Herpes Simplex Virus 1 infection: misleading findings in an infant with disseminated disease

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INTRODUCTION

Herpes Simplex Virus (HSV) infection is a major cause of morbidity and mortality in infants. The incidence of neonatal HSV infection is 31.2 per 100,000 live births (Brown et al., 2003), with a widely variable range (8 per 100,000-60 per 100,000 live births) (Corey et al., 2009). The clinical presentation of HSV infection in the neonatal period has been divided into three categories according to the extent of disease: skin, eyes and mouth disease (45% of cases of neonatal HSV), the central nervous system (CNS)-associated disease (30%), and disseminated disease (25%). HSV disseminated disease involves multiple organs; approximately 30% of affected infants die and surviving infants present neurological sequelae in 20% of cases (Kimberlin et al., 2001b).

Early high-dose intravenous Acyclovir at 60 mg/kg for 21 days has been found to reduce the mortality rate of neonatal HSV infection, and to improve the rate of infected infants without neurological impairment for the disseminated disease (Kimberlin et al., 2001a).

We describe a disseminated HSV-1 infection in a newborn who succumbed to severe pulmonary complications, liver dysfunction and coagulopathy.

SUMMARY

Neonatal Herpes Simplex Virus (HSV) infection is a serious illness with significant mortality and morbidity for disseminated disease. Clinical diagnosis of neonatal HSV infection is often difficult without evidence of HSV exposure, for example, absence of a rash or the presence of non-specified manifestations in an infant. Early recognition and treatment with high-dose Acyclovir may dramatically improve the short and long-term outcomes. We describe an infant with disseminated disease due to HSV-1 infection, who first presented clinical and radiologic features of pneumonia. The diagnosis was performed post-mortem by Real-Time Polymerase Chain Reaction (PCR) analysis of blood, cerebrospinal fluid and pleural liquid of the infant. Tissue PCR revealed a disseminated HSV-1 infection, with a high viral load detected in liver, lungs, brain, heart, striated muscle, kidneys, and thymus tissues. This case report highlights the need for neonatologists to raise awareness about the different clinical manifestations of disseminated neonatal HSV infection. HSV infections should be prominent in the differential diagnosis of an infant under four weeks of age with fever, pneumonia, unexplained seizures or sepsis-like disease, particularly if unresponsive to antibiotics. Early initiation of appropriate antiviral therapy for high-risk infants undergoing testing for HSV infection can be essential to prevent significant morbidity and mortality.

KEY WORDS: HSV-1, Disseminated disease, Neonatal infection.
A 40-week-old, 3620 g male infant was born to a 21-year-old primigravida by caesarean section, due to breech presentation, performed prior to rupture of membranes. Apgar scores were 10 and 10 at one and five minutes respectively. Physical examination at birth and after 16 hours was reported as normal.

The pregnancy was uncomplicated. Serological screening tests performed during pregnancy showed negativity for HBsAg, HCV and Syphilis antibodies; the mother was immune for Rubella, Toxoplasma gondii and Cytomegalovirus (CMV). Vaginal swab performed at 35 weeks of gestation was negative for *Group B streptococcus*.

At twenty-four hours post-delivery, the mother developed a fever without focal signs of infection; blood culture was negative for bacteria and fungi. The fever persisted for four days.

At 60 hours of life, the newborn developed fever associated with tachypnea and poor skin perfusion. The chest X-ray showed parenchymal opacity in the right lung (Figure 1). Laboratory data revealed a white blood cell count (WBC) of 10100 with 81.5% neutrophils, elevated C-reactive protein (CRP: 5.5 mg/dl), and normal aspartate aminotransferase (AST), alanine aminotransferase (ALT), blood glucose level and coagulative tests. A blood culture was obtained, and intravenous antibiotic therapy with ampicillin and gentamicin was initiated.

