4]. The finding of a sensory level on examination would in our opinion exclude AFM, but it may be quite difficult to identify this especially in young children [13,24]. Sensory deficits have been identified in cases of AFM, possibly associated with spinal cord edema, which may be seen in the acute phase [1].

Encephalopathy is uncommon in AFM and may point to a diagnosis of ADEM. In AFM, encephalopathy may occur due to respiratory failure or metabolic abnormalities. At onset of disease, it may be difficult to determine whether this explains the encephalopathy. Other features such as the pattern of weakness and MRI abnormalities may then help in differentiation between AFM and ADEM.

Bladder and bowel dysfunction has been reported in AFM as well as GBS, with bladder dysfunction being more common in the latter. However, in cases with predominant and persistent dysfunction a diagnosis of TM or MOGAD may be more likely, as this is associated with diffuse spinal cord involvement or significant involvement of the conus and caudal roots [1,25,26].

MRI

Abnormalities of the spinal cord grey matter on MRI are obligatory for a definite, probable or possible diagnosis of AFM, while their absence on adequately timed scans of sufficient quality would exclude AFM [4]. The spinal cord grey matter of the whole spinal cord may be involved in AFM and extensive lesions are common with the cervical cord being most often affected [4,27,28]. Isolated involvement of the conus is uncommon in AFM and should lead to consideration of another diagnosis such as MOGAD [29].

In our study, one child with a final clinical diagnosis of AFM associated with EV-D68 did not show MRI abnormalities even on repeated MRI-scans and after reassessment by experienced pediatric neuroradiologists. In another child with AFM the MRI was of insufficient quality to assess the presence of grey matter abnormalities leading to a classification as uncertain. While these scenarios may be rare and would lead to considering alternative diagnoses, our experience is that MRI abnormalities may be subtle especially early after onset of weakness. This urges the need for adequate and high-quality scans of the spinal cord in suspected AFM cases. Furthermore, MRI scans should be carefully assessed by radiologists with experience in spinal cord imaging.

CSF

CSF pleocytosis is identified in most patients with AFM, but is required for a classification as definite AFM, while the absence of pleocytosis would suggest an alternative diagnosis [4]. Similar to TM, pleocytosis may also be not yet found if CSF is examined in the first hours after onset of weakness. The presence of a significantly raised CSF protein (>100 mg/dL), especially in absence of pleocytosis, should lead to reconsidering the diagnosis, as this is more compatible with GBS [30].

Nerve conduction studies

Results from neurophysiology studies have not been included in the working group criteria, while others have suggested that the findings of a pure motor axonal neuropathy is supportive of the diagnosis [30,31]. While this finding is not exclusive for AFM in our cohort, but may also be seen in acute motor axonal neuropathy (AMAN) patients, we do believe that it supports the diagnosis, in particular in differentiating AFM from TM. On the other hand, NCS showing demyelinating features would exclude the diagnosis and point to a demyelinating variant of GBS [12,32]. Therefore the performance of NCS may be helpful in cases where differentiation remains difficult. It is not yet known what the optimal timing in AFM is.

Virology

Different viruses have been associated with AFM, including EV-D68 and EV-A71. EV-D68 is mostly identified in respiratory material, while EV-A71 is mostly found in fecal material, similarly to poliovirus. Associated viruses are only rarely identified in CSF [9,33,34]. The identification of an associated virus, in particular EV-D68, is not included in the current criteria, but it has been suggested as a confirmative item by other authors [31]. While different viruses have been associated with AFM, the evidence for EV-D68 as a cause for AFM has been increasing and therefore the identification of this virus in any material in a patient with AFP would in our opinion strongly support the diagnosis [2,3,8].

Autoantibodies

AQP4-antibodies, causing neuromyelitis optica spectrum disorder, and MOG-antibodies, present in MOGAD, need to be determined in any child with suspected myelitis [4].

In our cohort, AQP4-antibodies were not identified in any case, in line with the rare identification of these antibodies in children [35]. Its presence would however lead to the exclusion of AFM. MOG antibodies were identified in some patients in this study, most with a diagnosis of TM. While there is a spectrum of acquired demyelinating syndromes in which MOG antibodies may be seen, their significance is still being explored as they may also be seen in other conditions [29,36]. In our study, one patient with a clinical picture compatible with AFM showed weak positivity for MOG-antibodies. Therefore, while the presence of MOG-antibodies would suggest MOGAD, it does not exclude AFM, particularly with low titers.

Limitations

Our study is limited especially by the retrospective design in which a final classification was made based on expert opinion. While this final classification was carefully considered by experts in the field, this is still subjective, as there is no confirmative test for the diagnosis of AFM and as the features required for a definite diagnosis may not persist over time. This does however match clinical practice in which clinicians have to make a diagnosis based on clinical features and findings of further investigations.

The retrospective nature of this study leads to incompleteness of clinical data and investigations. In some cases, proper classification was therefore difficult or only possible by using clinical features at follow-up. This limits the recommendations made for early diagnosing AFM in clinical practice, but underlines the need for adequate testing.

In the selection process initially used for the epidemiological study, some cases of acute weakness may not have been included, because they did not have a diagnostic code matching the inclusion criteria. Furthermore, some cases, for example those with structural abnormalities, were excluded. At onset of disease, before imaging studies are performed, differentiation from other causes of AFP may be difficult. For these reasons, to confirm certain distinctive features of AFM found in this study and to explore further early diagnostic characteristics in children with AFP, a prospective study, ideally in a large, unselected cohort of children, is necessary.

CONCLUSIONS

The diagnostic criteria for AFM were created by the AFM Working Group, hoping to create uniformity in the diagnosis and management, as no confirmative test for the diagnosis exists [4]. The possibility of atypical features was commented on by the working group, underlining the difficulty in making a set of criteria covering all AFM cases [4]. Here we show, that the diagnostic criteria usually perform well, but that additional features may be required to distinguish AFM from other conditions that may present as AFP. These features were summarized and may help clinicians in establishing the challenging diagnosis of AFM. As early and adequate diagnostic tests are required to make a definite diagnosis, we provide a suggested clinical work-up for clinicians, which can be used when confronted with a case of AFP. (Table 3)

Table 3: Suggested investigation in a child with acute flaccid paralysis, adapted from Helfferich et al[16]**.** MOG: Myelin-oligodendrocyte glycoprotein, AQP4: Aquaporin 4, GM1: Ganglioside M1, GD1a: Ganglioside D1a, GQ1b: Ganglioside Q1b, CSF: Cerebrospinal Fluid. WNV: West Nile Virus, NCS: nerve conduction studies.

Suggested investigations in children with acute flaccid paralysis	
Blood	– Auto-antibodies (Anti-MOG IgG, anti-AQP4, anti-GM1, Anti-GD1a, Anti-GQ1b)
	– Oligoclonal bands and IgG (both serum and CSF)
	– Microbiology: Serology for enterovirus, Borrelia, WNV[1]
CSF	– Routine investigations (Cell count, protein, glucose)
	– Oligoclonal bands and IgG (both CSF and serum)
	– Virology: PCR for enterovirus
	– Serology for Borrelia, WNV[1]
Further microbiologic testing	– PCR for enterovirus of a respiratory sample, preferably a nasopharyngeal aspirate
	– PCR for enterovirus of a fecal sample, preferably a stool sample
Imaging	– Contrast enhanced MRI of the brain and spine
Neurophysiologic testing	– NCS with motor and sensory investigation of an affected limb

[1] For patients that have travelled to or live in areas where WNV is prevalent.

Conflict of interest

The authors declare no conflict of interest, relevant to this study.

Funding statement

This study was funded by the Ministry of Health, Welfare and Sport of the Netherlands.

Ethical statement

The 'Dutch Medical Research Involving Human Subjects Act' was considered not applicable for the study from which data was used.

Local approval of the initial study protocol was obtained by the ethical committees of the participating hospitals according to their individual institutional research policy requirements. Data sharing agreements were concluded for each participating hospital, in line with the General Data Protection Regulation.

Informed consent from the patients and/or parents described in the boxes with illustrative cases was obtained.

REFERENCES

1. Messacar K, Schreiner TL, Haren K Van, Yang M, Glaser CA, Tyler KL, et al. Acute flaccid myelitis: A clinical review of US cases 2012-2015. Ann Neurol. 2016 Sep;80(3):326–38.
2. Vogt MR, Wright PF, Hickey WF, De Buysscher T, Boyd KL, Crowe JEJ. Enterovirus D68 in the Anterior Horn Cells of a Child with Acute Flaccid Myelitis. Vol. 386, The New England journal of medicine. United States; 2022. p. 2059–60.
3. Hixon AM, Yu G, Leser JS, Yagi S, Clarke P, Chiu CY, et al. A mouse model of paralytic myelitis caused by enterovirus D68. PLoS Pathog. 2017 Feb;13(2):e1006199.
4. Murphy OC, Messacar K, Benson L, Bove R, Carpenter JL, Crawford T, et al. Acute flaccid myelitis: cause, diagnosis, and management. Lancet (London, England). 2021 Jan;397(10271):334–46.
5. Knoester M, Helfferich J, Poelman R, Leer-Buter C Van, Brouwer OF, Niesters HGM, et al. Twenty-nine Cases of Enterovirus-D68-associated Acute Flaccid Myelitis in Europe 2016: A Case Series and Epidemiologic Overview. Pediatr Infect Dis J. 2019 Jan;38(1):16–21.
6. Martin JA, Messacar K, Yang ML, Maloney JA, Lindwall J, Carry T, et al. Outcomes of Colorado children with acute flaccid myelitis at 1 year. Neurology. 2017 Jul;89(2):129–37.
7. Choudhary A, Sharma S, Sankhyan N, Gulati S, Kalra V, Banerjee B, et al. Midbrain and spinal cord magnetic resonance imaging (MRI) changes in poliomyelitis. J Child Neurol. 2010 Apr;25(4):497–9.
8. Messacar K, Asturias EJ, Hixon AM, Leer-Buter C Van, Niesters HGM, Tyler KL, et al. Enterovirus D68 and acute flaccid myelitis-evaluating the evidence for causality. LancetInfectious Dis. 2018 Feb;
9. Messacar K, Spence-Davizon E, Osborne C, Press C, Schreiner TL, Martin J, et al. Clinical characteristics of enterovirus A71 neurological disease during an outbreak in children in Colorado, USA, in 2018: an observational cohort study. Lancet Infect Dis. 2020 Feb;20(2):230–9.
10. Chong PF, Kira R, Mori H, Okumura A, Torisu H, Yasumoto S, et al. 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. Clin Infect Dis. 2018 Feb;66(5):653–64.
11. Tasker RC. Rapid Onset of Neuromuscular Paralysis or Weakness. Crit Care Clin. 2022 Apr;38(2):413–28.
12. Helfferich J, Roodbol J, de Wit MC, Brouwer OF, Jacobs BC. Acute flaccid myelitis and Guillain-Barré syndrome in children: A comparative study with evaluation of diagnostic criteria. Eur J Neurol. 2022 Feb;29(2):593–604.
13. Absoud M, Greenberg BM, Lim M, Lotze T, Thomas T, Deiva K. Pediatric transverse myelitis. Neurology. 2016 Aug;87(9 Suppl 2):S46-52.
14. Pohl D, Alper G, Haren K Van, Kornberg AJ, Lucchinetti CF, Tenembaum S, et al. Acute disseminated encephalomyelitis: Updates on an inflammatory CNS syndrome. Neurology. 2016 Aug;87(9 Suppl 2):S38-45.
15. Theroux LM, Brenton JN. Acute Transverse and Flaccid Myelitis in Children. Curr Treat Options Neurol. 2019 Dec;21(12):64.
16. Helfferich J, Knoester M, Van Leer-Buter CC, Neuteboom RF, Meiners LC, Niesters HG, et al. Acute flaccid myelitis and enterovirus D68: lessons from the past and present. Eur J Pediatr. 2019 Sep;178(9):1305–15.
17. Hayes LH, Hopkins SE, Liu S, Pardo CA, Garcia-Dominguez MA, Oleszek J, et al. Challenges in the clinical recognition of acute flaccid myelitis and its implications. J Pediatr. 2022 Sep;
18. Lazzarini LM, Werner JM, Perez IA, Seruya M, Ramos-Platt L, Tiongson E, et al. Does acute flaccid myelitis cause respiratory failure in children? Pediatr Pulmonol. 2022 Mar;57(3):682–5.
19. Rudy MJ, Frost J, Clarke P, Tyler KL. Neutralizing Antibody Given after Paralysis Onset Reduces the Severity of Paralysis Compared to Nonspecific Antibody-Treated Controls in a Mouse Model of EV-D68-Associated Acute Flaccid Myelitis. Antimicrob Agents Chemother. 2022 Aug;66(8):e0022722.

