Outbreak of Life-Threatening Coxsackievirus B1 Myocarditis in Neonates

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In the summer and fall of 2007, we observed a unique cluster of cases of severe coxsackievirus B1 (CVB1) infection among Chicago area neonates. Eight neonates had closely related strains of CVB1 that were typed at the Centers of Disease Control and Prevention; 2 other neonates had CVB infections, 1 of which was further identified as serotype CVB1. All had severe myocarditis; 1 neonate underwent heart transplantation, and 1 died of severe left ventricular dysfunction.

Enteroviruses can cause severe neonatal illness, including meningocencephalitis, sepsis, hepatitis, disseminated intravascular coagulation, and myocarditis [1]. In particular, group B coxsackieviruses, of which there are 6 serotypes, can cause myocarditis among neonates.

According to the National Enterovirus Surveillance System (NESS), coxsackievirus B serotypes 2–5 (CVB2–5) are frequently among the 15 most common serotypes identified [2] and are classically associated with severe neonatal disease [3]. Unlike cases of infection with these serotypes, no fatal cases of CVB1 infection were reported to the NESS from 1970 to 2005 [2], but CVB1 has caused sporadic neonatal myocarditis [4–11]. In the summer and fall of 2007, we observed 10 cases of severe community-acquired CVB myocarditis among neonates; CVB1 was identified in 9 patients.

Methods. From June to November 2007, there were 10 neonates who were hospitalized with severe enterovirus infection at our tertiary referral pediatric hospital. All patients presented with evidence of CVB myocarditis. Myocarditis was diagnosed clinically by (1) presenting signs and symptoms, (2) echocardiographic findings, (3) electrocardiographic findings, (4) elevated serum cardiac enzyme levels, and (5) lack of alternate explanation for cardiac dysfunction. All patients had clinical specimens that tested positive for acute enteroviral infection by use of culture and/or reverse-transcriptase polymerase chain reaction (RT-PCR).

Viral culture involved inoculation onto Rhesus monkey kidney cells, MRC-5 cells, and A549 cells and staining and grouping by indirect immunofluorescence. Cerebrospinal fluid was examined by use of RT-PCR (GeneXpert enterovirus assay; Cepheid). Serum samples were referred to Mayo Medical Laboratories for enterovirus RT-PCR. Nucleic acids were extracted and referred to the picornavirus laboratory at the Centers for Disease Control and Prevention for enterovirus RT-PCR and sequencing, as described elsewhere [12]. Clinical, laboratory, cardiac, and histopathologic data were extracted from the medical charts of the 10 patients. Interventions and discharge examinations and medications were documented (table 1).

Results. Enteroviral infections were identified by use of RT-PCR and viral culture, as shown in table 2. Enteroviral serotyping and VP1 genotyping of isolates at the Centers for Disease Control and Prevention demonstrated that all 8 patients with viral isolates available were infected with closely related strains of CVB1; the other 2 patients were identified as having CVB1 and CVB, respectively, but these infections were not further characterized.

The 10 patients were hospitalized during the period from June through November 2007, with 7 of the patients being hospitalized in August or September (table 1). Patients were unrelated. Five infants were born prematurely, and 8 by cesarean section. The causes of prematurity and indications for cesarean section were unknown because of limited obstetrical data. Age at onset of illness ranged from 1 h to 12 days (median age, 4 days). Median length of hospital stay was 31 days (range 13–122 days).