On day four of life, the infant was transferred to the Neonatal Intensive Care Unit, St. Orsola-Malpighi General Hospital, in Bologna, Italy. On admission he was febrile (37.8°C) and showed signs of respiratory distress; continuous nasal positive air pressure (nCPAP) was initiated at FIO2 of 0.35 supplemental oxygen.

Twenty-four hours later his oxygen requirement increased. A chest X-ray showed enlargement of opacity images in the right lung and a diffuse reticular, interstitial pattern of both lungs (Figure 1). The infant required mechanical ventilation, and surfactant was administered.

On the following days, the infant became unstable, developed mild hepatosplenomegaly, and further deterioration of respiratory dynamics. The fever subsided, the CRP value gradually decreased to normalization. Blood culture was negative. Liver function tests revealed AST/ALT values of 189/40 U/l on day five of life and 514/115 U/l on day six of life. The chest X-ray showed a progressive deterioration of the reticular, interstitial pattern bilaterally and alveolar infiltrates on the right superior lobe and retrocardiac area. Echocardiography was normal. Clinical examination showed mild hypertonia. For the clinical suspicion of HSV infection, treatment with Acyclovir 60 mg/Kg per day was initiated on the sixth day of life. A blood sample of the infant’s mother was collected for viral investigations.

On day eight of life, a chest X-ray showed a rightside pleural effusion (Figure 1). Pleural drainage produced 20 cc of serous-haematic liquid. Major hepatocellular failure was demonstrated by an AST/ALT value of 4334/985 U/l and impaired coagulation tests. Results of serological investigations showed: IgG positivity and IgM negativity for Toxoplasma gondii, CMV, Epstein-Barr virus (EBV), Enterovirus, Parvovirus B19, Herpes virus 6 (HHV 6), Varicella Zoster Virus (VZV). HSV IgG were negative. The infant’s urine was negative for CMV.

On the following day, the infant experienced seizures. Cranial ultrasound scanning (US) showed periventricular white matter hyperechogenicity. An electroencephalogram (EEG) demonstrated a reduction on the voltage of bioelectric activity and focal points in the left centrum-temporal regions. A lumbar puncture was done and the cerebrospinal fluid (CSF) biochemical and cytological examination showed normal protein concentration, low glucose level, and normal white cell count. The infant’s respiratory function continued to deteriorate. A chest X-ray showed a granular bilateral pulmonary opacification, and the persistence of the pleural effusion, with a normal cardiac silhouette (Figure 1). Liver and coagulation tests further worsened (AST/ALT value 1041/2062 U/l, prothrombin time 14%, activated partial thromboplastin time ratio 4.29). Despite full intensive care support, including mechanical ventilation, fresh frozen plasma transfusion and inotropic therapy, the infant succumbed on day nine of life. Autopsy was required.

Results of cultures, virological tests and specific metabolic tests performed during the hospital stay were available in the following days. Plasma amino acids, plasmatic acylcarnitine, urine organic acids were in the range of normality.
Cultures of pleural liquid, devices and CSF were negative for bacteria, fungi and Mycobacteria. Real-Time PCR in infant’s CSF and blood were negative for: HHV6, EBV, VZV, CMV, HSV2, and Enterovirus.

**Laboratory methods**

Enzyme immunoassay (EIA - Enzygnost, Siemens Healthcare Diagnostics, Marburg, Germany) was used for the quantitative determination of specific IgG and IgM antibodies in human serum. IgG or IgM index >1.3 was considered positive. DNA was extracted from maternal and neonatal samples using the NucliSens easyMAG System (bioMerieux, Marcy l’Etoile, France) according to the manufacturer's recommendations. DNA extraction from paraffin-embedded tissue was performed on five micron tissue slices using a BioSprint 15 DNA Blood Kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's package insert and one microgram DNA was used for PCR determinations. HSV-1, HSV-2, HHV6, EBV, VZV, CMV and Enterovirus were quantified using real-Time PCR assays (Nanogen Advanced Diagnostics SRL, Turin, Italy). Amplification, detection and analy-
sis were performed using the ABI PRISM 7300 platform (Applied Biosystems, Foster City, CA, USA). Viral load was reported as number of copies/ml or number of copies/microgram DNA. Immunohistochemical staining for HSV glycoprotein B (Advanced Biotechnologies Incorporated, Columbia, MD, USA) was performed to identify HSV-1 positive cells.

