Severe postnatally acquired cytomegalovirus infection presenting with colitis, pneumonitis and sepsis-like syndrome in an extremely low birthweight infant

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Abstract

Few cases of severe postnatally acquired cytomegalovirus (CMV) infection are reported in premature infants. We report on an extremely low birthweight (ELBW) preterm infant who presented with a sepsis-like syndrome and multiple organ involvement, notably pneumonitis and colitis. The course of infection was assessed by repeated analysis of urine, tracheal aspirates and blood. The patient was given intravenous ganciclovir. The clinical course was rapidly favorable. Development of neutropenia led to the discontinuation of the antiviral treatment after 28 days. Follow-up showed moderate white matter anomalies on cerebral MRI, a transient hypoacusis and a mild developmental delay at 18 months of corrected age. To the best of our knowledge, this is the first description of a severe combination of pneumonitis and colitis in postnatal CMV infection. Many issues remain controversial and are discussed. We propose that antiviral treatment should be considered in severe postnatal CMV infection in ELBW patients.
Severe Postnatally Acquired Cytomegalovirus Infection Presenting with Colitis, Pneumonitis and Sepsis-Like Syndrome in an Extremely Low Birthweight Infant

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Abstract
Few cases of severe postnatally acquired cytomegalovirus (CMV) infection are reported in premature infants. We report on an extremely low birthweight (ELBW) preterm infant who presented with a sepsis-like syndrome and multiple organ failure due to severe CMV infection. The infant survived with antiviral therapy and subsequent careful follow-up.

Established Facts
- Postnatal cytomegalovirus infection can lead to symptomatic disease in a preterm infant with a broad spectrum of clinical manifestations.
- Clinical course of the infection is difficult to assess.
- Knowledge about the efficiency of antiviral treatment is very limited.
- Many long-term issues remain uncertain.

Novel Insights
- Association of pneumonitis and colitis with severe clinical deterioration is a rare presentation of the disease in extremely low birthweight infants.
- Repeated analysis in urine, blood and tracheal aspirates were performed to assess the evolution of the infection.
- Intravenous ganciclovir should be considered for severe postnatal cytomegalovirus infection in extremely low birthweight patients.
- Careful long-term neurosensorial and developmental follow-up is needed.

Key Words
Cytomegalovirus · Preterm infant · Antiviral treatment · Ganciclovir · Colitis · Pneumonitis · Developmental outcome
involvement, notably pneumonitis and colitis. The course of infection was assessed by repeated analysis of urine, tracheal aspirates and blood. The patient was given intravenous ganciclovir. The clinical course was rapidly favorable. Development of neutropenia led to the discontinuation of the antiviral treatment after 28 days. Follow-up showed moderate white matter anomalies on cerebral MRI, a transient hyponocacus and a mild developmental delay at 18 months of corrected age. To the best of our knowledge, this is the first description of a severe combination of pneumonitis and colitis in postnatal CMV infection. Many issues remain controversial and are discussed. We propose that antiviral treatment should be considered in severe postnatal CMV infection in ELBW patients.

Introduction

Although cytomegalovirus (CMV) infection is well recognized as a leading cause of congenital pathology associated with important morbidity and mortality, less is known about CMV infection if acquired in the early postnatal period [1]. Postnatal CMV infection is most often transmitted through breastfeeding [2]. Other sources of perinatal and postnatal infection are cervical and vaginal secretions, blood transfusions and horizontal transmission by close contact. In contrast to the usually asymptomatic course in term neonates, postnatal infection in preterm infants can lead to a broad spectrum of potentially life-threatening problems [2, 3]. Only a few cases of such infections have been reported [4–7]. Major aspects like the natural evolution of the disease, therapeutic options or long-term outcome remain poorly recognized or controversial. We report here a case of postnatal CMV infection in an extremely low birthweight (ELBW) infant. He presented sudden clinical deterioration with sepsis-like symptoms, pneumonitis, colitis, hepatomegaly and thrombocytopenia, and showed rapid recovery under antiviral treatment. His neurodevelopmental follow-up until 18 months of corrected age is described.

