High Cytomegalovirus (CMV) DNAemia Predicts CMV Sequelae in Asymptomatic Congenitally Infected Newborns Born to Women With Primary Infection During Pregnancy

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Objective. We investigated the kinetics of cytomegalovirus (CMV) clearance in blood and urine and the relationship between the viral load in blood at birth and the development of late-onset sequelae in asymptomatic congenital CMV infection.

Methods. Thirty-three newborns with congenital asymptomatic CMV infection born to women with primary CMV infection during pregnancy were enrolled. CMV infection was monitored by polymerase chain reaction analysis of blood and urine. The follow-up examination was concluded at 6 years of age.

Results. Ten infants developed postnatal sequelae, whereas twenty-three infants remained asymptomatic. Fifty percent of babies cleared CMV in blood and urine within 3 and 36 months, respectively. Logistic multivariate regression revealed that the risk of neonatal clinical disease crossed the level of 50% with a DNAemia at birth of ≥12,000 copies/mL ($P = .0002$). The risk of hearing deficit crossed the level of 50% with a DNAemia at birth of ≥17,000 copies/mL ($P = .0001$). No significant difference was found between the kinetics of CMV clearance in asymptomatic children as compared to babies with late-onset disease.

Conclusions. Asymptomatic newborns with a CMV DNAemia at birth of ≥12,000 copies/mL were more likely to experience CMV-related sequelae. The risk of hearing deficit increased with a viral load in blood of ≥17,000 copies/mL.

Keywords. congenital CMV infection; CMV kinetics of clearance; DNAemia at birth; late-onset sequelae.

Human cytomegalovirus (CMV) is the most common cause of intrauterine infection and the main cause of psychomotor retardation and sensorineural hearing loss (SNHL) related to congenital infections in developed countries, affecting 0.3%–0.6% of all live births in Europe [1, 2]. Approximately 10%–15% of congenitally infected children exhibit clinical abnormalities at birth [3]. About 8%–15% of congenitally infected babies who are asymptomatic at birth may develop SNHL and neurodevelopmental disorders in the first years of life.

It is well known that children with clinical evidence of symptomatic CMV infection exhibit a higher amount of virus in blood than children with asymptomatic infection during the neonatal period [4–7]. In children with asymptomatic CMV infection, a definite association between the risk of hearing deficit and viral load in blood and urine during the neonatal period is still lacking, and prognostic markers of CMV-related sequelae have not been deeply investigated [5, 6].

Congenital CMV infections are characterized by prolonged excretion of the virus in urine after birth, even in asymptomatic neonates, and previous studies indicated that persistent viral replication was a possible explanation for the virus-related sequelae [8, 9]. There is also evidence that SNHL is associated with a shorter duration of CMV excretion in urine in congenitally infected babies [10], therefore the relationship between virus clearance and outcome in congenitally infected children remains incompletely understood. In addition, the
kinetics of CMV clearance in blood in congenital CMV infection has never been investigated.

In this study, we prospectively monitored the characteristics of viral replication in blood and urine in a group of asymptomatic congenitally infected babies born to women with primary CMV infection during pregnancy. First, we investigated the kinetics of CMV clearance in blood and urine. Second, by analyzing the association between viral load at birth and postnatal sequelae, we determined a viremia threshold predictive of late-onset CMV-related disease.

**METHODS**

**Study Population**

During January 2004–December 2007, newborns who were identified at birth with congenital CMV infection by isolation of the virus in urine within the first week [11] were regularly followed up in the Unit for Gestational and Congenital Infections of Padua University Hospital. Babies were selected on the basis of identification of primary CMV infection in the mother during pregnancy [12]. Primary maternal CMV infection was defined by seroconversion in previously seronegative mothers or by the presence of specific immunoglobulin G (IgG), specific immunoglobulin M (IgM), and low CMV-specific IgG avidity (<25%) [12]. We initially considered 37 newborns (17 males and 20 females) with congenital CMV infection born to mothers who experienced primary CMV infection during pregnancy. Four (11%) were symptomatic at birth and were treated intravenously with ganciclovir (6 mg/kg twice daily) for 6 weeks [13]. These babies were excluded from the analysis, to avoid the bias due to the antiviral therapy.