**Virological and autopsy diagnosis**

HSV1 DNA was found in the infant’s blood by Real-Time PCR (viral load>10,000,000 copies/ml).

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**FIGURE 2 -**

a) Adrenal histology, H&E, x250: adrenal cortex with multiple foci of necrosis of various size (arrows).
b) HSV immunostaining, x100: brown staining depicts necrotic spots and scattered adrenal cells.
c) Lung histology, H&E, x60: diffuse interstitial pneumonitis and blood vascular congestion.
d) Liver histology, H&E, x60: multiple foci of coagulative and haemorrhagic necrosis, mainly around central veins.
collected at day eight of life, drained pleural liquid (>10,000,000 copies/ml) collected at day eight of life and CSF (210,000 copies/ml) collected at day nine of life.

Viral investigations were performed on the maternal blood sample stored on day seven post-delivery, and showed slightly positive HSV IgG (EIA IgG index: 1.5) and positive HSV-1 DNA PCR (viral load 200,000 copies/ml). After one month, the mother was tested again for HSV-1, and viral investigations demonstrated an increased HSV IgG index (EIA IgG index: 3.2), and a HSV-1 DNA PCR of 800 copies/ml. Cervical and vaginal swabs obtained at this time were positive for HSV-1 DNA.

At post-mortem examination lung histology showed interstitial pneumonitis, hyperaemia and focal intralveolar haemorrhages. The liver showed a diffuse necrotizing hepatitis with confluent coagulation necrosis. Multiple foci of necrosis were present in the adrenal glands. The spleen revealed hyperaemia of the red pulp. Immunohistochemistry staining of the lungs, liver and adrenal glands were positive for HSV (Figure 2). HSV-1 DNA was extracted from all post-mortem specimens. Viral loads in each sample are reported in Table 1. The autopsy and virological findings were consistent with a severe disseminated systemic HSV-1 infection with necrotizing hepatitis and diffuse interstitial pneumonitis. The maternal antibody profile and the evidence of HSV-1 DNA in maternal blood and the cervical swab provided a diagnosis of primary HSV-1 infection which had occurred near term.

**DISCUSSION**

Neonatal HSV infection is a rare but severe illness. We described an uncommon onset of HSV-1 infection of an infant born by caesarean section presenting symptoms of pneumonia and only days later developed the typical features of disseminated disease. This case report highlights the difficulty of making an early diagnosis of HSV neonatal infection when the mother’s infection is not apparent and mucocutaneous lesions are absent in the infant.

The majority of reported neonatal HSV infections are caused by HSV-2 (Corey et al., 2009; Rudnick et al., 2002), since the passage through the infected birth canal is the principal route of transmission. However, in recent years, it has been shown that an increasing proportion of genital herpes is caused by HSV-1 (Lafferty et al., 2000; Westhoff et al., 2011). During pregnancy, the risk of transmission is significantly higher in mothers acquiring a primary HSV infection than mothers experiencing a reactivation of the virus (Brown et al., 1991)

The important aspect of this case report is that the mother acquired HSV primary infection near term. HSV has been transmitted to the infant in utero with intact fetal membranes by maternal viremia when protective IgG antibodies have not yet been produced by the mother. Diagnosis of HSV infection was performed post-mortem, by detection of HSV-1 DNA in the infant’s CSF, pleural liquid and blood. At autopsy, an extensive viral dissemination was demonstrated, with a massive involvement of lungs, liver, spleen, brain and adrenal glands. HSV-1 DNA was detected in all tested organs, even in striated muscle, kidneys, heart and thymus where histology did not show abnormalities.

