Lethal Parvovirus Myocarditis in an Infant

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We report a case of fulminant myocarditis in an 11-month-old female infant who had no other clinical signs of parvovirus infection. The patient presented with severe respiratory distress and died in sudden cardiac arrest 3 h after admission. The clinical presentation was similar to that of an asthmatic attack. Autopsy revealed signs of acute lymphocytic myocarditis. Parvovirus DNA was demonstrated by polymerase chain reaction (PCR) analysis of tissue sections obtained from the heart, lungs, liver, kidneys, and spleen. Transmission electron microscopy of myocardial tissue showed crystalline arrays with the appearance of parvovirus. The results of immunohistochemical analysis for the detection of parvovirus antigens were negative, and no viral inclusions were demonstrable. We suggest that the current diagnostic procedure underestimates the prevalence of parvovirus-associated myocarditis. PCR analysis should be used as a complement in suspected cases, to enhance the rate of detection of the infection and to reach a correct diagnosis.

Lymphocytic myocarditis is a risk factor for morbidity and mortality in the pediatric population, in which it occurs more frequently than in the adult population [1, 2]. Diagnosis of lymphocytic myocarditis is difficult, is often based on a certain degree of clinical suspicion, and is not always corroborated by adequate laboratory findings. Endomyocardial biopsy can be performed, but the results are often equivocal [2]. A variety of etiologies, including systemic diseases, drugs, and infectious agents, have been implicated. However, for a proportion of cases, the cause remains unknown. It is generally agreed that, in the majority of patients, the disease is due to a viral infection [2]. Among viral agents, enterovirus is most often associated with myocarditis, either on the basis of serological findings, or, more recently, by direct demonstration of enterovirus genome in the myocardial tissue of patients with lymphocytic myocarditis or in biopsy specimens from patients with clinically suspected myocarditis. However, it is becoming evident that infection with many other viruses, such as adenovirus [3, 4], mumps virus [3], influenza virus [3] and HIV [3, 5], can result in the clinical and histological presentation of myocarditis.

Parvovirus B19 is a small, single-stranded DNA virus that usually is associated with hydrops fetalis and other complications of pregnancy—notably, intrauterine fetal death [1, 6]. The virus has a predilection for infecting erythroid progenitor cells, specifically, although 2 reports [7, 8] have tentatively described tropism for myocardial cells also. Documentation of parvovirus-associated myocarditis in infants or children is extremely rare [9–14].

CASE REPORT

An 11-month-old female infant with severe respiratory problems was admitted to the pediatric emergency department. She had never been hospitalized previously, but she had been successfully treated for bacterial pneu-
monia 2 months before admission. During the week before admission, she had experienced upper respiratory tract symptoms of moderate severity, without fever. Earlier during the day of admission, she had appeared healthy, but, during the afternoon, she rapidly developed severe respiratory distress.

At presentation, the infant was grayish in color, pale, and in obvious discomfort. She was severely tachypneic and had wheezing with ronchi on auscultation. Oxygen saturation, as measured by a cutaneous pulse oxymeter, was 70%. The infant’s pulse was 120 beats/min, there was no heart murmur, and there were no enlarged organs in the abdomen. The patient had C-reactive protein levels that were in the normal range, a hemoglobin level that was considered to be slightly lower than the normal level for patients in her age group, and a low bicarbonate level suggesting metabolic acidosis.

Asthma was the preliminary diagnosis, and the patient was given inhaled salbutamol and adrenaline, as well as intravenously administered steroids and theophyllamine. The initial response to treatment was a moderate improvement in the infant’s general condition and an increase in oxygen saturation to 90%. However, the patient’s condition then deteriorated, and she had sudden cardiac arrest 2 h after admission. After resuscitation, she regained a normal electrocardiographic reading for 10 min but then lost all cardiac activity again. Despite resuscitation efforts, the heart activity never recovered, and the patient died 3 h after admission to the emergency department.

**RESULTS**

On macroscopic examination done at the time of autopsy, myocardial tissue in the left and right ventricle, as well as that in the interventricular septum, showed extensive, patchy, pale areas consistent with necrosis. Frozen-section analysis performed during autopsy revealed abundant infiltration of the myocardium by mononuclear cells and myocardial necrosis. Frozen-section analysis of lung tissue showed mild mononuclear cell infiltration in the interstitium, minor hemorrhages, and mild to moderate edema. No other pathologic changes or anatomical abnormalities were revealed at autopsy.

Histological examination of formalin-fixed sections of the myocardium verified the findings of frozen-section analysis. Corresponding to the pale areas, there was an intense, diffuse infiltration of mononuclear cells, mostly with the appearance of small lymphocytes. The lymphocytic infiltration was seen intermingled with areas of myocyteolysis. These findings thus fulfilled the Dallas criteria for acute myocarditis [2]. No significant granulocytic component or signs of muscle hypertrophy were evident. No viral inclusions were found.

