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Review Article

Enteroviral infection in neonates

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Abstract Enteroviruses generally cause mild and self-limited diseases, but they have been found to affect neonates much differently, and often more severely than older children. Clinical manifestations are difficult to differentiate from those of bacterial sepsis, such as fever, poor feeding, lethargy, respiratory distress and cardiovascular collapse. Severe life threatening complications, including hepatic necrosis with coagulopathy, meningoencephalitis and myocarditis, usually present during the first week of life. Factors affecting severity and outcome include virus serotype, mode of transmission, and presence or absence of passively acquired, serotype-specific maternal antibodies. Echoviruses and coxsackievirus B viruses are most common serotypes associated with the neonatal sepsis. An awareness of the clinical syndromes, recognition of the risk factors and monitoring parameters associated with severe cases and use of rapid reverse-transcriptase polymerase chain reaction test for viral load may help physicians in diagnosing severe cases in a timely manner. Prompt aggressive treatment including early intravenous immunoglobulin treatment may help in reducing morbidity and mortality. Enterovirus infections in neonates are common and should be routinely considered in the differential diagnosis of febrile neonates, particularly during enterovirus season. This article provides an overview of what is known about non-polio enteroviruses in neonates including epidemiology, transmission, clinical presentation, diagnosis, and treatment.

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Introduction

Enteroviruses and parechoviruses are RNA viruses that belong to the family *Picornaviridae*. Enteroviruses were formerly classified into enteric cytopathic human orphan (Echo) viruses, coxsackievirus A, coxsackievirus B viruses, polioviruses and newly identified serotypes. A new classification was developed based on genome sequencing. Enteroviruses are now subgroup into human enterovirus (HEV)-A, HEV-B, HEV-C, and HEV-D, based on the similarities in their viral structure protein gene.[1,2]

Enteroviruses have been found to affect neonates much differently, and often more severely than older children. Non-polio enterovirus infections are common in newborns and usually present with fever, poor feeding, lethargy, irritability, with or without a rash and occasionally can lead to sepsis, myocarditis, hepatitis with coagulopathy and meningoencephalitis.[3,4] Factors affecting severity and outcome include virus serotype, mode of transmission, and presence or absence of passively acquired, serotype-specific maternal antibodies.[5] The most commonly associated serotypes with severe neonatal infection are coxsackievirus (CV) B and Echovirus.[5] and both groups are now categorized as HEV-B.

Epidemiology

Nonpolio enteroviruses are distributed throughout the world and have a seasonal pattern causing infections during summer and fall in temperate regions and year-round in the tropics.[2] The circulating serotype vary over time and location and epidemics and outbreaks occur. Enterovirus activity was closely monitored by Taiwan Centers for Diseases Control. The most common reported serotypes changed year by year.[6–9] Between 2004 and 2012 in a Children's hospital in northern Taiwan, CVB3 was the most common serotype causing aseptic meningitis in infants younger than 3-month-old.[10] In an enterovirus outbreak in 2005, CVB3 caused various manifestations including, fever, meningitis, hepatitis, and sepsis in infants younger than 3-month-old.[11] In the United States, a markedly increased number of cases of CVB1 myocarditis in neonates were reported in 2007.[12] In 2018, an outbreak of Echo11 infections causing severe complications and mortality was noted in neonates and clusters in neonatal care units of hospitals or nursing homes occurred in Taiwan.[13]

Enterovirus infections are common in pregnant woman and in neonates. Forty-two percent of 1794 pregnant women from a 10-year seroepidemiologic survey were found to be infected with enteroviruses.[14] A prospective epidemiological survey at one to four weekly home visits until 1 month of age from New York, USA found that 12.8% of 586 infants tested were positive for enterovirus from throat or stool cultures during a typical enterovirus season.[15] Enterovirus is a frequent etiologic agent of nonspecific febrile illness in the young infants, accounting for 47%—63% of cases requiring hospitalization to rule out bacterial sepsis.[16,17]

Data from the National Enterovirus Surveillance System in the USA from 1983 to 2003 showed that of 26,737 enteroviruses detected, neonates accounted for 11.4% of those with known age. Echoviruses 11, CVB2, CVB5, Echovirus 6, Echovirus 9 and CVB4 were the most commonly identified serotypes in the neonates.[18] In Italy, the overall prevalence of enterovirus in 60 neonates with sepsis-like illness was 11.6%, which was similar to that in USA.[19]