20. Hixon AM, Clarke P, Tyler KL. Evaluating Treatment Efficacy in a Mouse Model of Enterovirus D68-Associated Paralytic Myelitis. J Infect Dis. 2017 Dec;216(10):1245–53.

21. Helfferich J, de Lange MM, Benschop KS, Jacobs BC, Van Leer-Buter CC, Meijer A, et al. Epidemiology of acute flaccid myelitis in children in the Netherlands, 2014 to 2019. Euro Surveill Bull Eur sur les Mal Transm = Eur Commun Dis Bull. 2022 Oct;27(42).

22. Murphy OC, Messacar K, Benson L, Bove R, Carpenter JL, Crawford T, et al. Acute flaccid myelitis: cause, diagnosis, and management. Lancet [Internet]. 2020 Dec 28; Available from: https://doi.org/10.1016/S0140-6736(20)32723-9

23. Roodbol J, de Wit MCY, van den Berg B, Kahlmann V, Drenthen J, Catsman-Berrevoets CE, et al. Diagnosis of Guillain-Barré syndrome in children and validation of the Brighton criteria. J Neurol. 2017 May;264(5):856–61.

24. Proposed diagnostic criteria and nosology of acute transverse myelitis. Neurology. 2002 Aug;59(4):499–505.

25. Chong PF, Kira R, Mori H, Okumura A, Torisu H, Yasumoto S, et al. 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. Clin Infect Dis. 2017 Oct;

26. Knoester M, Helfferich J, Poelman R, Van Leer-Buter C, Brouwer OF, Niesters HGM. Twenty-nine Cases of Enterovirus-D68-associated Acute Flaccid Myelitis in Europe 2016: A Case Series and Epidemiologic Overview. Pediatr Infect Dis J. 2019;38(1).

27. Maloney JA, Mirsky DM, Messacar K, Dominguez SR, Schreiner T, Stence N V. MRI Findings in Children with Acute Flaccid Paralysis and Cranial Nerve Dysfunction Occurring during the 2014 Enterovirus D68 Outbreak. Am J Neuroradiol [Internet]. 2014 Nov 20;36(2):245–50. Available from: http://www.ajnr.org/content/early/2014/11/20/ajnr.A4188.abstract

28. Okumura A, Mori H, Fee Chong P, Kira R, Torisu H, Yasumoto S, et al. Serial MRI findings of acute flaccid myelitis during an outbreak of enterovirus D68 infection in Japan. Brain Dev. 2018 Dec;41(5):443–51.

29. Bruijstens AL, Lechner C, Flet-Berliac L, Deiva K, Neuteboom RF, Hemingway C, et al. E.U. paediatric MOG consortium consensus: Part 1 - Classification of clinical phenotypes of paediatric myelin oligodendrocyte glycoprotein antibody-associated disorders. Eur J Paediatr Neurol. 2020 Nov;in press.

30. Elrick MJ, Gordon-Lipkin E, Crawford TO, Van Haren K, Messacar K, Thornton N, et al. Clinical Subpopulations in a Sample of North American Children Diagnosed With Acute Flaccid Myelitis, 2012-2016. JAMA Pediatr [Internet]. 2019 Feb 1;173(2):134–9. Available from: https://doi.org/10.1001/jamapediatrics.2018.4890

31. Kramer R, Lina B, Shetty J. Acute flaccid myelitis caused by enterovirus D68: Case definitions for use in clinical practice. Eur J Paediatr Neurol EJPN Off J Eur Paediatr Neurol Soc. 2019 Mar;23(2):235–9.

32. Hadden RD, Cornblath DR, Hughes RA, Zielasek J, Hartung HP, Toyka K V, et al. Electrophysiological classification of Guillain-Barré syndrome: clinical associations and outcome. Plasma Exchange/Sandoglobulin Guillain-Barré Syndrome Trial Group. Ann Neurol. 1998 Nov;44(5):780–8.

33. Messacar K, Asturias EJ, Hixon AM, Van Leer-Buter C, Niesters HGM, Tyler KL, et al. Enterovirus D68 and acute flaccid myelitis-evaluating the evidence for causality. Lancet Infect Dis [Internet]. 2018 Aug 1;18(8):e239–47. Available from: https://doi.org/10.1016/S1473-3099(18)30094-X

34. Harvala H, Broberg E, Benschop K, Berginc N, Ladhani S, Susi P, et al. Recommendations for enterovirus diagnostics and characterisation within and beyond Europe. J Clin Virol. 2018 Apr;101:11–7.

35. Tenembaum S, Yeh EA. Pediatric NMOSD: A Review and Position Statement on Approach to Work-Up and Diagnosis. Front Pediatr. 2020;8:339.

36. Amin M, Mays M, Polston D, Flanagan EP, Prayson R, Kunchok A. Myelin oligodendrocyte glycoprotein (MOG) antibodies in a patient with glioblastoma: Red flags for false positivity. J Neuroimmunol. 2021 Dec;361:577743.

SUPPLEMENTARY FILES

Supplementary Table 1: Clinical characteristics and investigations in patients initially classified as definite AFM.

Clinical characteristics			
	Definite AFM (n=7)	**TM (n=1)**	**ADEM (n=2)**
Demography			
Male: female (% M)	4:3	0:1	2:0
Age at diagnosis, median (IQR, full range), years	6 (3-8,1-10)	5[1]	5 (1-9)[2]
Prodrome			
Prodromal illness	7/7	0/1	2/2
Respiratory	3/7		½
Gastrointestinal	2/7		½
Fever	6/6		2/2
Time prodromal illness-onset, median (IQR, full range), days	6 (3-10, 3-17)[3]	NA	16 (5-26)[2]
Weakness			
Weakness arms	6/7	0/1	½
Proximal>distal	3/6		0/1
Distal>proximal	0/6		0/1
Global	3/6		1/1
Weakness legs	6/7	1/1	2/2
Proximal>distal	3/6	0/1	0/2
Distal>proximal	1/6	0/1	0/2
Global	2/6	1/1	2/2
Asymmetry	5/7	0/1	0/2
Time to nadir, median (IQR, full range), days	2 (1-3, 1-3)[3]	7	1 (1-1)[2]
Other clinical features			
Sensory abnormalities	2/5	1/1	1/1
Sensory level	0/2	0/1	0/1
Cranial nerve deficits	1/7	0/1	½
Hyporeflexia	7/7	1/1	½
Hyperreflexia	0/7	0/1	½
Pain	4/7	1/1	1/1
Autonomic problems	4/7	1/1	2/2
Bladder dysfunction	¾	1/1	2/2
Encephalopathy	1/7	1/1	½

Clinical characteristics			
Intubation required	3/7	0/1	½
ICU admisson	3/7	0/1	1/2
Follow-up			
Full recovery	0/7	0/1	1/2
Weakness arms	4/7	0/1	0/2
Weakness legs	2/7	0/1	0/2
Walking independently	4/7	1/1	½
Follow-up duration, median (IQR, full range), months	27 (1-54, 10-33)	25	13 (2-24)[2]
Investigations			
	Definite AFM (n=7)	**TM (n=1)**	**ADEM (n=2)**
MRI spinal cord			
Performed	7/7	1/1	2/2
Time after onset, median (IQR, full range), years	2 (1-5, 0-33)	7	1 (1-2)[2]
Abnormalities	7/7	1/1	2/2
Grey matter involvement	7/7	1/1	2/2
Root enhancement	1/4		0/1
second MRI spinal cord	4/7	1/1	½
MRI brain			
Performed	6/7	1/1	2/2
Abnormalities	3/6	0/1	2/2
Supratentorial	0/4		2/2
CSF			
Performed	7/7	1/1	2/2
Time after onset, median (IQR, full range), days)	2 (0-5, 0-33)	7	1 (0-1)[2]
Number of leukocytes, median (IQR, full range),	84 (17-250, 13-270)	63	136 (125-146)[2]
Pleocytosis	7/7	1/1	2/2
Protein, median (IQR, full range),	0.66 (0.35-0.74, 0.21-0.84)	0.37	0.43 (0.40-0.46)[3]
Raised protein	6/7	0/1	2/2
NCS			
Performed	1/7	0/1	0/2
Time after onset	250		
Abnormalities	1/1		
Axonal	1/1		
Demyelinating	0/1		

Clinical characteristics			
Auto-antibodies			
MOG	1/6	1/1	0/1
AQP-4	0/6	0/1	0/2
Virology			
CSF	7/7	1/1	2/2
Feces	3/5	0/1	1/2
Respiratory material	4/7	0/1	1/2
EV	4	0	0
EV-D68	3	0	0

A comparison between patients in patients who remained this label patients who did not. For the latter the clinical diagnosis is used for comparison.
[1]Data available for one patient. [2]Only full range is shown. [3]Data available for six patients
ADEM: Acute disseminated encephalomyelitis; AFM: Acute flaccid myelitis; AQP-4: Aquaporin-4; CSF: cerebrospinal fluid; EV: enterovirus; ICU: intensive care unit; IQR: Interquartile range MOG: myelin oligodendrocyte glycoprotein; MRI: Magnetic resonance imaging; NCS: Nerve conduction studies; TM: transverse myelitis;

Supplementary Table 2: Clinical characteristics and investigations in patients initially classified as probable AFM.

Clinical characteristics			
	Probable AFM (n=3)	**TM (n=6)**	**Other (n=2)**
Demography			
Male: female (% M)	2:1	3:3	1:1
Age at diagnosis, median (IQR, full range), years	12 (3-15)[1]	14 (11-16, 7-17)	10 (9-11)[1]
Prodrome			
Prodromal illness	2/3	4/6	0/2
Respiratory	1/2	2/4	
Gastrointestinal	0/2	0/4	
Fever	1/2	1/6	
Time prodromal illness-onset, median (IQR, full range), days	11 (6-14)[1]	8 (7-20)[1,2]	NA
Weakness			
Weakness arms	1/3	4/6	0/2
Proximal>distal	1/1	1/4	
Distal>proximal	0/1	1/4	
Global	0/1	2/4	
Weakness legs	3/3	5/6	2/2
Proximal>distal	1/3	0/5	1/2
Distal>proximal	0/3	1/5	0/2
Global	2/3	3/5	1/2
Asymmetry	2/3	3/5	1/2
Time to nadir, median (IQR, full range), days	1 (0-1)[1]	1 (0-4, 0-8)	0 (0-0)[1]
Other clinical features			
Sensory abnormalities	1/3	6/6	2/2
Sensory level	0/1	4/5	1/1
Cranial nerve deficits	1/3	1/5	0/2
Hyporeflexia	3/3	6/6	2/2
Hyperreflexia	0/3	0/6	0/2
Pain	3/3	3/5	0/2
Autonomic problems	2/3	5/6	1/1
Bladder dysfunction	2/2	5/5	1/1
Encephalopathy	0/3	0/6	0/2
Intubation required	1/3	2/6	0/2

Clinical characteristics			
ICU admission	1/3	2/6	0/2
Follow-up			
Full recovery	1/3	0/6	0/2
Weakness arms	1/3	4/6	0/2
Weakness legs	2/3	4/6	1/2
Walking independently	2/3	4/6	0/2
Follow-up duration, median (IQR, full range), months	23 (8-48)[1]	17 (8-34, 4-62)	9 (2-15)[1]
	Investigations		
	AFM (n=3)	**TM (n=6)**	**Other (n=2)**
MRI spinal cord			
Performed	3/3	6/6	2/2
Time after onset, median (IQR, full range), years	2 (-1-5)[1]	0 (-2-2, -2-4)[3]	1 (0-2)[1]
Abnormalities	3/3	5/6	2/2
Grey matter involvement	3/3	5/6	2/2
Root enhancement	1/2	0/4	0/1
second MRI spinal cord	1/3	5/6	1/2
MRI brain			
Performed	3/3	6/6	1/2
Abnormalities	0/3	0/6	0/1
CSF			
Performed	3/3	6/6	1/2
Time after onset, median (IQR, full range), days)	0 (-1-2)[1]	0 (-1-1,-2-1)	0 (0-0)1
Number of leukocytes, median (IQR, full range),	4 (1-4)[1]	1 (1-3, 0-5)	2[5]
Pleocytosis	0/3	0/6	0/1
Protein, median (IQR, full range),	0.35 (0.34-0.54)	0.23 (0.19-0.42, 0.18-0.47)[4]	0.18[5]
Raised protein	2/3	0/4	0/1
NCS			
Performed	1/3	1/6	0/2
Time after onset, median (IQR, full range), days)	4	NA	
Abnormalities	1/3	1/6	0/2
Axonal	1/1	0/1	

Clinical characteristics			
Demyelinating	0/1	0/1	
Auto-antibodies			
MOG	0/1	1/5	0/1
AQP-4	0/1	0/6	0/1
Virology			
CSF	3/3	4/5	1/2
Feces	1/3	0/5	0/2
Respiratory	2/3	1/5	0/2
EV	1	0	0
EV-D68	1	0	0

A comparison between patients in patients who remained this label patients who did not. For the latter the clinical diagnosis is used for comparison.
[1]Only full range is shown. [2]Data available for 3 patients. [3]Data available for 5 patients. [4]Data available for 4 patients. [5]Data available for 1 patient, IQR and full range not shown
ADEM: Acute disseminated encephalomyelitis; AFM: Acute flaccid myelitis; AQP-4: Aquaporin-4; CSF: cerebrospinal fluid; EV: enterovirus; ICU: intensive care unit; IQR: Interquartile range MOG: myelin oligodendrocyte glycoprotein; MRI: Magnetic resonance imaging; NCS: Nerve conduction studies; TM: transverse myelitis.