All 10 patients were moderately to very severely ill, requiring admission to the intensive care unit. Presenting symptoms included temperature abnormalities (6 patients), respiratory symptoms (4 patients), cyanosis (4 patients), poor feeding (4 patients), jaundice (4 patients), and seizures (2 patients) (table 1). One patient had an arrhythmia. Maternal illness was doc-
<table>
<thead>
<tr>
<th>Case</th>
<th>Month of presentation</th>
<th>Age at onset of illness</th>
<th>Gestational age, weeks</th>
<th>Symptoms at presentation</th>
<th>Type of maternal illness</th>
<th>Myocarditis</th>
<th>Coagulopathy</th>
<th>Hepatitis</th>
<th>CNS involvement</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 1</td>
<td>June</td>
<td>3 days</td>
<td>36</td>
<td>Fever, respiratory distress</td>
<td>Chorioamnionitis</td>
<td>Present</td>
<td>Likely present</td>
<td>Present</td>
<td>Likely present</td>
<td>(positive CSF PCR test result)</td>
</tr>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Death on day 10 of illness (13 days after birth)</td>
</tr>
<tr>
<td>Patient 2</td>
<td>July</td>
<td>4 days</td>
<td>35</td>
<td>Hypothermia, apnea, cyanosis, poor intake, jaundice</td>
<td>None</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>(positive CSF PCR test result and culture test results, CSF pleocytosis (WBC count, 107 cells/μL, and abnormal EEG) No medication at hospital discharge</td>
</tr>
<tr>
<td>Patient 3</td>
<td>August</td>
<td>9 days</td>
<td>37</td>
<td>Hypothermia, tachypnea, poor intake, fussiness, jaundice, seizure</td>
<td>None</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>Likely present</td>
<td>(seizure and positive CSF PCR test result) Lasix, aldactone, captopril, and digoxin at hospital discharge</td>
</tr>
<tr>
<td>Patient 4</td>
<td>August</td>
<td>4 days</td>
<td>38</td>
<td>Fever, jaundice</td>
<td>Postpartum fever</td>
<td>Present</td>
<td>Likely present</td>
<td>Present</td>
<td>Likely present</td>
<td>(positive CSF PCR test result; CSF pleocytosis (WBC counts of 55, 870, and 124 cells/μL) Lasix, captopril, and digoxin at hospital discharge</td>
</tr>
<tr>
<td>Patient 5</td>
<td>August</td>
<td>1 h</td>
<td>37</td>
<td>Fever, hypoxia, staring spell</td>
<td>Fever at delivery</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>(positive possible seizure, positive CSF PCR test result, elevated CSF protein level, and MRI finding of cerebral edema) Actigall but no cardiac medication at hospital discharge</td>
</tr>
<tr>
<td>Patient 6</td>
<td>September</td>
<td>12 days</td>
<td>40</td>
<td>Tachycardia, tachypnea, poor intake, respiratory distress, hypoxia</td>
<td>None</td>
<td>Present</td>
<td>Present</td>
<td>Not present</td>
<td>Present</td>
<td>(MRI finding of meningeal enhancement) Captopril at hospital discharge</td>
</tr>
<tr>
<td>Patient 7</td>
<td>September</td>
<td>20 h</td>
<td>34</td>
<td>Fever, poor intake, tachycardia, murmur, respiratory distress, jaundice</td>
<td>Placental abruption</td>
<td>Present</td>
<td>Not present</td>
<td>Present</td>
<td>Present</td>
<td>(positive CSF PCR test result) Lasix, captopril, and digoxin at hospital discharge</td>
</tr>
<tr>
<td>Patient 8</td>
<td>September</td>
<td>4 days</td>
<td>34</td>
<td>Lechage, poor intake, jaundice, bleeding from umbilical stump</td>
<td>Abdominal pain at delivery, postpartum fever, and lung infiltrates</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>Likely present</td>
<td>(positive possible seizure and non-specific EEG abnormalities) No medication at hospital discharge</td>
</tr>
<tr>
<td>Patient 9</td>
<td>September</td>
<td>1 day</td>
<td>39</td>
<td>Seizure</td>
<td>Fever, abdominal pain 1 week prior to delivery, fever postpartum</td>
<td>Present</td>
<td>Not present</td>
<td>Not present</td>
<td>Not present</td>
<td>(seizure, normal CSF finding, negative PCR test result, and normal EEG) Heart transplant on day 58 of illness (8 weeks old)</td>
</tr>
<tr>
<td>Patient 10</td>
<td>November</td>
<td>10 days</td>
<td>35</td>
<td>Irregular heart rhythm, hypotension</td>
<td>Fever during labor</td>
<td>Present</td>
<td>Not present</td>
<td>Present</td>
<td>Present</td>
<td>(positive CSF PCR test result and CSF pleocytosis (WBC count, 94 cells/μL) Lasix, captopril, and digoxin at hospital discharge</td>
</tr>
</tbody>
</table>

**NOTE.** CNS, central nervous system; CSF, cerebrospinal fluid; EEG, electroencephalogram; MRI, magnetic resonance imaging; PCR, polymerase chain reaction; WBC, white blood cell.
Table 2. Data on Enterovirus Strains and Methods of Diagnosis for Neonates with Coxsackievirus (CVB) Infection in Chicago, Illinois, 2007

<table>
<thead>
<tr>
<th>Enterovirus Strain</th>
<th>Methods of Diagnosis</th>
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<tbody>
<tr>
<td>CVB1</td>
<td>PCR, serology, culture</td>
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</table>

This table is available in its entirety in the online version of Clinical Infectious Diseases.