In a retrospective review of febrile infants aged younger than 60 days who had positive enterovirus PCR results from any site between January, 2015 and September, 2016 at Nationwide Children's Hospital, USA, 783 patients were tested, and 144 (18%) had a positive enterovirus PCR.[20] In a study based on detection of enterovirus RNA in blood samples showed that enteroviruses were responsible for 24% of 137 neonatal sepsis cases of the non-bacterial causes in Kuwait.[21] Another prospective cohort study on 334 febrile neonates, found the overall positivity of enterovirus RNA detection (stool and cerebrospinal fluid (CSF) samples, 58/334 if CSF positive) in febrile neonates was 39.22% during summer and fall in China.[22] A prospective observational study was conducted in a Children's Hospital, Hague, The Netherlands, during 2008 and 2012.[23] Of 353 infants $<$ 90 days of age presenting with a sepsis-like symptoms at emergent department, enterovirus RNA was detected in 132 infants (37%) by polymerase chain reaction (PCR) in plasma and/or CSF and peaked in summer. 22 of 23 specimens adequate for genotyping were CVB and echoviruses.

Thus, the incidence of neonatal enteroviral infection varied markedly in different countries/regions and is related to the serotypes of circulating enteroviruses (frequently due to CVB group and echoviruses) in the community, the age of infant population enrolled, the geographic regions and seasons studied and the detection methods used.

Transmission

Enteroviruses are transmitted predominately via fecal-oral and respiratory routes. Infections in the newborn may be acquired vertically before, during or after delivery, and possible transmission via breast milk; horizontally from family members, or nosocomial transmission in nurseries. A number of cases of neonatal enterovirus disease with onset in the first 2 days of life have been reported, suggesting that some neonatal enterovirus infections are acquired in utero.[24] Further evidence for antenatal transmission came from reports of isolation of virus from amniotic fluid or cord blood.[24–26] Recently, a prospective study was conducted in three French hospitals to estimate the prevalence of enterovirus infections in febrile syndromes in pregnant women.[27] During a four-month period, enterovirus infection was confirmed in four (12.9%) of 31 pregnant women with fever at gestational age from 16 to 38 weeks. Though no any adverse pregnancy outcome was recorded in these four cases, one baby developed viral enterovirus meningitis on day 5 of life followed by a severe cardiogenic shock following myocarditis with left ventricular dysfunction, leading to multi-visceral failure requiring intensive care.

The predominant mode of transmission of neonatal infection (63%) is intrapartum, at the time of delivery through contact with maternal blood, stool, amniotic fluid, or vaginal or cervical secretions.[24,25] Another mode of transmission is postnatal exposure to oral secretions from

close contacts with the baby after delivery.[24] Transmission from household members is relatively common.[28,29]

Enterovirus may be transmitted also from community outbreaks[30] or nosocomial outbreaks after birth. Nosocomial outbreaks in neonatal units and nurseries have frequently been reported. In an analysis of a worldwide database of healthcare-associated outbreaks up to January 2012,[31] 64 (10.8%) of 590 neonatal outbreaks were originated by viruses and enterovirus, after rotavirus and respiratory syncytial virus, was the third most common involving virus, accounting for 10 outbreaks (15.6%). An average of 10 cases were involved in each outbreak, with a case-fatality rate of 4.9%.[31] Echovirus 11 and CVB were the most common serotypes, but enterovirus 71 has also been reported.[31,32] Outbreaks were usually from newborn that had been vertically infected. The attack rate for infants at risk estimated to range from 22% to 53%.[24] Nosocomial acquired enterovirus infections are generally associated with less severe diseases and lower mortality rates than vertically acquired infections.[24]

Previous studies have found breastfeeding to be protective against symptomatic enterovirus infections due to the presence of neutralizing antibodies.[33] However, other studies have suggested that transmission of enteroviruses by breastfeeding may be possible. CVB3[34] were detected in breast milk by PCR and viral culture of two symptomatic mothers whose newborns are severely infected with hepatitis and meningitis. Echovirus 19[35] was also detected in the banked breastmilk by PCR and viral culture of a mother with skin rash of an infant with neonatal sepsis. Transmission by breastfeeding depends on the viral load in the breast milk is more than likely.[35] Although it was unclear that breast milk was the primary mode of transmission that led to disease in these neonates,[34] mothers with low or absent anti-CVB3 antibody before delivery correlated with more severe neonatal disease. It has been recommended that delivery be delayed if possible, such as delaying the scheduled cesarean section without laboring pain, for at least 5—7 days after the onset of symptoms suggesting maternal enterovirus infections.[34]