Supplementary Table 3: Clinical characteristics and investigations in patients initially classified as possible AFM.

Clinical characteristics			
	Possible AFM (n=3)	**TM (n=10)**	**ADEM (n=1)**
Demography			
Male: female (% M)	1:2	6:4	1:0
Age at diagnosis, median (IQR, full range), years	6 (0-15)[1]	12 (6-13,5-15)	11
Prodrome			
Prodromal illness	3/3	3/9	0/1
Respiratory	2/3	1/3	
Gastrointestinal	0/3	0/3	
Fever	1/1	2/9	0/1
Time prodromal illness-onset, median (IQR, full range), days	17[2]	6 (4-7)[1]	
Weakness			
Weakness arms	1/3	5/10	0/1
Proximal>distal	1/1	0/4	
Distal>proximal	0/1	2/4	
Global	0/1	2/4	
Weakness legs	3/3	10/10	1/1
Proximal>distal	0/1	3/9	0/1
Distal>proximal	0/1	1/9	0/1
Global	1/1	5/9	1/1
Asymmetry	1/3	6/10	0/1
Time to nadir, median (IQR, full range), days	7 (2-8)[1]	2 (1-2, 0-6)[3]	4
Other clinical features			
Sensory abnormalities	2/3	6/10	0/1
Sensory level	0/2	5/6	
Cranial nerve deficits	0/3	2/10	0/1
Hyporeflexia	0/3	2/10	0/1
Hyperreflexia	2/3	8/10	0/1
Pain	2/3	8/9	0/1
Autonomic problems	2/3	9/10	0/0
Bladder dysfunction	1/2	9/9	
Encephalopathy	0/3	0/10	1/1
Intubation required	0/3	1/10	0/1
ICU admission	0/3	2/10	0/1

Clinical characteristics			
Follow-up			
Full recovery	0/3	2/10	0/1
Weakness arms	1/3	3/10	0/1
Weakness legs	0/3	3/10	0/1
Walking independently	1/3	8/10	0/1
Follow-up duration, median (IQR, full range), months	32 (7-67)[1]	16 (12-30, 1-36)	23
	Investigations		
	Possible AFM (n=3)	**TM (n=10)**	**ADEM (n=1)**
MRI spinal cord			
Performed	3/3	10/10	1/1
Time after onset, median (IQR, full range), years	67 (2-91)[1]	1 (1-2,-1-6)	4
Abnormalities	3/3	10/10	1/1
Grey matter involvement	3/3	10/10	1/1
Root enhancement	1/3	0/10	0/1
second MRI spinal cord	3/3	8/10	1/1
MRI brain			
Performed	3/3	10/10	1/1
Abnormalities	0/3	5/10	1/1
Supratentorial		5/5	1/1
CSF			
Performed	2/3	8/10	1/1
Time after onset, median (IQR, full range), days)	38 (2-71)[1]	1 (1-2, -1-4)	8
Number of leukocytes, median (IQR, full range),	13 (5-21)[1]	23 (6-127,1-210)	86
Pleocytosis	1/2	5/8	0/1
Protein, median (IQR, full range),	0.50 (0.38-0.63)1	0.61 (0.28-1.05, 0.19-1.35)	0.45
Raised protein	2/2	5/8	0/0
NCS			
Performed	0/3	1/10	0/1
Time after onset, median (IQR, full range), days)		4[2]	
Abnormalities		0/1	
Auto-antibodies			
MOG	0/3	2/9	0/1

	Clinical characteristics		
AQP-4	0/2	0/10	0/1
Virology			
CSF	1/3	7/9	1/1
Feces	0/3	2/8	1/1
Respiratory	1/3	5/8	0/1
EV	0	0	0
EV-D68	0	0	0

A comparison between patients in patients who remained this label patients who did not. For the latter the clinical diagnosis is used for comparison.
[1]Only full range is shown. [2]Data available for 1 patient, IQR and full range are not shown. [3]Data available for 8 patients
ADEM: Acute disseminated encephalomyelitis; AFM: Acute flaccid myelitis; AQP-4: Aquaporin-4; CSF: cerebrospinal fluid; EV: enterovirus; ICU: intensive care unit; IQR: Interquartile range MOG: myelin oligodendrocyte glycoprotein; MRI: Magnetic resonance imaging; NCS: Nerve conduction studies; TM: transverse myelitis.

Supplementary Table 4: Clinical characteristics and investigations in patients initially classified as uncertain.

Clinical characteristics		
	Uncertain (n=11)	**GBS (n=31)**
Demography		
Male: female (% M)	8:3	20:11
Age at diagnosis, median (IQR, full range), years	7 (3-15,1-16)	7 (5-14,0-17)
Prodrome		
Prodromal illness	11/11	31/31
Respiratory	4/10	18/30
Gastrointestinal	4/10	8/30
Fever	4/10	20/27
Time prodromal illness-onset, median (IQR, full range), days	10 (4-22, 1-22)[1]	7 (5-12, 0-24)[3]
Weakness		
Weakness arms	6/11	23/31
Proximal>distal	2/4	5/21
Distal>proximal	1/4	4/21
Global	1/4	12/21
Weakness legs	11/11	28/30
Proximal>distal	2/10	4/27
Distal>proximal	5/10	7/27
Global	2/10	16/27
Asymmetry	1/11	1/30
Time to nadir, median (IQR, full range), days	7 (4-9, 0-10)	5 (3-7, 1-9)[4]
Other clinical features		
Sensory abnormalities	0/9	20/26
Sensory level		0/20
Cranial nerve deficits	3/11	19/31
Hyporeflexia	11/11	31/31
Hyperreflexia	0/11	0/31
Pain	8/11	27/31
Autonomic problems	4/10	12/24
Bladder dysfunction	1/4	5/12
Encephalopathy	0/11	2/31
Intubation required	1/11	5/31
ICU admission	1/11	16/31

Clinical characteristics		
Follow-up		
Full recovery	5/11	13/31
Weakness arms	1/11	4/31
Weakness legs	3/11	8/31
Walking independently	6/11	23/31
Follow-up duration, median (IQR, full range), months	6 (2-23, 0-41)	8 (1-15, 0-48)
Investigations		
	Uncertain (n=11)	**GBS (n=31)**
MRI spinal cord		
Performed	1/11	0/31
Time after onset, median (IQR, full range), years	6	
Abnormalities	NA	
Grey matter involvement	NA	
Root enhancement	NA	
second MRI spinal cord	1/11	
MRI brain		
Performed	4/10	6/31
Abnormalities	0/4	0/6
CSF		
Performed	9/10	29/30
Time after onset, median (IQR, full range), days)	6 (5-8, 1-8)	4 (2-7, 0-18)
Number of leukocytes, median (IQR, full range),	3 (2-5,1-38)	12 (1-8, 0-160)
Pleocytosis	1/9	3/29
Protein, median (IQR, full range),	0.76 (0.48-1.45, 0.11-2.50)	1.04 (0.45-1.55, 0.19-2.90)
Raised protein	8/9	25/29
NCS		
Performed	3/10	29/31
Time after onset, median (IQR, full range), days)	9 (8-11)[2]	11 (6-17, 2-260)[5]
Abnormalities	3/3	16/29
Axonal	2/3	2/16
Demyelinating	0/3	12/16
Auto-antibodies		
MOG	0/1	0/1
AQP-4	0/0	0/1

	Clinical characteristics	
Virology		
CSF	5/10	10/28
Feces	1/10	4/28
Respiratory	4/10	7/28
EV	0	0
EV-D68	0	0

A comparison between patients in patients who remained this label patients who did not. For the latter the clinical diagnosis is used for comparison. One patient diagnosed with probable functional limb weakness was not included.

[1]Data available for 9 patient. [2]Only full range is shown. [3]Data available for 29 patients. [4]Data available for 28 patients. [5]Data available for 16 patients

ADEM: Acute disseminated encephalomyelitis; AFM: Acute flaccid myelitis; AQP-4: Aquaporin-4; CSF: cerebrospinal fluid; EV: enterovirus; ICU: intensive care unit; IQR: Interquartile range MOG: myelin oligodendrocyte glycoprotein; MRI: Magnetic resonance imaging; NCS: Nerve conduction studies; TM: transverse myelitis.

9

SUMMARY AND GENERAL DISCUSSION

SUMMARY

In the studies reported in this thesis, we aimed to gain more insight in the epidemiology and clinical phenotype of acute flaccid myelitis (AFM). AFM is a polio-like disease characterized by acute flaccid weakness, combined with lesions of the spinal cord grey matter on MRI. It is included in the broad differential diagnosis of acute flaccid paralysis (AFP), defined by rapidly progressive weakness with low muscle tone. Poliomyelitis, caused by poliovirus, fulfills the diagnostic criteria for AFM, but is largely eradicated through effective vaccination campaigns. Other viruses have since been associated with AFM, of which enterovirus D68 (EV-D68) has been the most frequent after 2014.

In **Chapter 2**, a review on the published cohorts and case series of patients with AFM is presented to create an overview of the clinical and diagnostic features of AFM and its prototype poliomyelitis. We integrated the clinical and diagnostic features in a suggested work-up for cases of AFP. A major challenge lies in the propagation of adequate diagnostic procedures, required for an early diagnosis. Multidisciplinary collaboration is needed to meet the clinical and research challenges of AFM and EV-D68.

Following this review, the thesis is divided in three sections. The first one focuses on the epidemiology of AFM and EV-D68. In the second section, the differential diagnosis of AFM is considered and in the third section the applicability of diagnostic criteria for AFM is evaluated.

Section 1: Epidemiology

In **Chapter 3,** the clinical features of 29 cases of AFM associated with EV-D68 in Europe, are described, along with the results of enterovirus testing in several European laboratories. AFM cases were identified through an inventory within a European network of virologists and clinicians. The clinical features of these AFM cases from Europe largely resemble those from other countries. EV-D68 was mostly detected in respiratory samples only, illustrating the importance of collecting these samples to be able to identify this virus. The reported cases probably represent the tip of an iceberg, as in most European countries AFM is not a notifiable condition.

In **Chapter 4,** we tried to determine the incidence of AFM in the Netherlands by retrospectively identifying cases from a series of children presenting with acute weakness

in ten hospitals from 2014-2019. The diagnostic criteria described in Chapter 7 were used for classification of cases as not AFM, uncertain whether it is AFM, or possible, probable, or definite AFM. Cases classified as probable or definite were used to calculate the incidence in the Netherlands. AFM was shown to be a rare disease with an estimated mean incidence rate of 0.06/100,000 children/year. Cases clustered in periods of increased EV-D68 detection, further supporting the association between EV-D68 and AFM.

Section 2: Differential diagnosis

In the acute phase, it may be difficult to differentiate AFM from other causes of AFP, such as Guillain-Barré syndrome (GBS) and transverse myelitis (TM). In **Chapter 5,** we compared the clinical and diagnostic features of 26 children with AFM associated with EV-D68 (part of the cohort described in Chapter 3) with those of 156 children with GBS. Also, the diagnostic criteria of both conditions were evaluated. While there was overlap in clinical presentation, distinctive early clinical and diagnostic characteristics were identified which may help in the differentiation of AFM from GBS. The diagnostic criteria for AFM and GBS usually performed well, but some AFM cases fulfilled the criteria for GBS.

In **Chapter 6**, we followed a similar strategy for a study comparing AFM and TM. This showed a significant overlap in clinical presentation and diagnostic features, illustrated by a large percentage of AFM cases fulfilling the diagnostic criteria for TM and several TM cases fulfilling those for probable or definite AFM. Early clinical clues, such as the distribution of weakness and the presence of bowel- and/or bladder dysfunction, may help in the early differentiation between both conditions.

Section 3: Diagnostic criteria

In **Chapter 7,** a review of the clinical features, etiology, pathophysiology, and treatment of AFM by the international AFM-working group is presented. Consensus based diagnostic criteria are defined to promote homogeneity in making the correct clinical diagnosis. These criteria include both positive features and features which make an alternative diagnosis more likely.