Case Report

A 26-year-old primigravida vaginally delivered a male preterm infant at 24 6/7 weeks of gestation after preterm labor due to suspected chorioamnionitis. At the time of birth, pulmonary maturation with betamethasone had just been initiated. Birthweight was 780 g (P10–50th), body length 33.5 cm (P50–90th) and head circumference 23.5 cm (P50–90th). Apgar score was 1, 6 and 8 at 1, 5 and 10 min, respectively. The initial course was marked by a severe respiratory distress syndrome requiring intubation, repeated doses of exogenous surfactant and prolonged mechanical ventilation, relayed by nasal continuous positive airway pressure (nCPAP) starting on day of life (DOL) 50. Apneas were treated with caffeine citrate. Three courses of antibiotics were applied within the first month due to bouts of clinical deterioration for which no infectious agent could be isolated. Several erythrocyte transfusions were required between DOL 2 and 49. Enteral nutrition was started on DOL 2 giving a few milliliters of fresh colostrum, progressing with an increment of 10 ml/kg/day to reach 140 ml/kg/day of exclusive mother’s breast milk on day 14. All milk was frozen at –20°C at our nutrition center 2–30 days before administration. Pasteurization was not performed. Growth was initially retarded and then followed the 25th percentile.

On DOL 58 (corresponding to 33 1/7 weeks of gestational age) the infant presented bloody stools while maintaining a good general condition and a normal abdominal status. Table 1 summarizes the main relevant clinical events, laboratory findings and radiological studies. Blood count showed thrombocytopenia, lymphocytosis and neutropenia. Three days later, he showed abdominal distension, hepatosplenomegaly and bloody diarrhea, along with rapidly progressing respiratory distress and increased oxygen requirements. Simultaneously, his general condition deteriorated with apathy, hypotonia, grayish discoloration, fever, and many severe episodes of apnea and bradycardia requiring endotracheal intubation. The gastric tube was placed under intermittent aspiration. Laboratory findings revealed increasing inflammatory parameters (CRP 146 mg/l, leukocytosis with shifting to immature cells) and persistence of thrombocytopenia. Serum levels of transaminases and liver function remained within the normal range. Abdominal X-rays showed diffuse distension of the gastrointestinal tract with no signs of pneumatoasis or perforation. Chest X-ray revealed diffuse bilateral alveolar infiltrations with predominance on the right side. Empirical antibiotic therapy was initiated with a combination of cefazidime, amikacin and metronidazole and continued for 10 days. All cultures for bacterial pathogens (blood, urine, tracheal aspirates and stool) obtained before initiation of antibiotic treatment were found to be sterile. A lumbar puncture was not performed due to the child’s critical and unstable clinical condition. A CMV infection was suspected considering the infant’s unusual age and atypical clinical picture for necrotizing enterocolitis, the negative culture results together with the concomitant and persistent thrombocytopenia. CMV infection was confirmed by testing urine for CMV DNA with polymerase chain reaction (PCR), revealing a high viral load (581,100 copies/ml). DNA was extracted on a MagNA Pure LC® machine using the DNA Isolation Kit I (Roche Diagnostics, Mannheim, Germany) according to the manufacturer’s instructions and amplified for CMV pp65 (UL83) DNA using the forward primer CMVPP65_F (5’ACGGCTTTACGGTGTTGTC3’) and the reverse primer CMVPP65_R (GCAGGCCCAAATGCTCGTGTG). The reactions were performed in a final volume of 20 μl, including 0.2 μM of each primer, 0.1 μM Ctr_P probe, 10 μl 2× TaqMan Universal Master Mix (Applied Biosystems, Foster City, Calif., USA) and 5 μl DNA sample. Cycling conditions were 2 min at 50°C, 10 min at 95°C, followed by 45 cycles of 15 s at 95°C and 1 min at 60°C. Amplification and PCR product detection were performed with the ABI Prism 7900...
Sequence Detection system (Applied Biosystems). This PCR system has been validated clinically [8]. The infant’s blood obtained on DOL 58 was also found to be positive for CMV DNA by PCR. This analysis was performed retrospectively on a dried blood sample of a neonatal screening card which was performed on the same day. DNA was extracted using the QIAmp DNA mini kit (Qiagen, Hilden, Germany). CMV DNA analysis was performed using the TaqMan real-time PCR technique as described earlier [9]. Several complementary investigations were performed in an attempt to rule out a congenital infection and to date the acquisition of the virus by the child. The maternal serological status for CMV had checked positive for IgG but negative for IgM at 12 weeks of gestation. PCR testing for CMV on a specimen of blood obtained on DOL 1 before the first blood transfusion (kept as a dried sample on another neonatal screening card) was negative [10]. Reexamination of placental histology showed neither giant cells nor cell inclusions. Endotracheal secretions, that had been obtained at different time points throughout the whole hospitalization period, were retrospectively tested by PCR for CMV DNA. Viral DNA was undetectable on DOL 40 and detected on DOL 50, i.e. 10 days before the onset of respiratory symptoms. Weighing up psychological and ethical considerations (protect the mother from guilt feelings, preserve breastfeeding, and knowing that the clinical course, treatment and prognosis of the infant would not be influenced by the result), we intentionally did not perform CMV analysis in mother’s milk. However, we excluded the other important route of transmission by re-testing transfused blood samples. Due to the persistent critical condition, antiviral therapy with intravenous ganciclovir was introduced on DOL 62 (12 mg/kg body weight in 2 daily doses) and was associated with a rapid improvement in clinical, biological and radiological parameters. The patient was completely free of symptoms 1 week after starting antiviral treatment and could be extubated on the 8th day of therapy. Enteral feeding was resumed shortly thereafter without com-