Clinical manifestations

Manifestations of neonatal enterovirus infections may range from asymptomatic, non-specific febrile illness to severe fatal multi-system disease referred to as neonatal enterovirus sepsis. The clinical manifestations among 146 hospitalized young infants $<$3 months of age during a 9-year-period in Taiwan included 43 (29.5%) neonates with non-specific febrile illness, 61 (41.8%) with aseptic meningitis, and 42 (28.7%) with hepatic necrosis with coagulopathy.[4] Maternal history often reveals a recent viral illness with fever and frequently abdominal pain.[24,25] Symptoms may occur as early as day one of life, with those of severe disease generally within the first two weeks of life. Frequent findings include fever or hypothermia, irritability, lethargy, anorexia, rash, jaundice, respiratory symptoms, apnea, hepatomegaly, abdominal distension, emesis, diarrhea and decreased perfusion. Most have a benign course and fever resolves in an average of 3 days and other symptoms in one week.[1,2,36] Some have a severe disease and include sepsis, meningoencephalitis, myocarditis, hepatitis, coagulopathy, and pneumonitis.[1,2]

Neonatal myocarditis is most often caused by CVB 1—5. Signs of myocarditis include respiratory distress, tachycardia, cardiomegaly, arrhythmias and ECG sign of myocardial injury, then cyanosis and circulatory collapse develop rapidly. Myocarditis is often accompanied by disseminated viral infection involving central nervous system, liver, pancreas and adrenal glands. Mortality may be as high as 30—50%. Survivors may develop dilated cardiomyopathy and ventricular aneurysms.[1]

Central nervous system (CNS) disease may occur as isolated CNS disease or with myocarditis and hepatitis. Meningoencephalitis may be manifested by lethargy, seizures, bulging fontanelle, altered consciousness and CSF pleocytosis, although enteroviral meningitis without pleocytosis has been reported[37,38] and is not infrequently seen.[23] Complications include periventricular leukomalacia or extensive cerebral white matter injury.[39,40] Prognosis is variable; intellectual, motor, speech and language, and vision deficits and long-term seizure disorders may occur.[3]

Hepatitis and coagulopathy can occur alone or with myocarditis and/or meningoencephalitis, and is often associated with echovirus infections (types 5, 11, 6, 7, 9, 14, 17, 19, 21) and with CVB infections.[5,24,41] The initial symptoms are lethargy, poor feeding, apnea, and jaundice. Within 1—2 days, hypocoagulability with ecchymoses and bleeding at puncture sites appear, and anemia, prolonged prothrombin time (PT) and partial thromboplastin time (PTT) and extreme elevations of hepatic transaminase levels occur. Severe disease results in acute hepatic necrosis and fulminant liver failure with high mortality. Cirrhosis and chronic hepatic insufficiency can develop in survivors.[3]

Predictors of severe neonatal enteroviral disease

Understanding the risk factors for severe neonatal EV disease may help physicians become aware of infants at risk for adverse outcome and administer earlier and more aggressive management. Factors for hepatic necrosis with coagulopathy (HNC) were prematurity, maternal history of illness, early age of onset ($\leqq$7 days), higher white blood cell count (WBC$\geqq$15,000/mm^3) and lower hemoglobin ($\leqq$10.7 g/dL).[4] Of HNC cases, the case fatality rate was up to 24%. The most significant factors associated with fatality from HNC in a case series study were total bilirubin $>$14.3 mg/dL and concurrent myocarditis.[4] In another case series including 67 severe cases of neonatal enteroviral infection, the peak serum aspartate aminotransferase (AST) level was well correlated with the clinical outcome and patients with a serum AST level $>$ 1000 IU/L, particularly $>$ 2000 IU/L, had a significantly higher case-fatality rate.[42] In most of these severe cases of HNC, the serum AST level peaked within three days of illness onset, which suggests that frequent sampling for monitoring serum AST level within three days of illness onset provide opportunities to detect potentially severe cases of neonatal enterovirus disease.