In **Chapter 8**, we performed an evaluation of these diagnostic criteria by applying them to the AFM cases described in Chapter 4. This provides insight in the main differential

diagnosis of cases of rapidly progressive weakness in childhood. In several cases, additional clinical features beside those already included in the criteria were required for differentiating AFM from other causes of AFP and for making a final classification with respect to the certainty of the AFM diagnosis. We describe these features to help clinicians in making the correct diagnosis.

DISCUSSION AND FUTURE PERSPECTIVES

Acute flaccid myelitis (AFM) is characterized by rapidly progressive flaccid limb weakness and spinal cord grey matter abnormalities on MRI. This term was newly introduced after the upsurge of AFM cases in the United States in 2014 [1]. However, the syndrome had long been known before as poliomyelitis, which is defined by inflammation of the grey (Gr. πολιός) matter of the spinal cord (myelum). The main clinical feature of AFM, being acute flaccid paralysis (AFP), had been introduced for the surveillance of poliomyelitis, indicating rapidly progressive weakness with low muscle tone. Poliomyelitis is now strongly associated with poliovirus as the causative virus, but it took decades after the first detailed description of infantile paralysis by Heine in 1840, before the viral nature of poliomyelitis was shown [2,3]. Following the development and introduction of effective vaccines, poliomyelitis has been largely eradicated, although cases still occur in some countries and communities with low vaccination rates [4].

Whereas the number of cases of poliomyelitis, i.e. AFM caused by poliovirus, was minimized, in the last decades several other viruses have been associated with AFM. These include West Nile virus (WNV), and non-polio enteroviruses such as enterovirus A71 (EV-A71) and enterovirus D68 (EV-D68) [5–7]. The upsurge of AFM in recent years is believed to be mainly caused by EV-D68. The evidence for EV-D68 as a causative virus of AFM has been accumulating and includes the isolation of viral material from the spinal cord grey matter in autopsy material of an historic case of AFM [8,9]. This also again underlines the similarities in pathophysiology with poliovirus. For clarity, when speaking about AFM in this discussion, we consider modern variants of AFM, unless otherwise specified.

Epidemiology and surveillance

Only limited information is available on the incidence of AFM in most parts of the world, including Europe. Based on the current literature, including our study on the epidemiology of AFM in the Netherlands (Chapter 3) and the numbers from the Centers for Disease Control and Prevention (CDC) in the USA, AFM is a rare disease with a mean incidence rate of less than 1 per 100.000 children per year [7,10–13]. However, the impact on individual patients, mostly children, is often great, with persisting deficits even after many years

[14,15]. Therefore, the burden of disease, as determined by the financial consequences and the morbidity can be significant, even if the incidence is low.

To be informed of the burden of disease of AFM, it is important to keep track of its incidence by early detection of new cases. Another reason for obtaining reliable and current incidence numbers is the awareness of potential new outbreaks of AFM. Furthermore, these numbers would help to estimate whether the implementation of preventive strategies, including vaccination, should be considered.

As mentioned in the introduction of this thesis, surveillance for AFM is challenging, because there is no confirmative test for the diagnosis and because different viruses are associated with AFM. Different surveillance strategies are currently in place, including clinical surveillance, such as the AFM surveillance by the CDC, and laboratory surveillance for associated enteroviruses, as is performed in the Netherlands and many other European countries [16]. Both strategies have their benefits and disadvantages, as is further described below.

Clinical surveillance for AFP, which is much broader than AFM but at least includes most cases of AFM, is considered the gold standard in the worldwide campaign directed at the elimination of poliomyelitis. A case of poliomyelitis is confirmed when poliovirus is identified in a stool sample. The success of this surveillance strategy is determined by awareness amongst clinicians, who need to recognize a possible case, perform virological testing in stool samples and report a suspected case to public health authorities.

The CDC has started clinical surveillance for AFM after the large outbreak in the USA in 2014 [13]. The case definition of AFM which has been used for this purpose has been updated several times, but consistently included the presence of acute flaccid limb weakness (or AFP) and MRI abnormalities in the grey matter of the spinal cord [17]. This surveillance system has been effective in capturing upsurges of AFM in the USA [13]. Similar to the AFP surveillance to detect cases of poliomyelitis, the detection of cases relies on awareness and recognition by clinicians. However, while poliovirus may be detected for a longer period in feces and its detection can confirm the diagnosis, there is no confirmative test for AFM, as there are multiple associated viruses, which may be difficult to identify and may be found in various specimens. Therefore, the effectiveness of surveillance of AFM, not caused by the poliovirus, more heavily relies on the timely performance and adequate interpretation of diagnostic tests, such as MRI and CSF investigations.

In many European countries laboratory surveillance for EV has replaced AFP surveillance for poliomyelitis after this strategy had lost its effectivity after the eradication of poliovirus in Europe [16]. This EV-surveillance was initially installed to exclude poliovirus by subtyping samples in which an enterovirus is detected. EV-surveillance has the advantage that upsurges of different enteroviruses, independent of subtype and clinical phenotype, may be detected early. In this way, EV-D68 associated cases of severe respiratory disease and AFM may be identified. However, using this method of laboratory surveillance only a limited percentage (probably maximal 50 percent) of AFM cases will be recognized, as EVs are often not found in AFM and AFM may be related to other viruses [18–20]. Furthermore, the detection of AFM cases through EV-surveillance heavily depends on the performance of adequate and early virological tests by clinicians (diagnostic stewardship).

The effectiveness of this strategy for detecting outbreaks of enteroviruses, including EV-D68, is illustrated by the reports of European collaborative efforts, such as the European non-polio enterovirus network (ENPEN)[21]. After the upsurge reported in 2016, periods with increased detections of EV-D68 have been reported in European countries in 2018, 2019 and 2021 [22,23]. Only few patients with AFM were, however, identified among these EV-D68 positive cases, which is surely an underrepresentation of the real number, as (1) identification of EV-D68 in AFM may be difficult and depends on early and adequate testing and (2) other viruses may be associated with AFM. This illustrates the limitation of exclusive EV-surveillance to keep track of the incidence of AFM.

Other opportunities for monitoring enteroviruses associated with AFM lie in respiratory and environmental surveillance. Respiratory surveillance, in which patients with an acute respiratory infection (ARI) are tested with a multiplex-PCR panel, may be able to detect upsurges of EV-D68 associated respiratory disease, which often precedes AFM cases. If respiratory samples are routinely screened by first line health care providers, like in the ARI surveillance in the Netherlands, this approach is less dependent on voluntary testing [24–26]. It is important to notice that, while the focus of this thesis has been on AFM, the respiratory illness associated with EV-D68 may also be severe. While affected children usually recover well, some may require temporary ICU admission [27,28]. Therefore, respiratory disease will also contribute to the burden of disease associated with EV-D68.

Investigating wastewater samples for enteroviruses, as a way of environmental surveillance has been used to investigate the circulation of poliovirus for decades, and may also be useful for EV-D68 [29–31]. This way of surveillance will provide a less biased

approach, as it will also give information on asymptomatic or mild cases, who do not present to healthcare providers and are therefore not tested. Also, it provides a relatively easy way of continuous monitoring, with the potential of early detection of upsurges. The fact that EV-D68 is mainly a respiratory virus, may be a possible limitation for its effectiveness.

Recognition of AFM

The establishment of the diagnosis of AFM depends on the awareness and recognition by clinicians. They need to consider AFM early in the disease course, as the MRI abnormalities in the spinal cord and CSF pleocytosis, formally required to confirm the diagnosis, may no longer be detectable if testing is postponed [18]. Furthermore, in contrast to poliovirus, which is detected in stool samples for over two weeks, EV-D68 may only be detectable in respiratory material in the first days after onset of weakness [32,33].

An accurate diagnosis of AFM is important for several reasons. First, this is needed to acquire reliable incidence numbers and to relate the incidence of AFM to upsurges of circulation of associated viruses, as described in section 1. Second, children with AFM commonly show rapidly progressive deterioration with respiratory failure, which may be anticipated on through careful clinical monitoring [34]. Third, it is important for adequate counselling of patients and parents, which was identified as a need by parents of children with AFM [35]. Finally, studies from the mouse model of AFM suggest that there is an early window for treatment with monoclonal antibodies against EV-D68 and immunoglobulin [36,37]. To be able to investigate the effects of these treatments in patients with AFM, early recognition would be necessary.

It may be difficult to make a diagnosis of AFM and to differentiate it from other causes of AFP. (Figure 1) This is indicated by a retrospective study showing that in 72% of AFM patients, the diagnosis AFM was not considered at onset of disease, which may lead to suboptimal monitoring and treatment.[38] This is also illustrated by the dilemmas we encountered in the classification that had been performed in cases with acute onset weakness, as described in Chapter 8.

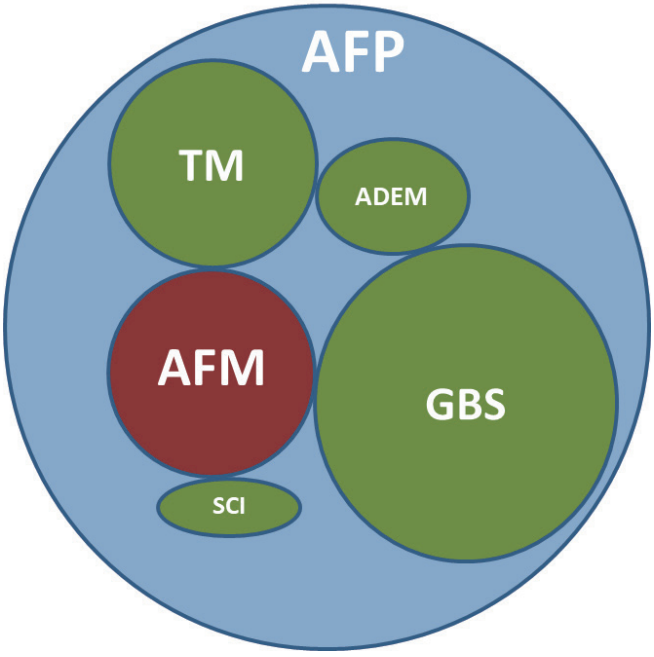

Figure 1: Differential diagnosis of acute flaccid paralysis (AFP).

The size of the different ovals corresponds with the incidence, relative to the other conditions in children based on the numbers described in Chapter 8. It needs to be emphasized that the differential diagnosis of AFP is broader and that this only provides a rough estimate as not all children with the mentioned conditions were included. ADEM: acute disseminated encephalomyelitis; AFM: acute flaccid myelitis; TM: transverse myelitis; GBS: Guillain-Barré syndrome; SCI: spinal cord ischemia

Especially early in the disease course, AFM may be mimicked by other conditions, which may present with AFP, such as Guillain-Barré syndrome (GBS) and transverse myelitis (TM) (Chapter 5 and 6). Also, atypical presentations of AFM exist, including those with pronounced cranial nerve deficits and bulbar weakness, without limb paralysis [18].

The first step in making the diagnosis of AFM is taking a medical history, with appreciation of prodromal symptoms and disease course. Then, a careful neurological examination is important to detect clues suggestive of AFM or an alternative diagnosis. For example, if weakness is asymmetric and/or predominantly proximal, this would make a diagnosis of AFM more probable. If AFM is considered, the next step will be the performance of adequate and complete investigations. At this point, involvement of virologists and infectiologists is important to be certain that appropriate virological examinations are done. Also,

neuroradiologists need to be instructed of the suspicion of AFM as abnormalities may be subtle [39].

These diagnostic steps and the clues provided from different studies may help in the differentiation and diagnosis of AFM. Still, AFM will only be correctly diagnosed if clinicians are aware of the syndrome and the diagnostic procedures. To improve and sustain awareness, clinicians who are taking care of children presenting with acute flaccid weakness need to be educated and alerted in periods that new cases of AFM may occur. National and international networks of virologists and clinicians, as well as public health authorities, have an important role in creating this awareness.

Diagnostic criteria

The first case definition for AFM was created by the CDC in 2014. A combination of rapidly progressive flaccid limb weakness (i.e. AFP) and spinal cord grey matter abnormalities was required for a definite diagnosis, while the combination of AFP and CSF pleocytosis was compatible with a probable diagnosis of AFM [40]. Since 2014, this case definition has been updated several times. In the most recent version of 2021, a confirmed diagnosis can be made based on (1) AFP in the absence of a clear alternative diagnosis attributable to a nationally notifiable condition, and (2) an MRI lesion in the spinal cord, predominantly affecting the gray matter, with exclusion of malignancy, vascular disease or anatomic abnormalities [17]. These case definitions were created for surveillance purposes and not specifically to guide clinicians in their diagnostic process. As mentioned before, the used definition was effective in detecting upsurges of AFM cases, but a certain 'background incidence' of other diseases may be detected [13]. The differences between cases classified as AFM in peak years (with circulation of EV-D68) and non-peak years, show similarities to the differences between AFM and ATM we found in the comparison study between both conditions in Chapter 6 [41]. This suggests that this 'background incidence' mainly consists of TM cases. The overlapping features which were more common in peak-years and in AFM in our comparison study include the presence of a prodromal illness and the involvement of the upper extremities only [41].