Table 1. Synopsis of clinical, radiological and laboratory data of the reported case

<table>
<thead>
<tr>
<th>DOL</th>
<th>Clinical signs</th>
<th>Radiology</th>
<th>Hematology</th>
<th>Chemistry</th>
<th>Virology (PCR)</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Blood negative</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>Tracheal secretions negative</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>Tracheal secretions positive 365 copies/ml</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>52</td>
<td>Leukopenia (5.6 × 10⁹/l) Thrombocytopenia (112 × 10⁹/l)</td>
<td>CRP &lt;2 mg/l</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>57</td>
<td>Neutropenia (0.97 × 10⁹/l) Lymphocytosis Thrombocytopenia (34 × 10⁹/l)</td>
<td>Urine positive 581,100 copies/ml</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>58</td>
<td>Bloody stool Good general condition Normal abdominal examination</td>
<td>Unchanged</td>
<td>Blood positive</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>61</td>
<td>Hypotonia, fever, gray color, apnea, increased oxygen requirements, painful abdominal distension, hepatosplenomegaly, bloody diarrhea</td>
<td>Unchanged</td>
<td>CRP 101 mg/l Intubation Gastric aspiration Antibiotics (10 days)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>62</td>
<td>Unchanged clinical picture</td>
<td>Cerebral US: normal</td>
<td>Leukocytosis (23.6 × 10⁹/l), left shift Anemia (hemoglobin 122 g/l) Thrombocytopenia (57 × 10⁹/l)</td>
<td>CRP 146 mg/l Normal hepatic values</td>
<td></td>
<td>Red cell transfusion Ganciclovir (28 days)</td>
</tr>
<tr>
<td>66</td>
<td>General condition improved, normalization of stool, liver and abdominal status</td>
<td>Thrombocytopenia (78 × 10⁹/l)</td>
<td>Tracheal secretions negative</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>69</td>
<td>Asymptomatic</td>
<td>Cerebral MRI: moderate diffuse anomaly of the white matter</td>
<td>Thrombocytopenia (97 × 10⁹/l)</td>
<td>CRP 6 mg/l Urine negative Nasal continuous positive airway pressure</td>
<td>Enteral refeeding</td>
<td></td>
</tr>
<tr>
<td>79</td>
<td>Neutropenia (0.57 × 10⁹/l)</td>
<td>Urine positive 3,700 copies/ml</td>
<td></td>
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</tr>
</tbody>
</table>