Table 3. Significant Laboratory, Electrocardiographic, and Echocardiographic Abnormalities among Neonates with Coxsackievirus B1 Infection in Chicago, Illinois, 2007

<table>
<thead>
<tr>
<th>Abnormality</th>
<th>Description</th>
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</thead>
<tbody>
<tr>
<td>Troponin I</td>
<td>Elevated in 7 of 7 patients tested</td>
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</table>

This table is available in its entirety in the online version of Clinical Infectious Diseases.

On the basis of abnormal electrocardiogram and echocardiogram findings, presence of arrhythmia, and elevated brain natriuretic peptide and troponin levels, it was determined that myocarditis was present in all 10 patients (table 3). Electrocardiogram findings included myocardial ischemia patterns and arrhythmia in 7 of the 10 patients. Echocardiography revealed that 9 of the 10 neonates had severe left ventricular dysfunction, and 7 of the 10 neonates had findings of myocardial infarction, including hypokinesis of the left ventricular free wall and/or septum. Troponin I was elevated in 7 of 7 patients tested, and the brain natriuretic peptide level was elevated in 8 of 8 patients tested.

Seven patients had significant thrombocytopenia (thrombocyte count, ≤65,000 cells/μL), and 6 patients were coagulopathic (table 3). Four patients developed significant bleeding, with 6 patients requiring transfusions of platelets, fresh frozen plasma, and/or cryoprecipitate.

Seven patients had markedly elevated serum transaminase levels (table 3). Direct hyperbilirubinemia developed in 3 patients, and 1 patient required long-term ursodiol therapy for cholestasis.

Three patients had a cerebrospinal fluid pleocytosis (white blood cell count, 54–870 cells/μL). Other central nervous system abnormalities are noted in table 1.

All 10 patients required intensive support, with 9 patients receiving pressors and 8 patients requiring intubation and ventilation. Five patients received corticosteroids, and 9 patients received intravenous immunoglobulin. No patients received plecanaril, an unlicensed candidate antienteroviral agent.

The cardiac disease of these infants was very severe. Patient 1 developed progressive congestive failure and life-threatening arrhythmias, including ventricular fibrillation, and died 10 days after initial presentation (13 days after birth). Patient 9 had persistent severe left ventricular dysfunction and received a heart transplant at 8 weeks of age. Both of these patients received treatment with intravenous immunoglobulin and steroids.

Five of the remaining 8 patients required postdischarge cardiac medications for ongoing myocardial dysfunction (table 1). Three patients had recovered normal myocardial function before hospital discharge.

Patient 1 died 10 days after presentation (at 13 days of life). Microscopic examination of the left ventricle at autopsy demonstrated lymphocytic infiltration with associated myocyte damage and necrosis (figure 1A); culture of the cardiac tissue yielded CVB1.

Patient 9 underwent an endomyocardial biopsy at 5 weeks, which revealed mild myocyte hypertrophy, focal calcium deposits, and increased sarcoplasmic granularity without inflammatory infiltrates or giant cells. Enteroviral PCR and culture results were negative; these tests were performed at Baylor College of Medicine in Houston, Texas. Patient 9 then underwent cardiac transplantation at 8 weeks old. Gross examination of the explanted heart showed a white rubbery area indicating extensive fibrosis in the left ventricle. Microscopic examination of the left ventricle revealed 2 large areas of calcification and interstitial fibrosis, with numerous multinucleated giant cells that were thought to be reactive to the calcium crystals. At the edges of repair, fibroblastic and mononuclear cell proliferation was seen. Cellular hypertrophy and increased granularity were noted throughout (figure 1B). No active myocarditis was evident, and molecular testing for enterovirus was negative. Findings were consistent with a previous myocardial infarct.