Apart from the case definition used by the CDC, several other disease criteria have been proposed. These include the case definitions for EV-D68 associated AFM for use in clinical practice, published in 2019 [42]. In these, the diagnosis is based on the combination of AFP

and findings from further investigations, including MRI and nerve conduction studies (NCS). The detection of EV-D68 is required for a confirmed diagnosis. These case definitions put emphasis on virological data and therefore rely on adequate and timely virological testing by clinicians. Also, these definitions focus on EV-D68, while the identification of other associated viruses, such as EV-A71, will not lead to a confirmed diagnosis [43–45].

In 2018, a more restrictive AFM definition was proposed [46]. This was intended to create homogeneous research cohorts with high specificity and possibly lower sensitivity compared to the CDC case definitions. In this definition, a combination of a prodromal viral syndrome, acute flaccid paralysis, and supportive findings from either MRI, NCS or CSF are required. Also, several factors, including sensory deficits and the presence of a definable alternative diagnosis, would lead to exclusion of AFM.

Many of the features used for this restricted definition of AFM were incorporated in the international clinical criteria published in 2020, described in Chapter 7 [47]. In these criteria, the combination of diagnostic items (AFP, MRI abnormalities of the spinal cord grey matter, and CSF pleocytosis) and features making an alternative diagnosis more probable is used. Also, a classification with different degrees of diagnostic certainty is included. This matches clinical practice where clinical and diagnostic findings make a diagnosis more or less probable. The study described in Chapter 8, does however indicate, that, when classifying cases according to these criteria, additional features may be required to differentiate AFM from other causes of AFP. Also, the diagnosis of AFM heavily relies on the presence of MRI abnormalities in the spinal cord grey matter, as their absence on an adequately timed MRI would lead to exclusion of the diagnosis [47]. In our experience, adequate imaging protocols and experienced neuroradiologists are often required to detect the MRI abnormalities in AFM.

The above considerations show the difficulties in drafting disease criteria. It illustrates that the content of criteria mainly depends on their purpose, and that every set of criteria will have its own pros and cons, balancing between sensitivity and specificity. The disease criteria for GBS and TM, used in chapter 5 and 6 respectively, were also not optimal in differentiating AFM from these conditions, indicating that these dilemmas are not unique to AFM [48,49].

As mentioned above, in clinical practice a diagnosis is based on positive and negative clinical findings, which may support it or make it less probable. Therefore, an approach, in which a combination of required and supportive features, together with items which would make the diagnosis less probable (orange flags) or exclude it (red flags), may be more

suitable (Table 1). Such a strategy has previously been proposed for GBS, for which, similarly to AFM, there is no single confirmative test [50]. To be able to use the approach in Table 1, adequate investigations have to be performed (Chapter 2).

Table 1: Suggested diagnostic approach for AFM in clinical practice

Required features for the diagnosis of AFM
Acute onset limb weakness
Low muscle tone in affected limbs
Supportive features for the diagnosis of AFM
Absent reflexes in affected limbs
Predominantly proximal weakness
Asymmetric weakness
Prodromal (respiratory) illness
Time course from prodrome till onset of < 5 days
EV-D68 or other associated enterovirus in any material
MRI lesions with predominant grey matter involvement
Pleocytosis in CSF (>5 leukocytes/L)
Features suggestive for axonal damage on NCS/EMG
Red flags excluding the diagnosis of AFM
Sensory level
Demyelinating features on NCS
AQP4- antibodies in serum
Orange flags raising doubts about the diagnosis of AFM
Sensory deficits
Symmetric weakness
Predominantly distal weakness
Predominant bladder- and bowel dysfunction
Hyperreflexia in affected limbs
Absence of MRI abnormalities in spinal cord
Isolated conus involvement on spinal cord MRI
Supratentorial abnormalities on brain MRI
Significantly raised CSF protein level, especially in absence of pleocytosis
MOG-antibodies in serum

The features included in Table 1 are a combination of items from the described sets of criteria and items, based on findings from the studies in this thesis. The presented approach may be helpful for the diagnosis of AFM in clinical practice, but it is not a validated tool.

Future perspectives

Significant progress has been made in the past decade on our knowledge of AFM, including the pathophysiology, clinical and diagnostic features, and epidemiology. This could only be achieved by international collaboration between clinicians, microbiologists, and public health officers.

Still, many questions considering AFM remain to be answered. Why do certain patients infected by EV-D68 develop AFM and what is the exact mechanism of anterior horn damage? Other questions consider the role of diagnostic tests, such as the role of nerve conduction studies and electromyography in the diagnosis and estimation of the prognosis, and the role of serology in determining the cause of AFM and seroprevalence of EV-D68. Other still unsolved issues involve the treatment of AFM, such as the role of immunoglobulin in the acute phase and the best way of rehabilitation in the subacute and chronic phase. To determine whether implementation of vaccination should be considered, it is, as mentioned before, important to keep track of the incidence of AFM and EV-D68 which requires adequate surveillance and proper recognition of cases.

A combined approach of clinical and laboratory surveillance, in which public health specialists collaborate with clinicians and microbiologists, may be able to tackle part of the limitations of the individual systems. This would improve opportunities to detect and associate upsurges of EV-D68 and AFM. Also, public health specialists and microbiologists would be enabled to make clinicians aware of possible new AFM cases in periods of increased rotation of EV-D68. Similarly, microbiologists can be alerted to perform adequate subtyping and sequencing of enteroviruses in periods of increased AFM case detections.

In Europe, the need for this combined approach already led to a currently emerging network of clinicians which aims to create a registry of new AFM cases and to function as a forum for expertise on AFM. This network will be collaborating closely with ENPEN, which is now largely an initiative of microbiologists and public health specialists [51]. An important question to be answered is how AFM cases will be found through this European network, as AFP surveillance is not effective in most countries. The reported incidence numbers would be too low to reinstall active surveillance. For the time being, passive reporting of cases in national networks seems the best alternative.

The studies in this thesis show that the diagnostic accuracy for AFM is not yet optimal. The diagnostic clues we provided may help to recognize AFM and to differentiate it from

other disorders but need further confirmation. Also, while GBS and ATM are important mimickers of AFM, the differential diagnosis of AFP is broader. (Figure 1) To confirm the differentiating features found in the comparison studies (Chapter 5 and 6) and to provide a more complete set of these features, a prospective study, including all cases of AFP can be of value. This study may also help to further describe the clinical features of AFM and to evaluate the applicability of the diagnostic criteria more extensively. Furthermore, if good national coverage is achieved, this study would help in obtaining more accurate incidence numbers of AFM [12]. The results of this study might create more insight in the spectrum of AFM, although it remains difficult to identify mild or rapidly recovering cases. Also, it will not answer the question which percentage of EV-D68 infected children develops AFM, which relies on adequate studies on the incidence and clinical spectrum of EV-D68 infections.

To conclude, it needs to be emphasized that being able to make a diagnosis of AFM depends on awareness by clinicians, as adequate and timely investigations are required for the diagnosis. As both clinical features and findings from additional investigations may help in securing the diagnosis, input from different disciplines is important. Therefore, the multidisciplinary collaboration, which led to the first diagnosis of AFM in the Netherlands in 2016, remains crucial, not only for the diagnosis, but also to move forward in meeting the further challenges of this often-devastating disease in children.

REFERENCES

1. Pastula DM, Aliabadi N, Haynes AK, Messacar K, Schreiner T, Maloney J, et al. Acute neurologic illness of unknown etiology in children - Colorado, August-September 2014. MMWRMorbidity Mortal Wkly Rep. 2014 Oct;63(40):901–2.
2. Heine J. Beobachtungen über Lähmungszustände der untern Extremitäten und deren Behandlung. Stuttgart, Germany: Köhler; 1840.
3. Landsteiner K. Ubertragung der Poliomyelitis acuta auf Affen. Z Immunitatsforsch. 1909;2:377–90.
4. Burki T. Vaccine-derived poliovirus cases exceed wild types. Lancet Infect Dis. 2019 Feb;19(2):140.
5. Glass JD, Samuels O, Rich MM. Poliomyelitis due to West Nile virus. N Engl J Med. 2002 Oct;347(16):1280–1.
6. Huang CC, Liu CC, Chang YC, Chen CY, Wang ST, Yeh TF. Neurologic complications in children with enterovirus 71 infection. N Engl J Med. 1999 Sep;341(13):936–42.
7. Haren K Van, Ayscue P, Waubant E, Clayton A, Sheriff H, Yagi S, et al. Acute Flaccid Myelitis of Unknown Etiology in California, 2012-2015. Jama. 2015 Dec;314(24):2663–71.
8. Messacar K, Asturias EJ, Hixon AM, Leer-Buter C Van, Niesters HGM, Tyler KL, et al. Enterovirus D68 and acute flaccid myelitis-evaluating the evidence for causality. LancetInfectious Dis. 2018 Feb;
9. Vogt MR, Wright PF, Hickey WF, De Buysscher T, Boyd KL, Crowe JEJ. Enterovirus D68 in the Anterior Horn Cells of a Child with Acute Flaccid Myelitis. Vol. 386, The New England journal of medicine. United States; 2022. p. 2059–60.
10. Kane MS, Sonne C, Zhu S, Malhotra A, Van Haren K, Messacar K, et al. Incidence, Risk Factors and Outcomes Among Children With Acute Flaccid Myelitis: A Population-based Cohort Study in a California Health Network Between 2011 and 2016. Pediatr Infect Dis J. 2019 Jul;38(7):667–72.
11. Sejvar JJ, Lopez AS, Cortese MM, Leshem E, Pastula DM, Miller L, et al. Acute Flaccid Myelitis in the United States, August-December 2014: Results of Nationwide Surveillance. Clin Infect Dis. 2016 Sep;63(6):737–45.
12. Helfferich J, de Lange MM, Benschop KS, Jacobs BC, Van Leer-Buter CC, Meijer A, et al. Epidemiology of acute flaccid myelitis in children in the Netherlands, 2014 to 2019. Euro Surveill Bull Eur sur les Mal Transm = Eur Commun Dis Bull. 2022 Oct;27(42).
13. Kidd S, Yee E, English R, Rogers S, Emery B, Getachew H, et al. National Surveillance for Acute Flaccid Myelitis - United States, 2018-2020. MMWR Morb Mortal Wkly Rep. 2021 Nov;70(44):1534–8.
14. Martin JA, Messacar K, Yang ML, Maloney JA, Lindwall J, Carry T, et al. Outcomes of Colorado children with acute flaccid myelitis at 1 year. Neurology. 2017 Jul;89(2):129–37.
15. Kirolos A, Mark K, Shetty J, Chinchankar N, Mcdougall C, Eunson P, et al. Outcome of paediatric acute flaccid myelitis associated with enterovirus D68: a case series. Dev Med Child Neurol. 2018 Nov;61(3):376–80.
16. Harvala H, Jasir A, Penttinen P, Pastore Celentano L, Greco D, Broberg E. Surveillance and laboratory detection for non-polio enteroviruses in the European Union/European Economic Area, 2016. Euro Surveill Bull Eur sur les Mal Transm = Eur Commun Dis Bull. 2017 Nov;22(45).
17. CSTE. Revision to the Standardized Case Definition, Case Classification, and Public Health Reporting for Acute Flaccid Myelitis. 2019.
18. Messacar K, Schreiner TL, Haren K Van, Yang M, Glaser CA, Tyler KL, et al. Acute flaccid myelitis: A clinical review of US cases 2012-2015. Ann Neurol. 2016 Sep;80(3):326–38.
19. Chong PF, Kira R, Mori H, Okumura A, Torisu H, Yasumoto S, et al. 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. Clin Infect Dis. 2017 Oct;

20. Ramsay M, Dunning J, Foulkes S, Lopez J, Antoaneta B, Nalini I, et al. An increase in reports of acute flaccid paralysis (AFP) in the United Kingdom, 1 January 2018-21 January 2019: early findings. Euro Surveill [Internet]. 2019 Feb 7;24(6):1900093. Available from: https://www.ncbi.nlm.nih.gov/pubmed/30755296

21. Fischer TK, Simmonds P, Harvala H. The importance of enterovirus surveillance in a post-polio world. Lancet Infect Dis. 2022 Jan;22(1):e35–40.

22. Knoester M, Scholvinck EH, Poelman R, Smit S, Vermont CL, Niesters HG, et al. Upsurge of Enterovirus D68, the Netherlands, 2016. Emerg Infect Dis. 2017 Jan;23(1):10.3201/eid2301.161313.

