CME CREDIT ***EDUCATIONAL OBJECTIVE:*** Readers will suspect enterovirus D68 infection in appropriate clinical scenarios

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Enterovirus D68: A clinically important respiratory enterovirus

ABSTRACT

Seasonal peaks of viral respiratory illnesses are common during late summer and early fall and have often been attributed to human rhinovirus. In the fall of 2014, the number of children hospitalized with severe lower respiratory symptoms and asthma suddenly increased, and the children tested positive by sequencing for enterovirus D68 (EV-D68). As the outbreak unfolded, a possible association was also observed between EV-D68 infection, polio-like acute flaccid paralysis, and cranial neuropathy in children.

KEY POINTS

EV-D68 is a respiratory virus that has genetic and biologic features that blur the distinction between the rhinoviruses and enteroviruses.

Recognition of EV-D68 as an important cause of viral lower respiratory tract illness in children underscores the role of specific strain typing in advancing our understanding of the epidemiology of respiratory virus infections.

Given the inability of commonly used clinical tests for rhinovirus to distinguish EV-D68 in the absence of strain-specific sequence data, caution needs to be used in attributing severe or acute lower respiratory illness to rhinovirus and in interpreting epidemiologic associations between asthma and rhinovirus.

Emerging data suggest that, in addition to its important role in pediatric respiratory illness, EV-D68 may cause systemic disease, especially acute neurologic disease.

doi:10.3949/ccjm.82a.14166

In the fall of 2014, the United States experienced an outbreak of severe respiratory illness due to a virus of emerging importance, enterovirus D68 (EV-D68). Here, we review the features of this virus and related viruses, the clinical syndromes this virus causes, the epidemiology of the recent outbreak, and its diagnosis and treatment.

THE ENTEROVIRUSES: AN OVERVIEW

Originally identified in 1962 from the throat swab of a child with pneumonia, human EV-D68 has unique genetic and clinical features that blur the typical division between human enteroviruses and rhinoviruses.[1–4] Enteroviruses and rhinoviruses are closely related species within the *Picornaviridae* family that are now classified together within the genus Enterovirus.[5] Picornaviruses are small, nonenveloped, positive-stranded RNA viruses of medical significance.

Poliovirus: The first enterovirus discovered

The first human enterovirus to be discovered was poliovirus.[6] Although sporadic cases of "infantile paralysis" occurred before the late 19th century, epidemic poliomyelitis abruptly appeared in Europe and the United States beginning around 1880. Before the introduction in 1955 of the inactivated poliovirus vaccine and then the oral poliovirus vaccine, polio was one of the most feared illnesses in the developed world. Outbreaks occurred primarily in cities during summer months. At its peak, epidemic polio killed or paralyzed more than half a million people a year.

One hypothesis to explain the sudden emergence of epidemic polio is that improved personal hygiene and public sanitation delayed the age at which children acquired this enteric infection.[7] Infections acquired after infancy occurred in the absence of maternal antibodies that may have protected against the virus's propensity to invade the nervous system.

Nonpolio human enteroviruses

In the decades since poliovirus was discovered, more than 100 nonpolio human enteroviruses have been recognized.[8] This group includes the coxsackieviruses, echoviruses, and the newer numbered nonpolio human enteroviruses classified into four species, designated *Human enterovirus A, B, C,* and *D*. The last of these, *Human enterovirus D,* includes three serotypes known to cause disease in humans: EV-D68, EV-D70, and EV-D94.[9]

As with poliovirus infection, most people infected with a nonpolio human enterovirus have a mild illness without distinctive features.[5] In temperate climates, enteroviral infections are most common during the summer and fall and are an important cause of the "summer cold." In tropical climates, the seasonal pattern is absent, and infections may occur throughout the year.

The clinical syndromes associated with a nonpolio human enterovirus can include nonspecific febrile illness; upper respiratory tract infection; pharyngitis; herpangina; hand, foot, and mouth syndrome; various skin exanthems; bronchiolitis; asthma exacerbation; gastrointestinal manifestations such as diarrhea and vomiting (which are especially common); more serious clinical syndromes such as hepatitis, pancreatitis, and cardiomyopathy; and neurologic illness, including aseptic meningitis, encephalitis, and polio-like paralytic disease.