Discussion. We describe 10 neonates with life-threatening myocarditis from diverse Chicago area locations during the summer and fall of 2007. Eight neonates had closely related strains of CVB1, which were determined by sequencing of the VP1 region of the capsid gene. An additional patient had CVB1 (not sequenced), and another had CVB not further characterized. These latter 2 patients were also likely infected with one of the closely related strains of CVB1. To our knowledge, this is the largest outbreak of community-acquired neonatal CVB1 infection that has been described.

CVB1 was the fifth most prevalent of the 6 group B coxsackieviruses in the United States during the period from 1970 to 2006 [2]. However, in 2007, CVB1 became the predominant enterovirus in the United States for the first time [13]. Phylogenetic analysis of strains submitted to the Centers for Disease Control and Prevention in 2007, including those obtained from 8 of our 10 patients, suggested a single genetic lineage that resembled a 2006 strain from Colorado but that was distinct from earlier strains [12]. Five neonates died of CVB1 infection, including our patient 1, and for the first time, fatal cases of CVB1 infection were reported to the NESS [13]. Two other clusters of cases of CVB1 infection in 2007 were noted in Los Angeles, California, and Kotzebue, Alaska. At least 32 neonates nationwide required intensive care, 27 of whom had multiorgan involvement.

Similar to patients described in prior reports, our patients...
Figure 1.  

A, Cardiac tissue at autopsy on day 10 of illness (patient 1). Focus of intense lymphocytic infiltration is accompanied by marked myocardial fiber damage and necrosis (hematoxylin and eosin stain; original magnification, ×100). B, Specimen of left ventricular wall after explantation, 8 weeks after onset of illness (patient 9). The upper portion of the figure shows an area of calcification in a background of fibrosis. The asterisk indicates an area of fibrosis. Relatively preserved myocardial fibers are present in the lower right quadrant of the image. A mild lymphocytic infiltrate is present in an area of fibrosis to the left of the myocardial fibers (hematoxylin and eosin stain; original magnification, ×100).

All presented with CVB1 infection in the first 2 weeks of life (7 patients [70%] in the first week); 5 patients (50%) were premature, and 6 patients (60%) had mothers who were ill [14, 15]. Early age at onset of disease (ie, <2 weeks) is thought to reflect transplacental or perinatal acquisition and is associated with more severe disease. Proposed explanations include increased viral load [16], increased viral receptors in neonatal host tissues [17], immaturity of neonatal cell-mediated immunity [18], and lack of transplacentally acquired antibody from a recently infected mother [19]. We hypothesize that mothers in our community did not transfer protective antibodies to these newborns because of the relative rarity of CVB1 infection in recent decades and because of the distinctiveness of this strain.

The cases of myocarditis in this cohort were very severe: 1 patient died, and 1 patient required a heart transplant. Many patients had elevated serum troponin levels that suggested myocardial ischemia, as well as electrocardiogram and echocardiographic findings classically associated with myocardial infarction. Previous studies have been unable to conclude whether neonatal enteroviral myocarditis mimics myocardial infarction or whether it actually causes frank myocardial ischemia [20, 21]. The progression of findings in the 3 types of pathologic specimens described here may clarify the evolution of cardiac damage in CVB myocarditis. At patient 1’s autopsy (day 10 of illness), cardiac tissue revealed active, primarily lymphocytic, inflammation, and culture was positive for CVB1. An endomyocardial biopsy of patient 9 at age 5 weeks (day 38 of illness) demonstrated no inflammation, and enteroviral PCR was negative. At 8 weeks of age (day 58 of illness), patient 9’s explanted heart exhibited calcification, fibrosis, and collections of multinucleated giant cells, without active myocarditis; enteroviral PCR was again negative. This suggests particularly virulent circulating strains of CVB1 in a susceptible population not com-
monly exposed to this virus. Following acute infection, myocardial infarction with significant tissue fibrosis and calcification can develop.

In summary, we report a unique cluster of cases of severe neonatal myocarditis caused by CVB1. This cluster proved to be part of a larger epidemic of CVB1 infection throughout much of the United States in the summer and fall of 2007. This suggests a particularly virulent circulating strain of CVB1 in a susceptible population not commonly exposed to this virus. Additional studies of the unique, closely related strains of CVB1 that caused this cluster of cases may provide insight into their ability to severely affect cardiac tissue.

Acknowledgments

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References