As previously reported,[28] maternal illness and early onset of disease in the first week of life were associated

with absence of serotype-specific transplacentally-acquired neutralizing antibody to the infecting serotype in the neonate[30] and would result in a higher case-fatality rate, suggesting that vertical transmission of enterovirus is a risk factor for severe neonatal disease. Infection by echovirus, particularly type 11, and CVB[18] are also associated with more severe disease in the newborn period. Other risk factors were prematurity, male sex, positive serum viral culture, and evidence of severe hepatitis and multisystem disease.[1,30]

Diagnosis of enterovirus infection

Diagnosis of neonatal enterovirus infection clinically is difficult without the help of laboratory tests to exclude other neonatal bacterial or viral sepsis. A high index of suspicion is the key to an early diagnosis of enteroviral infection in neonates and young infants. For a young infant with fever, and/or sepsis-like illness during the enterovirus season, enterovirus infection should be first considered and the diagnostic virologic methods for the detection of enterovirus should be applied. The specific diagnosis is best confirmed traditionally by viral culture, followed by immunofluorescence staining or typing with the use of antisera, which allows identification of the serotype. In neonates, highest yield are from rectum or stool (91—93%), cerebrospinal fluid (62—83%), and nasopharynx or throat (52—67%). Cultures from blood and urine are less common (24—47%), however, serum specimens grow virus more rapidly than other body fluids.[3] Viral culture is slow, and usually takes 3—8 days.

Recently, direct testing for nucleic acid overcomes the limited sensitivity and delayed results of viral culture. Reverse-transcriptase polymerase chain reaction (RT-PCR) provides a more rapid and increases the detection of enteroviral infections.[43] In patients with meningitis, from CSF samples, RT-PCR for enterovirus has 100% specificity and 94.7%—97% sensitivity, positive predictive value of 100% and negative predictive value of 98%.[44] Sensitivity and specificity of RT- PCR are high, and results are available within as short as 2—3 h. The enteroviral PCR test has become the gold standard for diagnosing enteroviral CNS infections.[39] Even, in a large multicenter cohort of 19,953 hospitalized infants undergoing evaluation for CNS infection, 945 infants with a positive enterovirus PCR test had a one-third shorter length of stay compared with infants with a negative enterovirus PCR test and no infant with a positive enterovirus PCR test had bacterial meningitis.[45] Although CSF abnormalities are common findings in neonates with enteroviral meningitis, lack of CSF pleocytosis has been reported[37,38] and is not infrequently seen.[23]

In neonates, PCR of serum and urine specimens are more sensitive than culture with a yield approaching 90%. RT-PCR can detect enteroviral RNA in blood and is useful for infants with sepsis-like illness.[46] Recently, a prospective study was conducted in 16 French hospitals to assess a method of detecting enterovirus in blood specimens by PCR[47] and found that detection of enterovirus was more frequent in blood samples than in CSF specimens of newborn babies (70 [99%] of 71 vs 62 [87%] of 71; $p = 0{\cdot}011$). They concluded that testing for enterovirus in blood by PCR should be an integral part of clinical practice guidelines for neonates and this testing could decrease the length of hospital stay and reduce exposure to antibiotics for low-risk patients admitted to the emergency department with febrile illness. Higher viral loads in blood are associated with infection in the first week of life and more severe disease, and viral nucleic acid may persist in blood of severely ill newborns for up to two months. Early diagnosis and predicting disease severity is now possible in severe neonatal enterovirus infection.[48]

Serology is generally not used for the diagnosis except when infection with a specific serotype is suspected. Acute and convalescent serum specimens are required, which make serology impractical for acute diagnosis. Sensitivity and specificity may be limited, and cross-reactivity among serotypes may occur.[3,36]

Therapy

Most enterovirus infections are self-limited, no specific therapy needed, and care is essentially supportive. However, for life-threatening infections, including neonatal infections, myocarditis, and disseminated infections, administration of intravenous immunoglobulin (IVIG) and antiviral drug pleconaril, if available, may be reasonable due to the high mortality.