Outbreaks caused by nonpolio human enteroviruses occur on a regular basis, may vary by strain from year to year, and often occur within a geographic region; multiple strains may circulate simultaneously. Occasionally, as with EV-D68 in August 2014 in the United States, epidemics can emerge suddenly and spread rapidly across the world, causing disease in hundreds or thousands of people, demonstrating the breadth of illness associated with particular strains.[10]

ENTEROVIRUS D68: AN EMERGING PATHOGEN

EV-D68 was first isolated in the United States from four children in Berkeley, California, who had lower respiratory tract symptoms (bronchiolitis and pneumonia) in 1962. The finding was published in the medical literature in 1967.[1] Since its initial identification, EV-D68 was infrequently reported as a cause of human disease, with the US Centers for Disease Control and Prevention (CDC) listing only 26 cases in the 36 years from 1970 through 2005.[11]

However, the past decade has seen EV-D68 emerge as a significant respiratory pathogen, with more reports of acute respiratory illness associated with it in North America, Europe, and Asia, especially in children.[12–17] A seasonal pattern may exist; a longitudinal survey of samples collected from New York City detected a focal outbreak in the fall of 2009.[18]

The observation that recent EV-D68 outbreaks have primarily been in children suggests that most adults have immunity to it. In this regard, seroepidemiologic studies from Finland demonstrated that most adults have neutralizing antibodies from previous infection.[9]

The genus Enterovirus includes the polioviruses; nonpolio enteroviruses A, B, C, and D; and rhinoviruses

The blurred line between enteroviruses and rhinoviruses

Enteroviruses and rhinoviruses are typically distinguished on the basis of the temperature at which they grow best (rhinoviruses grow better at lower temperatures, allowing them to replicate in the nose) and their sensitivity to acidity (enteroviruses are more resistant, enabling them to survive in the stomach).

The original ("Fermon") strain of EV-D68 isolated in 1962 was first classified as an enterovirus because it was resistant to low pH.[1] However, when molecular sequencing became available, EV-D68 was found to be identical to human rhinovirus 87 (HRV87), a phylogenetic outlier among the rhinoviruses that binds to cells at a receptor site distinct from that of other human rhinoviruses.[19]

Thereafter, further testing showed that both EV-D68 and HRV87 isolates were sensi-

tive to acid treatment by two different methods.[4] Moreover, unlike most enteroviruses, EV-D68 behaves like a rhinovirus and grows preferentially at 33°C, the temperature of the nose.[2]

How enterovirus D68 enters cells

Viral surface proteins, including hemagglutinin, from certain respiratory viruses have the ability to bind sugars on cells in the nose and lungs, which facilitates viral entry and replication. EV-D68 binds specifically to alpha 2-6 sialic acid, the predominant sialic acid found in the human upper respiratory tract.[19,20] The absence of EV-D68 binding affinity for alpha 2-3 sialic acid, present in ciliated epithelial cells of the lower tract, suggests that alternative mechanisms may be responsible for the severe lower respiratory disease associated with this virus.

Entry of EV-D68 into cells requires additional mediators. EV-D70 belongs to the same genetic cluster as EV-D68 and enters HeLa cells using decay-accelerating factor (DAF).[21] Evidence that EV-D68 also uses DAF for cell entry comes from experiments showing that monoclonal antibodies against DAF inhibit the cytopathic effects of this virus.[4] Virus-receptor interactions have been more thoroughly characterized for other enteroviruses.[22] In this regard, coxsackieviruses of group B use DAF as a coreceptor. Since DAF is expressed at high levels in both epithelial and endothelial cells, it may play an important role in the induction of the viremia that precedes the infection of specific tissues such as the heart or pancreas.