DOL = Day of life; CRP = C-reactive protein.
plication. Ganciclovir was discontinued after 4 weeks of therapy because of persistent neutropenia (min 0.4 × 10⁹/l). Urine test for CMV DNA became negative after 1 week of ganciclovir treatment but asymptomatic viral shedding reappeared 2 weeks after cessation of therapy. On DOL 66, tracheal aspirates were free of CMV DNA.

The infant remained on respiratory support with nCPAP during another 8 weeks, i.e. until 42 weeks of gestational age. Despite the diagnosis of severe bronchopulmonary dysplasia, he was discharged home at 44 weeks of gestational age, weighing 4,180 g (P10–50th), free of supplemental oxygen and medical treatment.

Cerebral ultrasound examination performed on a weekly basis remained normal. A first cerebral MRI was obtained on DOL 69 (i.e. at 34 weeks of gestational age) and repeated at term. Both examinations showed nonspecific lesions, with moderately high intensity of signal in the periventricular white matter and microhemorrhages in the posterior fossa.

The ophthalmologic examination revealed a persistent vitreous membrane which disappeared at term. A grade I retinopathy was described on DOL 108 but had spontaneously disappeared on checkup on DOL 139. Acoustic evoked potentials were moderately abnormal on DOL 132 (i.e. at 43 weeks of gestational age), with bilateral hearing loss (50 dB on the left and 30 dB on the right side). Follow-up examination at the corrected age of 4 months was normal on the right but unchanged on the left side. At 12 months it was normal on both sides. Neurological and developmental assessments performed at term, 6 and 12 months of corrected age showed mild abnormalities in muscle tone for which he benefited from early motor physiotherapy since discharged home. At 18 months, the child was mildly floppy and the developmental evaluation with the ‘Griffiths Scales of Infant Development’ (standardized at a developmental quotient of 100, with 1 SD = 15) revealed a homogeneous and global delay with a quotient of 74 [11]. The patient was then offered an early intervention program.

Discussion

On DOL 61 this very premature ELBW infant developed a severe sickle-like syndrome with multiple organ involvement including colitis, pneumonitis, thrombocytopenia, mild anemia, neutropenia and hepatosplenomegaly without hepatitis. CMV infection was proven by several PCR analyses in blood, urine and endotracheal secretions and antiviral therapy was started. Facing such a situation, the clinician is confronted with several questions.

Timing of Infection?

The isolation of CMV in a sample of urine obtained later than 2–3 weeks after birth cannot distinguish a congenitally from a postnatally acquired infection. However, a prenatal infection can be retrospectively diagnosed by a positive PCR analysis performed on a sample of dried blood from the early postnatal screening card (Guthrie test) [10]. In our case, negative CMV PCR on dried blood spot test from DOL 1 and normal placental histology reinforce the hypothesis of a postnatally acquired infection.

Source of Infection?

Transmission of CMV by the breast milk of seropositive mothers is well described [12]. CMV excretion in breast milk is at its peak from 3 to 5 weeks postnatally, with up to 97% of tested samples yielding CMV DNA [13]. Studies of CMV transmission through breast milk reveal a wide range of transmission rates [14, 15]. This likely reflects variations in CMV load of breast milk over time and of different standards of milk conservation. Colostrum seems to be at low risk of containing CMV [16]. Freezing milk to –20°C reduces but does not abolish the risk of infection, as shown by this patient who was exclusively fed with frozen mother’s milk except for a few milliliters of colostrum [5, 17, 18].