Sections of lung tissue showed mild edema and thickening of the interstitium with scattered mononuclear cells, reminiscent of pneumonitis. No plugging of the bronchioles or intra-alveolar granulocyte infiltration was present. Bone marrow sections showed hematopoiesis, which was considered to be a normal finding, given the patient’s age, and they did not reveal any signs of hypocellularity, dysmaturity, or blast infiltration. No viral inclusions were found. Sections obtained from other internal organs showed no obvious histopathological alterations.

Immunohistochemical analysis of sections from the myocardium, lungs, liver, kidney, and spleen was negative for parvovirus capsid protein; CD3, CD4, and CD8 antigens (Dako); and an antibody against the α/β T cell receptor (Dako).

PCR analysis was performed on frozen, stored tissue samples of the lung, liver, kidneys, and spleen, according to a standardized protocol described elsewhere [15]. Specific attention was given to avoiding the possibility of contamination occurring between the different steps of the procedure. Myocardial tissue for PCR examination was obtained from paraffin-embedded blocks after deparaffinization and was subsequently processed as previously described.

Material for transmission electron microscopy of the heart was obtained from paraffin-embedded myocardial tissue following routinely performed deparaffinization, 2 steps of fixation performed with 2% OsO₄ and 70 mM NaCaco (dimethylarsinic acid and sodium salt [sodium cacodylate]) solution, and subsequent dehydration. The ultrathin sections were examined in a Philips 420 model at 60 kV.

**MATERIALS AND METHODS**

An autopsy was performed ~10 h after the infant’s death, according to a standardized, detailed pediatric autopsy protocol that included examination of the CNS but not the spinal cord. Tissue specimens from all major organs were routinely processed, formalin fixed, paraffin embedded, and stained with hematoxylin-eosin. Immunohistochemical analysis was performed as described elsewhere [15] by use of an automated staining machine and monoclonal antibodies against parvovirus capsid protein; CD3, CD4, and CD8 antigens (Dako); and an antibody against the α/β T cell receptor (Dako).

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talline arrays of ~25 nm in size, consistent with the appearance of parvovirus particles (figure 1). The virus particles were observed in direct proximity to and, often, within areas of destruction and/or necrosis of muscle fibers.

Serological analysis was negative for enterovirus and adenovirus by complement binding. ELISA (for the detection of IgG and IgM) was negative for CMV, human herpesvirus 6 (HHV-6), herpes simplex virus, and Epstein-Barr virus, whereas serological tests for parvovirus B19 were positive for IgG but negative for IgM.

**DISCUSSION**

There currently is increasing awareness that the spectrum of parvovirus infection is much wider and multifaceted than previously was anticipated, both in immunocompromised individuals and in immunocompetent individuals [1, 18, 19]. Parvovirus specifically infects human cells through binding to its cellular receptor, the P antigen, which previously was widely assumed to reside only in erythroid precursors [6]. Recent studies have further demonstrated that P antigen is expressed on a variety of human cell types, such as trophoblast or endothelial cells, and that expression of the P antigen is necessary but is not sufficient for entry of parvovirus into the cells [20, 21]. In cases of hydrops fetalis, the presumed pathogenetic action of parvovirus is mediated via destruction of erythroid cells and fetal anemia, with subsequent cardiac failure. However, even this tenet may have to be reevaluated. Porter et al. [7] and Naides and Weiner [8] first demonstrated that myocardial cells can be a target for parvovirus infection. Soulié [22] summarized evidence showing the myocardial tropism of parvovirus and suggested that parvovirus-induced myocarditis may, per se, considerably contribute to cardiac failure and hydrops fetalis. In this study, we report an unusual case of parvovirus-associated myocarditis with a fulminant course. In contrast to most previously described patients, our patient showed no prodromal signs or serologic evidence of acute parvovirus infection.

Parvovirus-related myocarditis has been very rarely documented in adults. Chia and Jackson [23] and Orth et al. [24] each described a patient with acute perimyocarditis (a 43 year old and a 34 year old, respectively). The results of serological testing and PCR analysis of serum demonstrated parvovirus infection. Both patients previously had been healthy, and they survived the infection. Tsuda et al. [25] reported another case of acute myocarditis probably related to parvovirus and associated with the development of hemophagocytic syndrome.

Few cases of parvovirus-associated myocarditis in children
have been reported. Enders et al. [26] described 2 patients (a 7 year old and a 13 year old), one of whom died as a result of the infection and the other of whom was successfully treated with cardiac transplantation. Beghetti et al. [9] described 2 siblings (a 5 year old and a 9 year old) with merosin-deficient muscular dystrophy and parvovirus myocarditis. One patient died, and the other survived the infection. Recently, Murry et al. [11] described a 5-year-old patient with acute severe myocarditis that resulted in death; parvovirus was demonstrated in the myocardium and other tissues by use of PCR.

Similarly, among infants, parvovirus-related myocarditis has been documented as a rare event. Saint-Martin et al. [13] first reported a lethal case in a previously healthy 1-year-old infant who presented with facial erythema. Parvovirus was demonstrated by serological testing and immunohistochemical analysis of the myocardium. Schowengerdt et al. [4] examined cases of suspected or established myocarditis and found parvovirus DNA in 3 infants (of a total of 360 infants), one of whom died as a result of the infection. Nigro et al. [12] recently described 3 infants with parvovirus-related acute lymphocytic myocarditis; the infection was persistent in 1 of the 3 infants. Persistent parvovirus infection is well documented in children with immunodeficiency [19] or pediatric malignancies [18].