23. Benschop KS, Albert J, Anton A, Andrés C, Aranzamendi M, Armannsdóttir B, et al. Re-emergence of enterovirus D68 in Europe after easing the COVID-19 lockdown, September 2021. Euro Surveill Bull Eur sur les Mal Transm = Eur Commun Dis Bull. 2021 Nov;26(45).

24. Meijer A, Benschop KS, Donker GA, van der Avoort HG. Continued seasonal circulation of enterovirus D68 in the Netherlands, 2011-2014. Euro Surveill Bull Eur sur les Mal Transm = Eur Commun Dis Bull. 2014 Oct;19(42).

25. Meijer A, van der Sanden S, Snijders BEP, Jaramillo-Gutierrez G, Bont L, van der Ent CK, et al. Emergence and epidemic occurrence of enterovirus 68 respiratory infections in The Netherlands in 2010. Virology. 2012 Feb;423(1):49–57.

26. Donker G. Nivel Zorgregistraties Eerste Lijn - Peilstations 2018. 2019.

27. Ma KC, Winn A, Moline HL, Scobie HM, Midgley CM, Kirking HL, et al. Increase in Acute Respiratory Illnesses Among Children and Adolescents Associated with Rhinoviruses and Enteroviruses, Including Enterovirus D68 - United States, July-September 2022. MMWR Morb Mortal Wkly Rep. 2022 Oct;71(40):1265–70.

28. Messacar K, Abzug MJ, Dominguez SR. The Emergence of Enterovirus-D68. Microbiol Spectr. 2016 Jun;4(3):10.1128/microbiolspec.EI10-0018–2016.

29. Tedcastle A, Wilton T, Pegg E, Klapsa D, Bujaki E, Mate R, et al. Detection of Enterovirus D68 in Wastewater Samples from the UK between July and November 2021. Viruses. 2022 Jan;14(1).

30. Erster O, Bar-Or I, Levy V, Shatzman-Steuerman R, Sofer D, Weiss L, et al. Monitoring of Enterovirus D68 Outbreak in Israel by a Parallel Clinical and Wastewater Based Surveillance. Viruses. 2022 May;14(5).

31. Asghar H, Diop OM, Weldegebriel G, Malik F, Shetty S, El Bassioni L, et al. Environmental surveillance for polioviruses in the Global Polio Eradication Initiative. J Infect Dis. 2014 Nov;210 Suppl:S294-303.

32. Messacar K, Asturias EJ, Hixon AM, Van Leer-Buter C, Niesters HGM, Tyler KL, et al. Enterovirus D68 and acute flaccid myelitis-evaluating the evidence for causality. Lancet Infect Dis [Internet]. 2018 Aug 1;18(8):e239–47. Available from: https://doi.org/10.1016/S1473-3099(18)30094-X

33. GELFAND HM, LEBLANC DR, FOX JP, CONWELL DP. Studies on the development of natural immunity to poliomyelitis in Louisiana. II. Description and analysis of episodes of infection observed in study group households. Am J Hyg. 1957 May;65(3):367–85.

34. Lazzarini LM, Werner JM, Perez IA, Seruya M, Ramos-Platt L, Tiongson E, et al. Does acute flaccid myelitis cause respiratory failure in children? Pediatr Pulmonol. 2022 Mar;57(3):682–5.

35. Bove R, Werdal H, Olivera E. A Parental Perspective on Strengthening Knowledge After Acute Flaccid Myelitis. JAMA Pediatr. 2019 Feb;173(2):127.

36. Hixon AM, Clarke P, Tyler KL. Evaluating Treatment Efficacy in a Mouse Model of Enterovirus D68-Associated Paralytic Myelitis. J Infect Dis. 2017 Dec;216(10):1245–53.

37. Rudy MJ, Frost J, Clarke P, Tyler KL. Neutralizing Antibody Given after Paralysis Onset Reduces the Severity of Paralysis Compared to Nonspecific Antibody-Treated Controls in a Mouse Model of EV-D68-Associated Acute Flaccid Myelitis. Antimicrob Agents Chemother. 2022 Aug;66(8):e0022722.

38. Hayes LH, Hopkins SE, Liu S, Pardo CA, Garcia-Dominguez MA, Oleszek J, et al. Challenges in the clinical recognition of acute flaccid myelitis and its implications. J Pediatr. 2022 Sep;

39. Maloney JA, Mirsky DM, Messacar K, Dominguez SR, Schreiner T, Stence N V. MRI Findings in Children with Acute Flaccid Paralysis and Cranial Nerve Dysfunction Occurring during the 2014 Enterovirus D68 Outbreak. Am J Neuroradiol [Internet]. 2014 Nov 20;36(2):245–50. Available from: http://www.ajnr.org/content/early/2014/11/20/ajnr.A4188.abstract

40. CDC. Revision to the Standardized Surveillance and Case Definition for Acute Flaccid Myelitis. 2017.

41. McLaren N, Lopez A, Kidd S, Zhang JX, Nix WA, Link-Gelles R, et al. Characteristics of Patients with Acute Flaccid Myelitis, United States, 2015-2018. Emerg Infect Dis. 2020 Feb;26(2):212–9.

42. Kramer R, Lina B, Shetty J. Acute flaccid myelitis caused by enterovirus D68: Case definitions for use in clinical practice. Eur J Paediatr Neurol EJPN Off J Eur Paediatr Neurol Soc. 2019 Mar;23(2):235–9.

43. Gonzalez-Sanz R, Casas-Alba D, Launes C, Munoz-Almagro C, Ruiz-Garcia MM, Alonso M, et al. Molecular epidemiology of an enterovirus A71 outbreak associated with severe neurological disease, Spain, 2016. Euro Surveill Bull Eur sur les Mal Transm = Eur Commun Dis Bull. 2019 Feb;24(7).

44. Antona D, Kossorotoff M, Schuffenecker I, Mirand A, Leruez-Ville M, Bassi C, et al. Severe paediatric conditions linked with EV-A71 and EV-D68, France, May to October 2016. Euro Surveill. 2016 Nov;21(46):10.2807/1560-7917.ES.2016.21.46.30402.

45. Messacar K, Spence-Davizon E, Osborne C, Press C, Schreiner TL, Martin J, et al. Clinical characteristics of enterovirus A71 neurological disease during an outbreak in children in Colorado, USA, in 2018: an observational cohort study. Lancet Infect Dis. 2020 Feb;20(2):230–9.

46. Elrick MJ, Gordon-Lipkin E, Crawford TO, Van Haren K, Messacar K, Thornton N, et al. Clinical Subpopulations in a Sample of North American Children Diagnosed With Acute Flaccid Myelitis, 2012-2016. JAMA Pediatr [Internet]. 2019 Feb 1;173(2):134–9. Available from: https://doi.org/10.1001/jamapediatrics.2018.4890

47. Murphy OC, Messacar K, Benson L, Bove R, Carpenter JL, Crawford T, et al. Acute flaccid myelitis: cause, diagnosis, and management. Lancet (London, England). 2021 Jan;397(10271):334–46.

48. Sejvar JJ, Kohl KS, Gidudu J, Amato A, Bakshi N, Baxter R, et al. Guillain-Barré syndrome and Fisher syndrome: case definitions and guidelines for collection, analysis, and presentation of immunization safety data. Vaccine. 2011 Jan;29(3):599–612.

49. Transverse Myelitis Consortium Working Group. Proposed diagnostic criteria and nosology of acute transverse myelitis. Neurology. 2002;59(4):499–505.

50. Willison HJ, Jacobs BC, van Doorn PA. Guillain-Barre syndrome. Lancet (London, England). 2016 Aug;388(10045):717–27.

51. Harvala H, Broberg E, Benschop K, Berginc N, Ladhani S, Susi P, et al. Recommendations for enterovirus diagnostics and characterisation within and beyond Europe. J Clin Virol. 2018 Apr;101:11–7.

NEDERLANDSE SAMENVATTING

'Acute flaccid myelitis' (AFM) is een zeldzame aandoening die vooral bij kinderen voorkomt en waarbij in korte tijd ernstige spierzwakte ontstaat door beschadiging van voorhoorncellen. Deze cellen bevinden zich in de grijze stof van het ruggenmerg en zijn belangrijk voor de aansturing van de spieren. De meest bekende vorm van AFM is poliomyelitis ('poliomyelitis anterior acuta'), ook wel polio genoemd, wat wordt veroorzaakt door het poliovirus. Dit komt wereldwijd door effectieve vaccinatiecampagnes vrijwel niet meer voor. Bij de huidige patiënten met AFM lijken vaak andere zogenaamde non-polio enterovirussen verantwoordelijk voor de beschadiging van voorhoorncellen.

Bij kinderen die getroffen worden door AFM, is er sprake van een binnen enkele dagen in ernst snel toenemende slappe verlamming ('acute flaccid paralysis' (AFP)), vaak voorafgegaan door een bovenste luchtweginfectie. Niet alleen de spieren van de armen of benen kunnen verlamd raken, maar ook ademhalingsspieren, spieren in het gelaat en kauwspieren. Kinderen met AFM herstellen vaak onvolledig en houden ernstige zwakte van een of meer ledematen. Soms blijven zij ook afhankelijk van een beademingsapparaat.

De diagnose AFM kan worden gesteld op basis van de verlammingsverschijnselen, gecombineerd met typische MRI-afwijkingen in de grijze stof van het ruggenmerg. Daarnaast wordt er bij AFM vaak een ontstekingsreactie in het hersenvocht gevonden. De term AFM werd geïntroduceerd nadat er in 2014 in de Verenigde Staten een grote groep kinderen met dit ziektebeeld werd gezien en dit samenviel met een toegenomen circulatie van enterovirus D68 (EV-D68). EV-D68 is een in de luchtwegen voorkomend virus dat meestal alleen een luchtweginfectie veroorzaakt. Sinds 2014 is EV-D68 ook het virus dat het meest met AFM wordt geassocieerd. Minder vaak zijn andere virussen betrokken, zoals enterovirus A71 (EV-A71).

In Nederland werd in 2016 het eerste kind met AFM en EV-D68 gediagnosticeerd. De diagnose werd pas gesteld nadat virologen EV-D68 hadden aangetoond in luchtwegmateriaal van dit kind. De ziektegeschiedenis van deze eerste patiënt

vormde de aanleiding voor dit proefschrift. Doel van de daarop volgende studies, beschreven in dit proefschrift, was om meer inzicht te krijgen in de epidemiologie van AFM en EV-D68. Ook wilden we onderzoeken hoe beter onderscheid gemaakt kan worden tussen AFM en andere aandoeningen die acute (slappe) verlammingsverschijnselen bij kinderen kunnen veroorzaken.

In **Hoofdstuk 2** geven we een overzicht van de verschillende groepen patiënten met AFM die in de wetenschappelijke literatuur zijn beschreven. We beschrijven de klinische kenmerken en de bevindingen bij het aanvullend onderzoek en vergelijken AFM veroorzaakt door EV-D68 met AFM veroorzaakt door andere virussen, zoals het poliovirus. Ook doen we een aanbeveling welk aanvullend onderzoek uit te voeren bij kinderen met acute verlammingsverschijnselen. Het is belangrijk om zich te realiseren dat EV-D68 bij patiënten met AFM hoofdzakelijk in materiaal uit de luchtwegen, bijvoorbeeld verkregen met een neusspoelsel, wordt gevonden. Ook is de kans om dit virus te vinden het grootst wanneer het materiaal vroeg in het ziektebeloop wordt afgenomen. Daarom moeten artsen, die kinderen met acute verlammingsverschijnselen behandelen, alert zijn op het verrichten van vroege en juiste virologische diagnostiek. Een goede samenwerking tussen de microbioloog/viroloog en de behandelend arts is dus essentieel. Deze samenwerking is ook belangrijk om de wetenschappelijke vragen op het gebied van AFM en EV-D68 beter te kunnen beantwoorden.

Het vervolg van dit proefschrift opgedeeld in drie delen. Het eerste deel richt zich op de epidemiologie van AFM en EV-D68 in Nederland en Europa. In het tweede deel worden de differentiële diagnose van AFM en de onderscheidende kenmerken tussen AFM en andere oorzaken van AFP onderzocht. In het derde deel worden de diagnostische criteria van AFM beschreven en de toepasbaarheid ervan onderzocht.

Deel 1: Epidemiologie

Om inzicht te krijgen in de consequenties voor de volksgezondheid en om goed voorbereid te zijn op mogelijke nieuwe uitbraken, is het belangrijk om te weten hoe vaak AFM voorkomt. Omdat er geen simpele test bestaat die de diagnose

AFM met zekerheid kan bevestigen, is het een uitdaging om de incidentie ervan te onderzoeken.