Effect of IVIG therapy in neonatal enterovirus infection is varied. A prospective study was conducted to evaluate the virology and serological response in 16 neonates.[49] In the only randomized trial in neonates, administration of IVIG at a dose of 750 mg/kg was associated with modest boosts of serum neutralizing antibody titers to viral isolates of patients, subtle clinical benefits, and faster cessation of viremia and viruria in patients who received a high titer ($\geq$1:800) of neutralizing antibody to their own viral isolates.[49] In another study, in cases of hepatic necrosis, no correlation of IVIG with clinical outcomes was found.[4]

However, favorable outcome after IVIG treatment was reported in one neonate with disseminated echovirus 11 infection with hepatitis, pneumonitis, meningitis, disseminated intravascular coagulation, decreased renal function, and anemia.[50] Successful treatment of myocarditis with IVIG in two neonates was reported among five neonatal enterovirus infections with CNS and cardiac complications.[39] Recently, we conducted a retrospective analysis of 67 cases of culture-confirmed neonatal enterovirus infection with severe hepatitis and coagulopathy.[42] Forty-one infants received IVIG and 29 infants received early IVIG therapy within the first 3 days of illness onset. We found early IVIG therapy was independently associated with survival. The timing of IVIG administration is crucial and early IVIG may be beneficial for survival in severe cases. In addition, IVIG must contain high titers of antibodies against the infecting serotypes, but these are not usually measured.[51]

The antiviral drug pleconaril offers hope for the treatment of enteroviral infections. Pleconaril is a capsid inhibitor, which prevents the virus from attaching to cellular receptors and therefore prevents uncoating and subsequent release of viral RNA into the host cell. The drug exhibits potent in vitro activity against many enterovirus and rhinovirus serotypes and a favorable pharmacokinetic profile when administered orally.[52] In a clinical trial in

adolescents and adults with enterovirus meningitis, pleconaril reduced the duration of headache when given within 2 days of onset.[53] Experience in 38 patients, either immunocompromised with persistent enteroviral infections or those with serious, potentially fatal infections, including 6 neonates, receiving compassionated usage of pleconaril suggested substantial clinical benefit, including recovery in four infants, development delay in one infant and death in one infant.[54] In a small case series, 2 of 3 neonates with life-threatening enteroviral hepatitis recovered fully after treatment with pleconaril.[55] In a randomized trial of 61 neonates with suspected enterovirus disease who were assigned to seven days of pleconaril or placebo, there was a trend toward more rapid viral clearance (median 4 vs 7 days) and lower overall mortality among pleconaril-treated infants (23 versus 44% with placebo).[56] Thus, shorter times to culture and PCR negativity and greater survival among pleconaril recipients support potential efficacy and warrant further evaluation. However, pleconaril is not currently available for systemic administration.

Pocapavir, also known as SCH 48973 and V-073, is a potent, selective anti-enteroviral agent only available as an emergency investigational drug. The drug is a capsid inhibitor, administered orally and is highly protein-bound and excreted exclusively in feces. Pocapavir was used and successfully treated several severe cases of neonatal enteroviral infection, even with myocarditis.[57–59]

Since neonates with severe enteroviral myocarditis may rapidly progress to cardiovascular collapse, which may be refractory to conventional medical treatment, extracorporeal membrane oxygenation (ECMO) may have a role in these patients. Lately, Cortina et al.[60] reported seven neonates with cardiovascular collapse within the first 10 days after birth and required ECMO support. Four patients survived ECMO and three survived to hospital discharge. All three survivors showed complete cardiac recovery after a median follow-up of 34 months. They also reported 35 additionally previously reported cases of EV myocarditis supported with ECMO and the survival rate was 34%.

Conclusions

Enterovirus infections in neonates are common and should be routinely considered in the differential diagnosis of febrile neonates, particularly during enterovirus season. The most commonly associated serotypes with neonatal enteroviral sepsis are coxsackieviruses B and echoviruses. Manifestations range from unapparent infection to overwhelming systemic illness and even death. Severe enteroviral diseases in neonates include hepatic necrosis with coagulopathy, meningoencephalitis and myocarditis. An awareness of the clinical syndromes, recognition of the risk factors and monitoring parameters associated with severe cases and use of rapid RT-PCR test for viral load may help physicians in diagnosing severe cases in a timely manner. Prompt aggressive treatment including early IVIG administration may help to reduce morbidity and mortality. It is advisable that delivery be delayed if possible for at least 5–7 days after onset of symptoms suggesting maternal enterovirus infections, allowing the fetus time to passively acquire protective antibodies.

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