Two cases of acute severe flaccid myelitis associated with enterovirus D68 infection in children, Norway, autumn 2014

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Citation style for this article:

Article submitted on 25 February 2015 / published on 12 March 2015

Enterovirus D68 (EV-D68), phylogenetic clade B was identified in nasopharyngeal specimens of two cases of severe acute flaccid myelitis. The cases were six and five years-old and occurred in September and November 2014. EV-D68 is increasingly associated with acute flaccid myelitis in children, most cases being reported in the United States. Awareness of this possible neurological complication of enterovirus D68 infection is needed.

An unexpectedly high proportion of children were admitted for severe respiratory infections at the Oslo University Hospital, Ullevål, Norway, during September and October, 2014 [1]. Enterovirus was detected in 66 (22%) of 303 samples from children hospitalised with acute respiratory infection, and in five of 51 samples received from outpatient clinics. Enterovirus D68 (EV-D68) was verified in 33 of the enterovirus-positive samples from hospitalised patients, and in one of the outpatients.

We report two cases of severe acute flaccid myelitis (AFM) associated with EV-D68 infection that occurred in September and November 2014 in Norway.

Case 1
A six year-old girl was referred to a paediatric department in the Oslo area, Norway, on 20 September 2014. She was previously healthy and fully vaccinated according to the Norwegian child vaccination programme, including polio vaccine. She reported a sore throat, neck pain, headache and occasional vomiting for two days. Fever (38.6°C) occurred on the second day and she became increasingly tired with tachypnoea, coughing and abdominal pain. She presented with a faint voice, reduced general condition, neck pain, but no nuchal rigidity. General and neurological examination was normal. Leucocyte count was 12.9 × 10^9 cells/L (norm: 5.0–15.5) with neutrophilocytes accounting for 74%. C-reactive protein (CRP) was 4 mg/L (norm: 0.0–4.0). Viral upper airway infection was suspected, but PCR analysis of a nasopharyngeal specimen was negative for common respiratory viruses, and the patient was discharged. She was readmitted two days later with further deteriorated general condition, general muscle weakness, mainly proximal and more severe in both upper extremities and neck, and weak/absent deep tendon reflexes. A chest X-ray showed an atelectasis in the left lower lobe but a lower respiratory specimen was not secured. Severe respiratory failure, due to diaphragmatic paresis, resulted in respirator treatment. Meningitis treatment with cefotaxime, ampicillin and aciclovir was started. Acute disseminated encephalomyelitis (ADEM) was suspected, and methylprednisolone instituted.

Cerebrospinal fluid (CSF) pleocytosis (173 × 10^6 cells/L (norm: 0–4), 94% mononuclear) and a slightly increased CSF protein concentration (0.45 g/L (norm: 0.000–0.450)) were found on Day 4. On Day 6, before intravenous immunoglobulin infusion or plasmapheresis, only a slight pleocytosis (23 × 10^6 cells/L) was found, however, an increased IgG index indicated intrathecal IgG production but oligoclonal bands were not detected. CSF cell count had normalised on Day 44, with CSF protein still slightly increased (0.648 g/L).

PCR analyses of the CSF revealed no intrathecal herpes simplex virus, human herpes virus 6 or 7, varicella-zoster virus, Epstein-Barr virus, cytomegalovirus, human parechovirus or enterovirus. Bacterial culture, including for Listeria monocytogenes, was negative. No intrathecal antibodies against Borrelia burgdorferi were detected. Serological analysis showed no infection with herpes simplex virus, varizella-zoster virus, Epstein-Barr virus, cytomegalovirus, Mycoplasma...
pneumoniae, Borrelia burgdorferi, Cryptococcus neoformans or Mycobacterium tuberculosis. Two faecal specimens, collected on Days 21 and 22, showed no growth of enterovirus. Antibodies associated with autoimmune encephalitis could not be detected. Magnetic resonance imaging (MRI) on Day 4 showed oedema of mainly grey matter in a longitudinal, transverse pattern, extending from the pedunculi cerebri to the thoracolumbar level. Day 8 there was regression of the oedema, and MRI was normal on Day 44. Electroencephalography (EEG) was normal on Day 3 and Day 16, but compatible with encephalitis/encephalopathy on Day 5.

Following reports from the United States (US) and Canada on EV-D68, a nasopharyngeal specimen taken on Day 2 was tested with a generic real-time RT-PCR for enteroviruses targeting the 5' non-coding region, and was positive. Sequencing of the PCR product suggested EV-D68, which was confirmed by a real-time RT-PCR EV-D68 assay (ct value < 30 cycles). PCR for EV-D68 was negative in serum (Days 4 and 12), faeces (Day 9) and CSF (Days 4 and 44), and in tracheal secretion (Day 30).