The onset of the HSV disseminated disease was on day 3 of life, that is clearly earlier than usual. Disseminated HSV disease presentation is usually around days 10 to 12 of life (Kimberlin, 2007), but based on antiviral treatment trials and case series, there is a widely variable range from birth to the fourth week of life (Campbell et al., 1983;

**TABLE 1 - HSV-1 load detected by Real-Time PCR in post-mortem specimens.**

<table>
<thead>
<tr>
<th>Tested organ</th>
<th>HSV-1 load (copies/microgram DNA)</th>
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<tbody>
<tr>
<td>Lung</td>
<td>68,000,000</td>
</tr>
<tr>
<td>Spleen</td>
<td>7,000,000</td>
</tr>
<tr>
<td>Kidney</td>
<td>1,800,000</td>
</tr>
<tr>
<td>Brain</td>
<td>1,000,000</td>
</tr>
<tr>
<td>Thymus</td>
<td>220,000</td>
</tr>
<tr>
<td>Heart</td>
<td>214,000</td>
</tr>
<tr>
<td>Striated muscle</td>
<td>130,000</td>
</tr>
<tr>
<td>Liver</td>
<td>8,000</td>
</tr>
</tbody>
</table>
Withley et al., 1991; Kimberlin et al., 2001b; Kropp et al., 2006; Long et al., 2011; Catlin et al., 2012).

No single association of presenting symptoms and signs identifies all infants with HSV disseminated disease: the most common features are skin vesicles, fever, lethargy, respiratory distress, disseminated intravascular coagulopathy, seizures, and poor feeding. Most cases of HSV infection in infants under 21 days of age have a nonspecific presentation (Long et al., 2011). This case report shows an early uncommon manifestation of the HSV disseminated disease. Although the presence of pulmonary infiltrates at the onset of infection is rare, it has been previously reported (Campbell et al., 1983; Lissauer et al., 1984; Langlet et al., 2003; Knezevic et al., 2007). This means that early and rapidly progressive pneumonia might be considered one of the different initial manifestations of disseminated neonatal HSV. The early appearance of the respiratory distress, the consolidation on the chest radiography and the elevated CRP value which decreased after antibiotic therapy, lead to the clinical suspicion of bacterial sepsis. However, the progressive radiological findings were suggestive of HSV-pneumonia: normal lung in the first stage, reticular and interstitial pattern in the second, alveolar infiltrates without hyperinflation in the third and total pulmonary opacification with pleural effusion in the last stage (Dominguez et al., 1984). On day nine of life the infant developed seizures as a manifestation of CNS disease without relevant cranial US abnormalities, but with abnormal bioelectric activity of the temporal lobe at EEG examination. However, the involvement of the temporal lobe is considered to be typical of HSV encephalitis after the neonatal period (Kimberlin, 2007).

Clinical deterioration in spite of antibiotic treatment, low CRP and increasing transaminase levels detected from day 5 of life might have helped in making a differential diagnosis. The clinical course of the infant described in this case report emphasizes the need for neonatologists to raise awareness about the different ways that disseminated HSV infection may present in the neonatal period. Acyclovir treatment was delayed due to a misinterpretation of the presenting symptoms as bacterial pneumonia. The finding that early manifestations of HSV infection are often nonspecific should be considered when weighing management strategies of an acutely ill infant under 21 days of age: empiric acyclovir strategy narrowly restricted to infants with onset of illness under 21 days of age has been reported to capture 90% of HSV cases and to anticipate the rate of CNS involvement similar to that of bacterial meningitis (Long et al., 2011).

In our opinion, infants under four weeks of age with fever, pneumonia, unexplained seizures or sepsis-like disease, particularly if unresponsive to antibiotics, should be promptly evaluated for HSV infection. Early initiation of appropriate antiviral therapy for high-risk infants can be essential to prevent significant morbidity and mortality.

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REFERENCES