During the last decade, EV-D68 has emerged as a significant respiratory pathogen

Different strains exist

EV-D68 strains can be divided into three genetic groups based on the sequence of the capsid-coding VP1 region, the most variable genome region of enteroviruses.[23]

Investigators have explored whether emergent EV-D68 strains differ in their antigenicity and receptor-binding properties in comparison to the Fermon strain isolated in 1962.[20] Using antisera generated from various strains of EV-D68, significant differences were observed in terms of hemagglutination inhibition and neutralization titers both between emergent strains and the original Fermon strain and among the emergent strains.

Viremia in systemic disease

Like other enteroviruses, EV-D68 has the ability to infect lymphocytes.[9] This may provide a mechanism by which the virus is transported during the viremic phase to secondary target organs. Indeed, EV-D68 was detected in the serum of 12 (43%) of 28 pediatric patients with pneumonia and positive nasopharyngeal swabs.[24]

Interestingly, whether EV-D68 was detected in the serum varied with age. Viremia was not detected in the serum of children younger than 1 year, an observation suggesting that maternal antibodies protect against viremia.

The role of viremia in systemic disease associated with EV-D68 is intriguing, especially since delayed acquisition of polio infection beyond infancy is hypothesized to have contributed to disease severity.[7]

ENTEROVIRUS D68 CAUSES SEVERE LOWER RESPIRATORY DISEASE

While identification of large numbers of patients with respiratory illnesses due to EV-D68 in a single season is unique to 2014, clusters of EV-D68-related respiratory illnesses have previously been recognized.[25,26]

As with EV-D68 outbreaks in other parts of the world, the outbreak in the US Midwest in August 2014 primarily involved children, many of whom needed to be admitted to the hospital because of severe lower respiratory symptoms.[10] In the 30 children admitted to two children's hospitals described in the initial report, difficulty breathing, hypoxemia, and wheezing were common. A minority of patients (23%) presented with fever. Of hospitalized children, 67% required admission to the intensive care unit. Two patients required intubation, including one who required extracorporeal membrane oxygenation. Six required bilevel positive airway pressure therapy.

Cleveland Clinic experience

At Cleveland Clinic during the same time, nearly 45% of patients identified with a respiratory enterovirus infection required intensive care.

For patients previously diagnosed with asthma, chronic lung disease, or wheezing, essential supportive care measures included continuing the inhaled steroids the patients

were already taking, early use of short-acting beta agonists, and, in those with previously diagnosed asthma, consideration of a systemic steroid. Many of our patients with previously diagnosed asthma had an unusually long prodrome of an increase in mild symptoms, followed by a rapid and severe decline in respiratory status.

At the later phase, supportive care measures that were needed included maintenance of hydration and monitoring of oxyhemoglobin saturation with use of supplemental oxygen as necessary, as well as close observation of clinical indicators of respiratory distress, such as development of crackles, asymmetric air exchange, and progression in wheezing or in use of accessory muscles. In an attempt to avoid invasive ventilatory support in patients with asthma or other comorbid conditions, some patients were treated with aerosolized epinephrine, ipratropium, heliox, and noninvasive positive pressure ventilatory support.

NEUROLOGIC DISEASE: ACUTE FLACCID PARALYSIS

Although EV-D68 causes primarily respiratory illness, systemic disease occurs, especially neurologic involvement.

Before the recent outbreak of EV-D68, two cases of neurologic involvement from EV-D68 were reported. The first of these, mentioned in a 2006 enterovirus surveillance report issued by the CDC, was in a young adult with acute flaccid paralysis and EV-D68 isolated from the cerebral spinal fluid.[11] In the second case, from 2010, a 5-year-old boy developed fatal meningomyeloencephalitis. The child had presented with pneumonia and acute flaccid paralysis. EV-D68 was identified in his cerebral spinal fluid by polymerase chain reaction (PCR), and histopathologic study of the meninges, cerebellum, midbrain, pons, medulla, and cervical cord demonstrated extensive T-cell lymphocytic meningomyelitis and encephalitis, characterized by prominent neuronophagia in motor nuclei.[27]

At the same time as the recent outbreak of EV-D68 respiratory disease, neurologists throughout the United States observed an increase in the number of children with polio-like acute flaccid paralysis. On September 26, 2014, the CDC issued an alert describing acute neurologic illness with focal limb weakness of unknown etiology in children, possibly associated with EV-D68.[28] The report described nine cases of an acute neurologic illness in children ages 1 through 18 years (median age, 10) hospitalized in Colorado between August 9 and September 17, 2014. Common clinical features included acute focal limb weakness and paralysis and acute cranial nerve dysfunction, with no altered mental status or seizures. Pain before the onset of weakness was also identified as a common complaint.