The patient now walks steadily, but head control is poor. Motor impairment is worse proximally and still pronounced in the proximal upper extremities and neck where muscular atrophy is evident. She is partly fed through a gastrostomy tube and speaks with a thin voice.

Sequence analysis for Case 1
PCR for sequencing was performed as described in Nix et al. [2]. Sequencing was not possible in for Case 2, due to low amounts of virus and insufficient sample material. The sequence from Case 1 was aligned with the sequences of one reported AFM case from 2014 in France as well as other European strains, including the sequences of one reported AFM case from 2014 in the Oslo region [1].

The outcome is with severe paresis and atrophy in the proximal left arm and left upper limb girdle.

Discussion
EV-D68 is mainly associated with respiratory disease and was first isolated in California in 1962 from children with airway infection [3,4]. Until 2009, EV-D68 was rarely isolated [5]. Since then, outbreaks of respiratory disease with EV-D68 have occurred worldwide [6-9]. EV-D68 infections in the Dutch population is increasing [10]. In autumn 2014, EV-D68 was found in 11% of children hospitalised for airway infection in the Oslo region [1]. Of the 34 patients, 32 were younger than seven years.

During an outbreak of respiratory EV-D68-disease in the US in autumn 2014, nine children presented with AFM; EV-D68 was detected in four of eight nasopharyngeal specimens [11]. From 2 August 2014 to 2 March 2015, the American Centers for Disease Control and Prevention (CDC) verified reports of 115 children in 34 US states who developed AFM. These cases are
Phylogenetic cluster analysis of enterovirus D68 partial VP1 sequences (207 nt) from an acute flaccid myelitis case, Norway, September 2014

Phylogenetic and cluster analysis with currently available EV-D68 sequences in GenBank (n = 231). Maximum parsimony clustering (BioNumerics, Applied Maths) of EV-D68 clade B viruses are shown together with neighbor-joining (Kimura-model) (Mega v.6) subtrees containing Norwegian viruses marked in red. Case 1 is marked in purple. Other AFM cases are underlined in purple. Viruses are named with GenBank accession numbers followed by strain name.
currently being investigated [12]. A single European case of EV-D68-associated AFM is reported [13]. This virus was of Clade B, closely related to Dutch, Spanish and Italian types from 2009 to 2014. From outbreaks in Asia and Australia, enterovirus-71 (EV-A species) is known to cause AFM [14,15]. However EV-D68 has rarely been reported to cause AFM, and almost never been isolated from CSF [5,14].

In the two AFM cases we report here, the virus was only detected in the early nasopharyngeal specimens, highlighting the importance of obtaining this specimen early. Pleocytosis, increased intrathecal IgG index, increased CFS-proteins, spinal grey matter-oedema and denervation on neurophysiology may indicate direct CNS infection and neuron destruction. A causal relation is however not proven since no direct evidence of EV-D68 infection in the CNS has been found. Detection of EV-D68-virus may have been coincidental and due to the high incidence of the virus [9]. However, the two cases share striking clinical and imaging similarities [16] and no other pathogen has been detected. Intrathecal EV-D68 antibody detection would be indicative of EV-D68 being the neurotrophic pathogen, however this analysis is not available in our hospital and was therefore not performed.

Sequencing of the VP1 gene obtained from Norwegian cases showed a similar genotype in the AFM case and non-AFM cases. While a statistical increase is difficult to prove for such a rare disease, the authors are of the opinion that there is an increase in AFM cases associated with enterovirus D-68 as has been reported from the US [17]. On basis of our findings we can speculate that this may not be caused by increased neurotropism in a single genotype, but perhaps due to an increased number of EV-D68-infected individuals. It is possible, however, that mutations in non-VP1 regions are responsible for increased neurotropism.

As we found an identical VP1-sequence in Case 1 and in a child with solely respiratory disease, host-related factors are likely to be of importance for the individual risk of developing AFM. At least three different EV-D68 strains were circulating in Norway in the autumn of 2014, suggesting three separate introductions. No differences in the partial sequence of the VP1 region were observed between the AFM case and non-AFM cases. International collaboration is needed to confirm the association between EV-D68 and AFM, and to achieve knowledge on treatment and outcome. It is important to recognise this disease, which may become life-threatening.

Acknowledgements

We are grateful for technical assistance by Pundharika Barkved performing the PCR analysis and sequencing.

Conflict of interest

None declared.

Authors’ contributions

HCVP drafted the paper, collected information on the patient, analysed and interpreted it, as well as examined the patient and coordinated the work. KB developed the EV-D68 PCR and sequencing protocol and carried out the sequence analysis followed by the phylogenetic analysis. KV and SGD cultivated virus samples. HCVP, MKS, HMD, PKK, MSC, MHP, KV, KB, SGD, AMBK, HCMR assisted in analysis, interpretation of data for the work, revised it critically for important intellectual content, and final approval of the version to be published. All authors approved the final article.

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