Other potential sources of CMV infection include the following: (1) Exposure to infected genital secretions in the birth canal resulting in a disease starting within the first 6 postnatal weeks. (2) Infection through transfusion of contaminated blood products. This way of transmission can be effectively reduced by transfusing blood from CMV-negative donors and/or by transfusing blood after leukofiltration. Both approaches are practiced in our center for all neonates needing blood transfusions. (3) Nosocomial transmission from congenitally infected hospitalized neonates, or infected caregivers or family members [1].

Clinical Presentation?

Shedding of CMV in breast milk almost invariably results from reactivation of preexistent CMV infection in seropositive mothers. The neonates of these mothers are usually protected by transplacental transfer of maternal antibodies. Ingestion of breast milk containing CMV is therefore considered harmless in term neonates. However, this protective mechanism does not apply to preterm infants in whom the risk of developing an early and symptomatic postnatal CMV infection is directly proportional to the degree of immaturity [19]. Indeed, depending on the child’s maturity, the rate of symptomatic infection shows a wide variation going up to 75% [20, 21]. Individual factors of susceptibility are suspected but so far unknown. The onset of relevant signs of postnatal CMV infection is reported from DOL 22 to 209 [19]. Manifestations can vary from hematological signs like neutropenia or lymphocytosis, anemia and thrombocytopenia to organ involvement like hepatosplenomegaly with or without hepatitis and cholestasis, pneumonitis, or colitis. In
most severe cases, the clinical picture is a severe sepsis-like disease. A case of hemophagocytic lymphohistiocytosis-like syndrome has recently been described [22]. Furthermore, our patient presented a multiorgan involvement. Pneumonitis was suggested by apnea, respiratory distress, increasing oxygen and ventilatory needs. Chest X-ray initially showed a bilateral reticulo-granular pattern compatible with CMV pneumonitis. We were able to document the presence of CMV in periodic tracheal aspirate samples, interestingly, from 10 days before to 5 days after the onset of symptoms, while all bacterial cultures remained sterile, suggesting but not proving an etiological role for this virus. Additionally, the patient manifested concomitant signs of a hemorrhagic colitis with important abdominal distension and bloody diarrhea, which might be a hint to the enteral route of infection. An etiologic role of CMV infection in enterocolitis has recently been suggested [23]. We hypothesized that an enterocolitis due to CMV infection could have favored bacterial translocation and infection via an altered intestinal mucosa. This could explain the unusually high CRP value [3]. However, as all blood and stool cultures remained negative we cannot prove this hypothesis.

**Antiviral Therapy?**

No controlled study has yet evaluated the impact of antiviral treatment on the outcome of postnatally acquired CMV infection and no treatment recommendations exist so far. There are, however, encouraging data about ganciclovir treatment in congenital infections [24]. Positive effects of treatment have also been described in anecdotal reports of postnatal infections [25, 26]. Commonly reported side effects of ganciclovir therapy include neutropenia and technical problems with the venous access. Not rarely, one of these problems is responsible for early interruption of the planned long-term treatment. Among the potential long-term side effects of this treatment are the reduced male fertility observed in animal studies and neurological effects described in adult patients [27]. Valganciclovir, the oral bioavailable prodrug of ganciclovir, and maribavir, a new oral anti-cytomegalovirus drug might be potential alternatives in the future [28, 29]. Recent pharmacokinetic and clinical studies of valganciclovir in the neonatal population with congenital infection are quite promising [30–32]. Specific immunoglobulin preparations do not seem to be a valuable approach [33]. As the clinical situation remained critical despite intensive care and broad-spectrum antibiotic therapy, in our patient we opted for starting intravenous ganciclovir-based antiviral therapy. Over the next days, we observed an impressive clinical improvement and a rapid disappearance of CMV DNA detectable by PCR in urine and tracheal aspirates.