Specific findings on magnetic resonance imaging of the spinal cord consisted of nonenhancing lesions largely restricted to the gray matter and in most cases spanning more than one level of the spinal cord. In patients with cranial nerve dysfunction, correlating nonenhancing brainstem lesions were observed.

Most children experienced a febrile respiratory illness in the 2 weeks preceding the onset of neurologic symptoms. In most cases, cerebrospinal fluid analyses demonstrated mild or moderate pleocytosis consistent with an inflammatory or infectious process, with normal to mildly elevated protein and normal glucose levels. In six of the eight patients tested, nasopharyngeal specimens were positive for rhinovirus-enterovirus. Of the six positive specimens, at least four were typed as EV-D68.

Neurologists observed an increase in the number of children with polio-like acute flaccid paralysis

The CDC also reported a second cluster of cases of acute flaccid paralysis with anterior myelitis on magnetic resonance imaging, in 23 children (mean age 10 years) in California from June 2012 to June 2014.[29] No common cause was identified, although clinical and laboratory findings supported a viral etiology. Two patients tested positive for EV-D68 from upper respiratory tract specimens. Common features among the clinical presentations included an upper respiratory or gastrointestinal prodrome less than 10 days before the onset of the paralysis (83%), cerebrospinal fluid pleocytosis (83%), and absence of sensory deficits (78%). Ten patients (43%) also had concomitant mental status changes, and eight (34%) had cranial nerve abnormalities.

Details regarding outcomes from these paralytic illnesses remain unclear, although it would appear that time to recovery has been

prolonged in many cases, and the degree of recovery remains uncertain.

TREATMENT IS SUPPORTIVE

The treatment of EV-D68 infection is mainly supportive, as no specific antiviral therapy is currently available for any of the enteroviruses. Critically ill patients require organ-specific supportive care.

Potential targets for novel antienteroviral therapies exist; some of the experimental compounds were initially evaluated for their activity against polioviruses or rhinoviruses.[30]

TESTING MAY HAVE A ROLE

In general, testing does not play a role in the management of patients with mild disease, but it may be indicated for epidemiologic purposes or for specific diagnosis in critically ill patients. Molecular techniques are commonly used to detect respiratory viruses from clinical samples, either as discrete tests or as a multiplex viral panel.

Since patients with EV-D68 infection typically have respiratory symptoms, the virus is generally tested for in nasal wash samples. However, depending on the clinical presentation, it may be appropriate to attempt to detect the virus from other sites using either PCR or culture.

Many clinical laboratories use real-time PCR assays designed to detect both rhinoviruses and enteroviruses, but these tests do not distinguish between the species. While more specific real-time PCR assays are available that generally distinguish rhinoviruses from enteroviruses,[31] during the recent outbreak our laboratory observed that confirmed EV-D68 samples cross-reacted with rhinovirus. Most clinical laboratories do not routinely perform viral sequence analysis to specifically identify EV-D68, but this test may be obtained through state health departments and the CDC on a case-by-case basis.

Recently, the CDC's enterovirus laboratory announced the development of a real-time PCR assay specifically for EV-D68, which may make specific detection more readily available.

INFECTION PREVENTION

The routes by which EV-D68 is transmitted are not fully understood. In contrast to most enteroviruses, which are spread in a fecal-oral manner, it is possible that EV-D68 is also spread through close respiratory or mucous contact.

For this reason, interim infection prevention guidelines issued by the CDC recommend that hospitals use droplet precautions along with contact or standard precautions, depending on the scenario.[32] In our children's hospital, we use droplet and contact precautions for hospitalized patients.

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