The neonatal general evaluation included a physical examination, determination of blood cell and platelet counts, measurement of aminotransferase levels, serologic testing for CMV-specific IgG and IgM, and quantitative polymerase chain reaction (PCR) analysis of CMV DNA in blood and urine [14]; brain transfontanellar ultrasonography; evaluation of brain stem auditory evoked responses; and visual assessment. Medical examinations of infants were scheduled at 1, 3, 6, 12, 18, 24, 30, and 36 months of life and then annually until 6 years of age. Follow-up included clinical, virological, audiological, neurodevelopmental, and visual assessments. The study was approved by the ethics committee of the Padua General Hospital.

**Clinical Definitions**

Symptomatic congenital CMV disease at birth was defined by the presence of ≥1 of the following findings: neurologic involvement as microcephaly (head circumference below the fifth percentile), hypotonia, hemiparesis and seizures, SNHL and deafness, chorioretinitis, cholestasis, and hepatosplenomegaly with an elevated alanine aminotransferase (ALT) level. Periventricular calcifications, cortical dysplasia, ventricular enlargement, and cerebellar hypoplasia on brain computed tomography or magnetic resonance imaging supported the clinical diagnosis of cytomegalic disease.

Late-onset sequelae were defined by the presence of ≥1 of the following findings: hemiparesis, hypertonia or hypotonia, psychomotor retardation, and unilateral or bilateral SNHL.

**Virological Tests**

CMV DNA was detected in blood and urine by quantitative real-time PCR as described previously [14]. The sensitivity of the assay has been determined to be approximately 100 copies per 1 mL of whole blood. The conversion factor for the viral load values to international units per milliliter is 1. CMV DNA in blood was performed every 3–4 weeks until it was no more detectable. Tests for detection of CMV DNA level in urine were performed during every medical examination.

**Audiological Assessment**

The audiological assessment consisted of a formal audiological follow-up, according to which all CMV-infected children underwent newborn audiological screening, using automated transient evoked otoacoustic emissions and auditory brainstem response, followed by an audiological evaluation that was scheduled twice a year until the age of 3 years and once a year until the age of 6 years. This audiological assessment included pure-tone threshold evaluation by testing air conduction (250–8000 Hz) and bone conduction (250–4000 Hz). Outer hair cell function was tested using distortion product otoacoustic emissions. Middle-ear function was assessed by tympanometry and on the basis of acoustic reflex thresholds. SNHL was defined as a unilateral or bilateral hearing threshold of >25 dB for at least 2 of the frequencies tested (from 500 to 4000 Hz). Conductive hearing loss was diagnosed in children with normal bone conduction thresholds (<20 dB) and with an airborne gap of ≥15 dB, averaged over 500, 1000, and 2000 Hz [15].

**Neurodevelopmental Assessment**

Neurologic assessment and evaluation with the Bayley III scale (which includes cognitive, language, motor and social-emotional scales) were performed in children up to 36 months of life. The NEPSY II scale was used to assess neuropsychological development in children ranging from 4 to 6 years old.

**Visual Assessment**

Fundus oculi examination was performed soon after birth and at 6 and 12 months of life.

**Statistical Analysis**

A time-to-event (survival) analysis was performed by using the termination of CMV viremia and viruria as a reference event (outcome). The termination of CMV excretion corresponded to the first negative result of a CMV DNA test of blood or
We investigated the kinetics of the CMV clearance in blood and urine in the 33 babies with congenital CMV infection. Fifty percent of the babies cleared CMV DNA in blood within 3 months of life. All 33 infants were negative for CMV in blood within 15 months of life. The mean time of viremia clearance was 4.09 months (95% confidence interval [CI], 2.98–5.20 months). Fifty percent of the babies cleared CMV DNA in urine within 36 months of life. The mean time of viruria clearance was 38.39 months (95% CI, 31.84–44.94 months). We analyzed separately the kinetics of the CMV clearance in blood and urine in children who developed postnatal sequelae and in asymptomatic children (Figure 1). No statistically significant difference emerged between the 2 groups ($P = .96$ and $P = .88$, respectively).