In 2016 was er een duidelijke toename van de circulatie van EV-D68 in Europa. In dat jaar werd, zoals hierboven vermeld, ook de eerste patiënt met AFM in Nederland beschreven. Om een indruk te krijgen van het vóórkomen van AFM in Europa, werd binnen een netwerk van virologen en clinici gevraagd om patiënten met AFM, waarbij EV-D68 was aangetoond, te rapporteren. Deze inventarisatie leverde 29 patiënten op, waarvan de klinische kenmerken in **Hoofdstuk 3** worden beschreven. Ook komen in dit hoofdstuk de methoden van opsporing en detectie van enterovirussen in verschillende Europese laboratoria aan de orde. Van de 29 patiënten waren er 26 kinderen en 3 volwassenen. Zij kwamen uit 12 verschillende Europese landen. De klinische kenmerken kwamen overeen met die van eerder beschreven patiënten met AFM. Patiënten hadden vaak asymmetrische zwakte van de ledematen, zwakte van de spieren in het gelaat en moeite met slikken of spreken. Ook was er vaak zwakte van de ademhalingsspieren, waardoor bij twee-derde van de patiënten beademing nodig was. De MRI-scan toonde een afwijkend signaal van de grijze stof van het ruggenmerg en de hersenstam. Bij zenuwgeleidingsonderzoek werd een beeld passend bij schade aan de voorhoorncellen gezien. Slechts drie van de patiënten herstelden volledig en twee patiënten overleden. EV-D68 werd bij deze patiënten vooral in materiaal uit de bovenste luchtwegen aangetoond. Dit laat nog eens zien dat het belangrijk is om dit materiaal te onderzoeken bij patiënten verdacht van AFM. Waarschijnlijk is deze groep patiënten slechts een klein deel van het werkelijke aantal in Europa, deels door de manier waarop dit onderzoek is opgezet, maar ook omdat er regelmatig geen virus kan worden aangetoond bij patiënten met AFM. Door het systematisch opsporen en melden van patiënten van AFM moet het mogelijk zijn beter zicht te krijgen op het daadwerkelijke aantal. Eerder was er in veel landen een actief AFP-meldsysteem om gevallen van poliomyelitis op te sporen, maar dit is nu nog maar in weinig landen operationeel en effectief.

In **Hoofdstuk 4** hebben we gepoogd om een antwoord te krijgen op de vraag hoe vaak AFM in Nederland voorkomt. Dit hebben we gedaan door terug te kijken in

dossiers van kinderen die in de periode van 2014 tot 2019 in tien Nederlandse ziekenhuizen waren gezien in verband met acute spierzwakte. Deze ziekenhuizen waren geselecteerd op de aanwezigheid van kinderneurologen en een zo groot mogelijke landelijke dekking. Na een aantal vooraf vastgelegde selectieronden werden de kinderen die overbleven ingedeeld in de volgende diagnosegroepen: (1) andere diagnose meer waarschijnlijk, (2) onzeker of sprake is van AFM, (3) mogelijk AFM, (4) waarschijnlijk AFM, (5) zeker AFM (deze classificatie wordt uitgebreider beschreven in hoofdstuk 7). Het aantal kinderen dat als waarschijnlijk of zeker AFM werd geclassificeerd, werd gebruikt om een schatting te maken van de incidentie. Uit dit onderzoek bleek dat AFM in deze periode, met acht zekere en drie waarschijnlijke gevallen in Nederland, vrij zelden voorkwam. De geschatte incidentie was 0,06/100.000 kinderen per jaar. AFM kwam vaker voor in periodes waarin EV-D68 circuleerde, wat het verband tussen AFM en EV-D68 verder ondersteunt. De diagnose AFM werd niet meteen bij alle kinderen gesteld. Bij een deel werd eerst gedacht aan een andere aandoening zoals myelitis transversa (MT), een auto-immuun aandoening die gepaard gaat met een ontsteking van het ruggenmerg. Om te zorgen dat de juiste diagnose wordt gesteld, is het belangrijk dat artsen bij kinderen met acute verlammingsverschijnselen aan AFM denken, zodat ze adequate diagnostiek inzetten. Goed inzicht in de incidentie van AFM is alleen mogelijk, wanneer artsen de goede diagnose stellen en nieuwe gevallen van AFM worden geregistreerd.

Deel 2: Differentiële diagnose

Het stellen van de juiste diagnose is niet alleen belangrijk om een goed beeld te krijgen van de incidentie, maar ook omdat de behandeling, het beloop en de prognose van andere aandoeningen die gepaard gaan met acute verlammingsverschijnselen heel anders kunnen zijn. Twee hiervan zijn het Guillain-Barré syndroom (GBS), een immuun-gemedieerde ziekte van de zenuwen en zenuwwortels, en de eerder genoemde myelitis transversa (MT).

Om kenmerkende verschillen tussen deze aandoeningen en AFM te vinden hebben we groepen patiënten met de verschillende aandoeningen vergeleken. In **Hoofdstuk**

5 vergeleken we de klinische kenmerken en bevindingen bij aanvullend onderzoek van een groep van 156 kinderen met een zekere diagnose GBS, met die van een groep van 26 kinderen met AFM. Ook pasten we bestaande diagnostische criteria voor beide aandoeningen toe op beide groepen om te zien of deze voldoende onderscheidend waren. We vonden dat er vooral in de beginfase veel overlap tussen beide aandoeningen is, maar ook dat er belangrijke onderscheidende kenmerken zijn. Bij AFM is de zwakte bijvoorbeeld sneller progressief en vaker asymmetrisch en treden er geen gevoelsstoornissen op. Om een zeker onderscheid te maken is ook aanvullend onderzoek van belang, waarbij liquoronderzoek en een MRI-scan van het ruggenmerg een belangrijke rol spelen. Kinderen met GBS hebben een veel betere prognose dan kinderen met AFM en herstellen vaak volledig. De diagnostische criteria voor beide aandoeningen zijn redelijk goed onderscheidend, maar enkele kinderen met AFM voldoen ook aan de criteria voor GBS. Dit laat nog eens zien dat het belangrijk is om tijdig de diagnose AFM te overwegen, zodat gericht aanvullend onderzoek ingezet kan worden.

In **Hoofdstuk 6** gebruikten we dezelfde opzet om AFM met myelitis transversa (MT) te vergelijken. Een groep van 36 kinderen met MT werd vergeleken met een groep van 21 kinderen met AFM. Er was een belangrijke overlap in de klinische kenmerken en bevindingen bij aanvullend onderzoek tussen beide groepen. Een aanzienlijk deel van de kinderen met AFM voldeed aan de diagnostische criteria voor MT en een deel van de kinderen met MT voldeed aan de criteria voor waarschijnlijke of zekere AFM. Ook hier vonden we vroege onderscheidende kenmerken: kinderen met AFM hadden vaak meer betrokkenheid van de armen en meer proximale zwakte, terwijl kinderen met MT vaker gevoelsstoornissen, verhoogde spierrekkingsreflexen en een gestoorde blaasfunctie hadden. Een belangrijke reden om beide aandoeningen van elkaar te onderscheiden is het verschil in behandeling. Bij AFM is er geen bewezen effectieve behandeling, maar worden intraveneuze immuunglobulinen (IVIG) aanbevolen. Bij MT is prednison de eerste keuze behandeling. In een muismodel van AFM hadden muizen behandeld met prednison uiteindelijk een slechtere uitkomst met meer zwakte dan onbehandelde muizen, wat wijst op een mogelijk nadelig effect bij AFM.

Deel 3: Diagnostische criteria

De diagnostische criteria van AFM zijn reeds aan bod gekomen in de vorige hoofdstukken. De diagnose AFM werd oorspronkelijk gesteld op basis van een definitie opgesteld door de 'Centers for Disease Control and Prevention' (CDC) in de Verenigde Staten. Deze definitie is een aantal keren aangepast, was vooral bedoeld voor registratiedoeleinden en werd minder geschikt geacht voor toepassing in de kliniek. Daarom heeft een internationale werkgroep zich ingezet om klinische diagnostische criteria op te stellen. Met deze diagnostische criteria, beschreven in een overzichtsartikel in **Hoofdstuk 7**, wordt de diagnose AFM gesteld op basis van klinische kenmerken in combinatie met bevindingen van aanvullend onderzoek. Bovendien worden er kenmerken benoemd die een diagnose AFM juist minder waarschijnlijk maken of uitsluiten. In het overzichtsartikel wordt daarnaast de meest actuele kennis op het gebied van de klinische kenmerken, etiologie, pathofysiologie en behandeling van AFM beschreven. Ook worden aanbevelingen gedaan voor therapeutische interventies in de verschillende fasen van de ziekte.

De in hoofdstuk 7 genoemde diagnostische criteria zijn nog niet geëvalueerd. In **Hoofdstuk 8** geven we hiertoe een eerste aanzet door de criteria toe te passen op het Nederlandse cohort van kinderen met in korte tijd ontstane verlammingsverschijnselen, beschreven in hoofdstuk 4. In dit cohort werden naast AFM de diagnoses GBS en MT frequent gesteld. Kinderen met MT voldeden vaak aan de criteria voor mogelijke of waarschijnlijke AFM, terwijl kinderen met GBS vaak voldeden aan de criteria voor een onzekere diagnose AFM. Meestal lukt het om op basis van de aanvullende kenmerken uit de criteria, die een andere diagnose waarschijnlijker maken, het onderscheid met AFM te maken. Soms zijn er echter andere kenmerken nodig voor dit onderscheid, zoals de uitslagen van virologische diagnostiek, zenuwgeleidingsonderzoek en uitslagen van liquoronderzoek. Deze kenmerken kunnen artsen helpen om AFM te onderscheiden van andere oorzaken van snel ontstane verlammingsverschijnselen. Het is wel van belang hiervoor de juiste diagnostiek te verrichten.

Het proefschrift eindigt in **Hoofdstuk 9** met een samenvatting en discussie van

de bevindingen, waarbij ook nagedacht wordt over toekomstig onderzoek. Een belangrijk initiatief in die richting is het opzetten van een Europees AFM-register binnen een samenwerkingsverband van clinici, virologen en medewerkers uit de publieke gezondheidszorg. Hierin worden nieuwe gevallen van AFM geregistreerd en gerelateerd aan de circulatie van geassocieerde virussen. Een dergelijke samenwerking tussen clinici en virologen, die ook leidde tot de eerste AFM-diagnose in Nederland, blijft van groot belang om de vele klinische en wetenschappelijke uitdagingen betreffende dit bijzondere ziektebeeld aan te gaan.

DANKWOORD

In dit proefschrift is al vaak het belang van goede samenwerking benadrukt. Zonder samenwerking tussen neurologen, radiologen en virologen kan de diagnose AFM niet worden gesteld. Zonder samenwerking tussen verschillende onderzoekers waren de verschillende projecten in dit proefschrift, niet tot stand gekomen. Zonder samenwerking had ik dit proefschrift niet kunnen schrijven. Graag wil ik dan ook iedereen die heeft bijgedragen aan het tot stand komen van dit proefschrift van harte bedanken.

Beste prof. dr. O.F. Brouwer, beste Oebo, ongeveer zeven jaar geleden zaten we aan tafel met de virologen van het UMCG om voor het eerst over AFM en enterovirus D68 te praten. Dit gesprek vormde het begin van dit avontuur, wat je wel met me aan durfde, zoals je toen aangaf. Dat zo'n gesprek de aanleiding vormde voor dit proefschrift, past goed bij de manier waarop je me leerde om wetenschappelijk onderzoek te doen. Je gaf me mee dat iets wat je tegenkomt in de kliniek vaak een goede en blijvende motivatie geeft voor het doen van wetenschappelijk onderzoek. Daarnaast leerde je me dat de vruchtbaarste discussies vaak tot stand komen tussen specialisten van verschillende disciplines, die samen gemotiveerd zijn om een probleem op te lossen.

Zowel voor als na je pensioen was je altijd laagdrempelig benaderbaar om te spreken over onze projecten, over casuïstiek en over kinderneurologie in het algemeen. Het is inspirerend om te zien, hoe veel plezier je nog steeds beleeft aan het bespreken van complexe of interessante casuïstiek. Het was ontzettend leuk op Curaçao nog een week met je samen te werken, toen we daar waren met Nienke en Nynke. Ik hoop dat we, ook in de volgende jaren, nog regelmatig contact zullen hebben.

Beste Bert, ondanks alle tijd en aandacht die het coronavirus van je opeiste in de afgelopen jaren, heb je steeds gelegenheid gevonden om me te leren over virologie. Je betrok me bij het project van Marjolein en bracht me in contact met nationale en internationale experts op het gebied van EV-D68, bijvoorbeeld tijdens de bijeenkomst op Schiphol in 2017. Je haalde Kevin naar Groningen om van hem te leren over AFM in de Verenigde Staten, bracht me in contact met het internationale AFM netwerk en betrok me bij het Nederlandse onderzoek naar AFM, wat vanuit het RIVM werd geïnitieerd. Zonder deze samenwerking en contacten was het onderzoek wat leidde tot dit proefschrift niet mogelijk geweest.