**Outcome?**

Although postnatally acquired CMV infection shows a spontaneous resolution in the vast majority of term infants, mortality is estimated to be up to 10% in severely symptomatic preterm patients infected through transfusion. Long-term effects of postnatally acquired CMV infection in premature neonates are a matter of debate. Some data suggest an increased risk of bronchopulmonary dysplasia, however, this is contradicted by others [25, 34, 35]. Neurological sequelae that are frequently reported in congenitally infected patients (progressive chorioretinitis, permanent neurosensorial hearing loss, seizures or developmental delay) have so far not been clearly demonstrated in postnatally acquired infections [36]. A case control study from the 1980s did not show an increase in the risk of moderate or major handicap in CMV-infected low birthweight patients. Nevertheless, the risk of severe handicap was greater in very preterm infants when CMV infection was acquired early (within the first 8 postnatal weeks) [37]. In contrast, in a recent report comparing 22 postnatally infected very preterm patients with 22 matched counterparts, no difference in the rate of sensorineural hearing loss, neurological anomalies or developmental delay was found at the age of 2.5–4 years [38]. In this series, 3 of the 4 patients presenting with sepsis-like symptoms had some abnormalities on follow-up. However, all these patients, like the case reported here, were the most immature of the series, with a gestational age of <25 weeks. Our patient showed transient hypoaosisis over the first few months which resolved at 1 year of corrected age, a finding which is not uncommon in the very premature population. A similar observation was reported recently by Takahashi et al. [4]. Intracerebral calcifications were described in 2 preterm infants following postnatal infection [39]. In our patient, repeated cerebral ultrasound examinations remained normal, but 2 cerebral MRIs showed moderate anomalies of the periventricular white matter and micro-hemorrhages in the posterior fossa. A causal relationship with the infection is uncertain as these findings are unspecific and rather common in ELBW infants. However, so far there is no literature available on characteristic cerebral MRI findings in postnatally acquired CMV infection. The neurodevelopmental course of the patient presented here is common to ELBW infants, with no clear developmental sequels of CMV infection so far. In the absence of conclusive literature as to the innocu-
ousness of postnatal CMV infection in premature patients, careful and long-term neurodevelopmental follow-up, with auditory monitoring, should be offered. Onset of late and progressive manifestations as described in congenital CMV disease should be considered.

**Prevention?**

Freezing of breast milk can reduce the viral load but not prevent the risk of CMV infection [2, 5, 40]. Standard pasteurization procedures (30 min at 62.5°C) are efficient to inactivate CMV but disturb important enzyme activity and reduce the nutritional and immunological quality of human milk [17]. There is increasing evidence that short pasteurization procedures might be of similar efficiency to reduce CMV infectivity with the advantage of altering the immunological and nutritional components of the breast milk to a lesser degree [41]. General precautions in blood transfusions and in hand hygiene of caregivers should be applied. Intensive research is ongoing regarding a CMV vaccine, but so far none is available [42].

**Conclusion**

The report of this severe case of postnatally acquired CMV infection aims at raising the index of suspicion towards its highly heterogeneous and unspecific clinical picture which can lead to under-, mis- or delayed diagnosis. We suggest that postnatal CMV infection should be considered in all breast milk-fed preterm infants of seropositive mothers presenting during the first 12 postnatal weeks with infectious symptoms and accompanying manifestations such as thrombocytopenia, hepatosplenomegaly, cholestasis, pneumonia, enterocolitis and/or sepsis-like signs. Diagnosis can be made by PCR detection of CMV DNA in urine, blood or tracheal aspirates. A PCR assay can also be retrospectively performed on an early sample of blood (e.g. on dried blood of metabolic screening cards) in an attempt to differentiate between prenatal and postnatal infection. Although the prognosis of postnatally acquired infection seems better than that of the congenital disease, treatment with intravenous ganciclovir may be considered in the very sick preterm infant. Additional data are needed to determine whether this treatment is of benefit for controlling the acute infection and also preventing long-term neurosensorial sequel.

**Acknowledgement**

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Postnatal CMV Infection