The mean viral load in blood at birth was 1770 copies/mL (95% CI, 960–3262 copies/mL) in asymptomatic babies and 17 045 copies/mL (95% CI, 6164–47 133 copies/mL) in babies who developed late-onset disease. The difference between the 2 mean values was significant, as determined by the Student $t$ test ($P = .0002$).

The risk of developing postnatal sequelae was evaluated using logistic regression. Multivariate logistic regression was performed using either neonatal clinical disease or hearing deficit as dependent variable and (1) CMV DNAemia at birth (as a log$_{10}$-transformed quantity), (2) maternal age, or (3) gestational weeks as predictor covariates. With both outcomes, a significant result was obtained for DNAemia only. Thereafter, the analysis was repeated after excluding nonsignificant covariates and maintaining neonatal clinical disease versus DNAemia and hearing deficit versus DNAemia. As a result, the risk of neonatal clinical disease crossed the level of 50% with a DNAemia at birth of ≥12 000 copies/mL (the log$_{10}$ DNAemia corresponding to 50% risk was 4.077; $P = .0002$; Figure 2). As shown in Figure 2, the risk of CMV-related sequelae has a sigmoidal shape at a CMV DNAemia of >1000 copies/mL, and it is quasi-linear in the range of 3000–30 000 copies/mL. The risk of hearing deficit crossed the level of 50% with a CMV DNAemia of ≥17 000 copies/mL ($P = .0001$).

**DISCUSSION**

The first aim of this study was to analyze the kinetics of CMV viremia and viruria clearance in postnatal life after primary CMV intrauterine infection. A median period of 3 months for blood and 36 months for urine was required for CMV clearance. Prolonged viral shedding implies that babies with prenatal CMV infection are a source of virus throughout their first years of life and represent an important risk factor for the infection of pregnant women.

A statistically significant difference in the duration of viremia and viruria was not observed in children with late-onset symptomatic infection, compared with healthy babies. We speculate

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**Table 1. Late-Onset Sequelae and Age of Appearance Among Babies With Congenital Cytomegalovirus Infection**

<table>
<thead>
<tr>
<th>Patient, Sequelae</th>
<th>Age of Appearance</th>
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<tbody>
<tr>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Severe bilateral sensorineural hearing loss</td>
<td>6 mo</td>
</tr>
<tr>
<td>Psychomotor retardation</td>
<td>8 mo</td>
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<tr>
<td>2</td>
<td></td>
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<tr>
<td>Right-hand paresis</td>
<td>9 mo</td>
</tr>
<tr>
<td>Right-side hemiparesis</td>
<td>16 mo</td>
</tr>
<tr>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Severe bilateral sensorineural hearing loss</td>
<td>6 mo</td>
</tr>
<tr>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Psychomotor retardation</td>
<td>12 mo</td>
</tr>
<tr>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Severe left-ear sensorineural hearing loss</td>
<td>3 y</td>
</tr>
<tr>
<td>Severe right-ear sensorineural hearing loss</td>
<td>5 y</td>
</tr>
<tr>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Left-ear sensorineural hearing loss</td>
<td>12 mo</td>
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<td>7</td>
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<td>Left-ear sensorineural hearing loss</td>
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<td>8</td>
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<tr>
<td>Severe bilateral sensorineural hearing loss</td>
<td>18 mo</td>
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<td>9</td>
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<tr>
<td>Right-ear sensorineural hearing loss</td>
<td>3 mo</td>
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<td>Right-ear sensorineural hearing loss</td>
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that virus clearance is mostly affected by the newborn immune system response. The long persistence of CMV in body fluids could be due to the immaturity of the immune system in newborns, who are unable to mount an effective cellular immune response. Recent studies showed impaired interferon γ secretion by fetal CD8+ T cells in CMV-infected fetuses and defective expansion of the pp65-specific cytotoxic T-lymphocytes pool in response to CMV antigen [8, 16]. This was associated with decreased viral clearance. Elsewhere, the development of CMV-specific cellular immunity has been correlated with the termination of viral excretion [9].