The case report that we present here illustrates several intriguing aspects of parvovirus infection. From the clinical point of view, the course of the disease was unusually severe and rapid, leading to fulminant myocarditis and death within a few hours of the presentation of symptoms. Moreover, the clinical presentation of the disease was unclear and inconclusive; it closely mimicked the presentation of an asthmatic attack, and, in fact, the disease was interpreted as such. The correct diagnosis of acute myocarditis was only determined at the time of autopsy. The patient did not present any of the prodromal signs associated with parvovirus infection, such as rash or fever, in contrast with most previously reported similar cases. However, ~50% of parvovirus infections are asymptomatic in all children. It is a matter of speculation whether our patient’s recent history of pneumonia, which presumably was of bacterial origin and obviously was treated successfully, could have contributed to a subtle immunologic imbalance, predisposing to the development of the subsequent severe viral infection. The clinical history suggested no sign of immune abnormality or deficiency, the results of histological examination of bone marrow was normal.

On the basis of the cases reported in the literature, it is apparent that parvovirus myocarditis can manifest as a severe, life-threatening disease in fully immunocompetent children and adults [4, 11, 27]. The severity of the infection in our patient may have been the result of a long-standing parvovirus infection, because the child had parvovirus B19 IgG antibodies but no IgM antibodies. Parvovirus B19 IgM antibodies usually persist for 2–4 months after an acute infection develops. Chronic or persistent parvovirus infections have also been speculated as the cause of intrauterine fetal deaths in late pregnancy [15]; B19 has been found in the cardiac tissue of the affected fetuses. Rohayem et al. [27] recently described a 11-year-old child with a case of fatal myocarditis that obviously was associated with acute coinfection with parvovirus B19 and HHV-6. No serological response to either virus was detected. The authors suggested that HHV-6 induced severe immunosuppression that enhanced the dissemination of parvovirus B19.

Although myocarditis due to parvovirus is, in general, rarely reported, it apparently is more common in infants than in older children or adults, as previously discussed. This could be because of the inherent susceptibility to viral infection that is common during the neonatal period and infancy as a result of the delayed maturation of component(s) of the immune system. Maternal antibodies acquired through transplacental passage usually disappear by the end of the first year of life, further contributing to the susceptibility of the infant’s immune system.

As previously discussed, serological tests could not verify evidence of acute parvovirus infection in our patient. On the other hand, we could demonstrate parvovirus DNA in several tissues, including the heart. Furthermore, electron microscopic examination of myocardial tissue revealed virus particles with the crystalline appearance of parvovirus either in close proximity to or within the disrupted myofibrils. The routine histological presentation was that of classical acute lymphocytic myocarditis. In sum, we believe that there is good evidence that parvovirus was the cause of myocarditis in our patient. However, we were unable to show viral antigens in any tissue by immunohistochemical analysis, and the typical parvovirus inclusions were absent. This finding is in accordance with previous observations made in reported cases of parvovirus-associated myocarditis [4, 27]. Moreover, in a recent study of parvovirus-associated fetal death in the second and third trimesters of pregnancy, we observed a similar paucity of demonstrable viral antigens and a poor correlation between the results of immunohistochemical analysis and the presence of parvovirus DNA in tissues [15]. The reasons for this phenomenon are not clear, but they could be either virus related and/or immune system related.

The mechanism or mechanisms of virus-induced myocarditis are not entirely understood. A direct viral effect is implied, but an autoimmune component, feasibly involving γ/δ T cells, has been suggested [28]. Using a quantitative PCR method, Murry et al. [11] found lesser amounts of parvovirus DNA in the heart, compared with other organs, in a patient with fatal myocarditis. They proposed that parvovirus may not affect the heart per se but, instead, may initiate some kind of cross-reactive immunologic reaction. Our findings on electron microscopy were suggestive of a direct cytopathic effect of the virus. On the other hand, we
recently have shown that parvovirus can specifically recruit CD8\(^+\) T cells with appropriate effector function [17]. In the patient described in the present report, we actually observed increased relative amounts of CD8\(^+\) T cells in myocardial tissue. In accordance with this finding, Koga et al. [16] described recruitment of CD8\(^+\) T cells infiltrating the myocardium in 2 cases of fulminant myocarditis in previously healthy children.

In conclusion, we report a rare case of lethal, fulminant, parvovirus-associated lymphocytic myocarditis in a previously healthy infant. The condition can be difficult to recognize clinically and may be misdiagnosed as either asthma or cardiac and/or respiratory failure of other origin [29]. The case presented in this report suggests that the prevalence of parvovirus-associated myocarditis is probably underestimated and that the disease entity might be missed altogether. PCR analysis for the detection of parvovirus should be considered as a complementary test in cases of myocarditis of unknown etiology in infants.

References