Prof. Kremer, prof. Verhagen en prof. Kroes, hartelijk dank voor het lezen en beoordelen van dit proefschrift. Beste Berry, bedankt voor de mogelijkheden die je me gaf om naast het klinische werk ook aan dit proefschrift te werken.

Beste collega's van het RIVM, Marit, Kim, Adam en Margreet, bedankt voor de bijzondere samenwerking. Bedankt ook voor het mede mogelijk maken van mijn reis naar de Verenigde Staten en de financiële ondersteuning voor het maken van dit proefschrift. Jullie enthousiasme om 'tussen alle corona door' over AFM en EV-D68 te praten motiveerde om onze projecten tot een succes te maken. Hopelijk kunnen we hier nog verder op bouwen in de toekomst.

Beste Marjolein en Coretta, bedankt voor de leuke samenwerking. Ik heb veel van jullie geleerd over de virologie en jullie 'virologische input' op de stukken in dit proefschrift. Bedankt voor het betrekken bij het Europese project over AFM en EV-D68, wat aan de basis staat van veel van de projecten in dit proefschrift. Ik hoop dat we ook in de toekomst samen kunnen blijven werken en af en toe sparren over neuro-virologische casuïstiek.

Beste Bart, tijdens een borrel van het Erasmus MC kwam het idee op om AFM en het Guillain-Barré syndroom bij kinderen met elkaar te vergelijken. Je brede oriëntatie, waarbij je ook graag specialisten op andere gebieden betrekt in onderzoek en kritische blik op de inhoud en relevantie van bevindingen voor de praktijk, zijn voor mij een inspiratie om te gebruiken in (onze) verdere projecten.

Beste Rinze, het idee voor een stage kinderneurologie in Rotterdam kwam tijdens de bijeenkomst in Schiphol. Naast dat het een leerzame en verrijkende stage was, kwam ook het idee op voor een vergelijking tussen AFM en inflammatoire myelitis. Naast kennis en kunde op het gebied van de neuro-inflammatoire aandoeningen bij kinderen, heb je ook een goed oog voor kansen op het gebied van kinderneurologisch wetenschappelijk onderzoek in Nederland.

Beste Joyce en Arlette, het was ontzettend leuk om met jullie samen te werken aan de verschillende projecten. Ik denk dat we elkaar op een fijne manier aanvulden en konden motiveren.

Dear Kevin, thank you for involving me in the international working group and for sharing your insights in enterovirus D68. I am impressed by your knowledge and creativity in analyzing and predicting the epidemiology and characteristics of virus infections in children. Thank you, and your family and colleagues, for your hospitality during my visit to Colorado in the fall of 2022. Not only did I learn a lot from EV-D68 surveillance and research at your

hospital, but also I watched my first American football game and learned how to ride slippery slides on a horseback.

Dear Carlos, Matt, Cristina, Janet and others at John's Hopkins, thank you for the hospitality when I visited you. Thank you for showing me the great work you are doing for children and their parents, and of course for teaching me the art of eating crabs. Carlos, I am sure you can appreciate the statement on the terminology of 'transverse myelitis'. Thank you for involving me in the AFM working group and the organization of the always instructive meetings.

Dear European collaborators, thank you for sharing your data and insights on AFM. Hopefully our collaboration will continue together with ENPEN to learn more from the epidemiology and impact of AFM in Europe. Heli, Kim and Thea, I am looking forward to move forward in these efforts.

Dank aan alle patiënten en hun ouders die hun gegevens hebben willen delen om hiervan te kunnen leren. Gert-Jan en Gerda, zeven jaar geleden ontmoetten wij elkaar voor het eerst en sindsdien hebben we vaak contact gehad. De uitdagingen die jullie tegenkwamen om de beste zorg voor jullie zoon te organiseren, waren voor mij een motivatie om dit proefschrift tot een succes te brengen.

Beste Annemarie, Deborah, Ineke en Maartje, bedankt dat jullie me in het team van de kinderneurologen in het UMCG hebben opgenomen en mij langzamerhand in mijn rol laten groeien. Bedankt voor de ruimte die jullie me hebben gegeven om tijd te besteden aan het proefschrift, ondanks alle vraag die er is vanuit de kliniek. Ook aan de andere collega's in het UMCG, dank voor de ondersteuning en samenwerking in de afgelopen jaren. Saloua, leuk en gezellig om samen met jou een kamer te delen en de mooie en minder mooie dingen van het begin van onze carrière als neuroloog met elkaar te delen.

Lieve Niek en Linda, wat ontzettend leuk dat jullie vandaag naast me staan vandaag. Onze vriendschap begon al aan het begin van de geneeskunde studie en we mochten al vele reizen, mooie momenten en life-events samen beleven. Vandaag voegen we er nog maar eentje aan toe en ik weet zeker dat er, samen met Eline, Dirk en Daniëlle nog veel zullen volgen.

Lieve pap en mam, zonder de goede basis en jullie nuchtere kijk op het leven, was ik nooit zover gekomen. Jullie hebben me altijd de ruimte gegeven om mijn dromen na te streven, maar leerden me ook om goed na te denken voor ik ergens aan begon.

Lieve Joop en Lydia, bedankt dat jullie altijd voor ons gezin klaar staan en ons helpen als we jullie nodig hebben. Mooi om te zien wat een hechte band jullie met onze kinderen hebben opgebouwd.

Lieve Daniëlle, wat ben ik blij dat jij er altijd voor me bent. Wat hebben we samen een geluk met onze prachtige dochters Elin en Lise. Als ik gefrustreerd was of mopperde, kon jij me helpen om te relativeren of met me mee mopperen, wat me ruimte gaf om verder te gaan. Je gaf me de ruimte om mijn proefschrift te schrijven en hielp me om ook keuzes te maken om dingen soms ook niet te doen, zodat er ruimte blijft voor de (andere) belangrijke dingen in het leven. Ik zie er naar uit om het einde van mijn promotie-traject samen met jou te vieren!

Jelte

CURRICULUM VITAE

Jelte Helfferich werd op 8 maart 1990 geboren in Sneek. In 2017 behaalde hij zijn VWO-diploma aan het Greijdanus College in Zwolle. Daarna begon hij aan de studie Geneeskunde aan de Rijksuniversiteit Groningen. Hij deed zijn wetenschappelijke stage in het laboratorium van Peter Crino aan de University of Pennsylvania in Philadelphia. Gedurende een kort coschap kinderneurologie in het UMCG werd de interesse voor dit vak gewekt. Deze interesse werd verder aangewakkerd tijdens een stage kinderneurologie in het Skåne ziekenhuis in Lund en Malmö te Zweden. Na het afronden van de opleiding geneeskunde in april 2014, werkte hij een klein jaar als ANIOS neurologie in de Isala klinieken in Zwolle. Daarna begon hij aan de opleiding tot neuroloog en kinderneuroloog in het Universitair Medisch Centrum Groningen. Tijdens de opleiding deed hij onder andere een half jaar stage op de afdeling kinderneurologie in het Erasmus MC te Rotterdam in 2018. Onder begeleiding van prof. dr. O.F. Brouwer, combineerde Jelte zijn opleiding met wetenschappelijk onderzoek. In het begin van de opleiding deed hij een studie naar laaggradige gliomen. Vanaf 2016 begon hij met onderzoek op het gebied van ‘acute flaccid myelitis’. Na het afronden van de opleiding in juli 2021 is hij als kinderneuroloog in het UMCG blijven werken. Ook in de toekomst hoopt hij wetenschappelijk onderzoek en klinisch werk te blijven combineren.

LIST OF PUBLICATIONS

Helfferich J, Bruijstens AL, Knoester M, Brouwer OF, Neuteboom RF; 2016 EV-D68 AFM Working Group and the Dutch Study Group for Pediatric Multiple Sclerosis and Acute Disseminated Encephalomyelitis. Comparison of acute flaccid myelitis and transverse myelitis in children and evaluation of diagnostic criteria. Eur J Neurol. 2023 May 12. Online ahead of print.

Helfferich J, Neuteboom RF, de Lange MMA, Benschop KSM, Van Leer-Buter CC, Meijer A, Bakker DP, de Bie E, Braakman HMH, Brandsma R, Niks EH, Niermeijer JM, Roelfsema V, Schoenmaker N, Sie LT, Niesters HG, Te Wierik MJM, Jacobs BC, Brouwer OF. Pediatric acute flaccid myelitis: Evaluation of diagnostic criteria and differentiation from other causes of acute flaccid paralysis. Eur J Paediatr Neurol. 2023 May;44:28-36.

Helfferich J, de Lange MM, Benschop KS, Jacobs BC, Van Leer-Buter CC, Meijer A, Bakker DP, de Bie E, Braakman HM, Brandsma R, Neuteboom RF, Niks EH, Niermeijer JM, Roelfsema V, Schoenmaker N, Sie LT, Niesters HG, Brouwer OF, Te Wierik MJ. Epidemiology of acute flaccid myelitis in children in the Netherlands, 2014 to 2019. Euro Surveill. 2022 Oct;27(42):2200157. doi: 10.2807/1560-7917.ES.2022.27.42.2200157.

Helfferich J, Roodbol J, de Wit MC, Brouwer OF, Jacobs BC; 2016 Enterovirus D68 Acute Flaccid Myelitis Working Group and the Dutch Pediatric GBS Study Group. Acute flaccid myelitis and Guillain-Barré syndrome in children: A comparative study with evaluation of diagnostic criteria. Eur J Neurol. 2022 Feb;29(2):593-604.

Helfferich J, Bruijstens AL, Wong YYM, Danielle van Pelt E, Boon M, Neuteboom RF; Dutch Study Group for Pediatric Multiple Sclerosis and Acute Disseminated Encephalomyelitis. Prognostic factors for relapse and outcome in pediatric acute transverse myelitis. Brain Dev. 2021 May;43(5):626-636.

Murphy OC, Messacar K, Benson L, Bove R, Carpenter JL, Cwarfor T, Dean J, DeBiasi R, Desai J, Elrick MJ, Farias-Moeller R, Gombolay GY, Greenberg B, Harmelink M, Hong S, Hopkins SE, Oleszek J, Otten C, Sadowsky CL, Schreiner TL, Thakur KT, Van Haren K, Carballo CM, Chong

PF, Fall A, Gowda VK, **Helfferich J**, Kira R, Lim M, Lopez EL, Wells EM, Ye EA, Pardo CA; AFM Working group. Acute flaccid myelitis: cause, diagnosis, and management. Lancet. 2021 Jan 23;397(10271):334-346.

Helfferich J, Knoester M, Van Leer-Buter CC, Neuteboom RF, Meiners LC, Niesters HG, Brouwer OF. Acute flaccid myelitis and enterovirus D68: lessons from the past and present. Eur J Pediatr. 2019 Sep;178(9):1305-1315

Knoester M, **Helfferich J**, Poelman R, Van Leer-Buter C, Brouwer OF, Niesters HGM; 2016 EV-D68 AFM Working Group. Twenty-nine Cases of Enterovirus-D68-associated Acute Flaccid Myelitis in Europe 2016: A Case Series and Epidemiologic Overview. Pediatr Infect Dis J. 2019 Jan;38(1):16-21.

Helfferich J, Kingma EM, Meiners LC, Schölvinck EH, Mulder HD, Brouwer OF. Acute slappe verlamming na een luchtweginfectie. Ned Tijdschr Geneeskd. 2017;161:D1566

Helfferich J, Meiners LC, Brouwer OF. Acute flaccid weakness associated with enterovirus D68. (Comment) Eur J Paediatr Neurol. 2017 May;21(3):594-595.

Helfferich J, Nijmeijer R, Brouwer OF, Boon M, Fock A, Hoving EW, Meijer L, den Dunnen WF, de Bont ES. Neurofibromatosis type 1 associated low grade gliomas: A comparison with sporadic low grade gliomas. Crit Rev Oncol Hematol. 2016 Aug;104:30-41

Helfferich J, Crino PB. Ganglioglioma, mTOR Activation, and Epileptogenesis, chapter 17, page 147 in Tumors of the Central Nervous System, Volume 9 edited by M.A. Hayat.

Parker WE, Orlova KA, Parker WH, Birnbaum JF, Krymskaya VP, Goncharov DA, Baybis M, **Helfferich J**, Okochi K, Strauss KA, Crino PB. Rapamycin prevents seizures after depletion of STRADA in a rare neurodevelopmental disorder. Sci Transl Med. 2013 Apr 24;5(182).