We investigated the viral load in blood at birth to determine possible prognostic indicators in asymptomatic newborns born after primary CMV infection in pregnancy. A statistically significant difference was found between the mean viral loads in blood at birth in babies who developed late-onset diseases and babies who remained asymptomatic during the 6-year follow-up period. Furthermore, we were able to define a threshold of DNAemia above which the risk of late and progressive CMV-related sequelae in asymptomatic neonates is high (≥12 000 copies/mL). The curve assumes a steep uphill shape in the range of 3000–30 000 copies/mL, indicating that the risk of developing late-onset sequelae raises dramatically. Since the hearing deficit represents the most important CMV-related sequelae, a predictive threshold of SNHL (≥17 000 copies/mL) was also defined.

These thresholds may be useful for the early identification of congenitally infected babies who are at higher risk of SNHL and psychomotor retardation, who can then be monitored carefully with audiological and neurodevelopmental assessments. At present, antiviral therapy with (val)ganciclovir is restricted to neonates with symptomatic congenital CMV disease involving the central nervous system, and it is administered in the first month of life to prevent progressive hearing loss and neurodevelopmental retardation at 1–2 years of age [17]. Antiviral therapy is not administered to infants who present with disease later in life. An early prognosis, identifying babies that are at high risk of late-onset sequelae, could be crucial for planning potential antiviral treatment to prevent the disease in asymptomatic newborns.

The possibility of a threshold (ie, nonlinear) relationship between CMV-related disease and viral load was first implied by the results of Stagno et al in 1975, using cell culture, and was confirmed by Walter et al in 2007, using PCR of dried blood.

Figure 1. Kaplan–Meier analysis of the time course of viremia (A) and viruria (B) clearance in 33 babies with congenital cytomegalovirus infection.

Figure 2. Logistic regression analysis of the risk of developing postnatal sequelae in relation to cytomegalovirus (CMV) DNAemia at birth. The gray area denotes the 95% confidence interval.
spots (DBS) [18, 19]. The latter observed that, in 34 children with congenital CMV infection, the risk of SNHL increased with DBS viral load [19].

Nevertheless, contrasting data exist about the association between high viral load in blood in early infancy and risk of hearing loss. Bradford et al observed that an increase in viral load in blood was not more predictive of hearing loss than qualitative identification of viremia [7]. However, in their study population only symptomatic newborns with congenital CMV infection involving the central nervous system were considered [7].

In a cohort of 58 asymptomatic infants with congenital CMV examined by Boppana et al, babies with SNHL had a significantly greater amount of CMV in urine and blood during infancy than those with normal hearing [5]. In 2009, Ross et al expanded this cohort of asymptomatic infected children. By studying children <2 months of age they found that the median CMV DNAemia was not different between children with SNHL and the group of children with normal hearing [6].

The different results obtained in our study could be due to the timing of blood sample collection from newborns, which was done in the first days of life.

Our results reveal also that the CMV load is associated with late-onset disease in congenital CMV infection. Consequently, it would be worth investigating the presence of the virus in the inner ear fluid in children with sensorineural hearing loss from larger populations [20].

In conclusion, we analyzed in depth the kinetics of CMV clearance in blood and urine and the viral load in blood at birth in asymptomatic congenitally infected infants born to women with primary CMV infection during pregnancy. These observations are important for the elucidation of CMV infection resolution, which raises epidemiological, social and prophylactic issues.

Notes

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Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References


