Congenital Cytomegalovirus Infection: Prognostic Value of Maternal DNAemia at Amniocentesis

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Background. Human Cytomegalovirus (HCMV) is the most common cause of childhood hearing loss and can lead to neurodevelopmental delay. The aim of our study was to ascertain if HCMV DNA in the peripheral blood of pregnant women with primary HCMV infection at the time of amniocentesis may have a prognostic value in terms of congenital infection and neonatal symptomatic disease.

Methods. We performed a prospective observational study of pregnant women referred to our maternal-fetal medicine division with suspected HCMV infection. Primary infection was diagnosed based on seroconversion for HCMV and/or HCMV immunoglobulin M–positive and low or moderate HCMV immunoglobulin G avidity. At the time of amniocentesis, maternal blood samples were collected and analyzed by means of real-time polymerase chain reaction to determine the presence of viral DNAemia. Fetuses and newborns were evaluated for the presence of congenital infection and symptomatic disease.

Results. A total of 239 pregnant women were enrolled; 32 blood samples (13.4%) were positive, and 207 (86.6%) were negative for HCMV DNA. The overall rate of transmission was 23.4%. Fifteen infected patients (26.8%) were symptomatic. Vertical transmission occurred in 14 women (43.8%) with positive and 42 (20.3%) with negative results for HCMV DNAemia (P = .006; odds ratio, 3.06; 95% confidence interval, 1.41–6.64). Symptomatic infection occurred in 6 (42.9%) infected fetuses or newborns from women with and in 9 (21.4%) from women without viral DNAemia (P = .16).

Conclusion. Maternal viremia at amniocentesis is associated with a 3-fold greater chance of congenital infection, but it is not correlated with symptomatic disease.

Keywords. Human Cytomegalovirus Primary Infection in Pregnancy; congenital infection; maternal viremia; prenatal diagnosis; symptomatic congenital disease.

Human cytomegalovirus (HCMV) is a leading cause of congenital infections in developed countries, where it is the most common nongenetic cause of childhood hearing loss and an important cause of neurodevelopmental delay [1–3]. The incidence of primary HCMV infection during pregnancy is approximately 1%–7% [4, 5], with a vertical transmission risk from 14.2% to 52.4% (combined prevalence, 32.4%) [5, 6]. Symptoms are present at birth in 10%–15% of congenitally infected infants, with a perinatal mortality rate of about 10% and a 70%–80% risk of major neurological sequelae. Despite infection, 85%–90% of infants have no symptoms at birth, but 10%–15% will suffer delayed injury [3, 7, 8].

Amniocentesis, performed after 20 weeks [9] of gestation and at least 6–8 weeks after maternal infection [10], can identify infected fetuses with high sensitivity and specificity rates [11–16], but it does not provide information on the clinical features of neonatal infection. Unfortunately, the prognosis for an infected fetus with either no ultrasonographic (US) features or nonsevere US anomalies is difficult to establish until late in pregnancy, and some prognostic laboratory parameters require the collection of fetal blood by an invasive procedure [17].

In the past, HCMV DNA in maternal blood at the time of amniocentesis was investigated as a risk factor for iatrogenic transmission of infection [18, 19]. These studies showed that invasive procedures performed in pregnant women with viral DNAemia do not represent a significant risk factor for iatrogenic transmission of HCMV to the fetus.

To date, few studies have examined the correlation between maternal viremia and congenital HCMV infection [18, 20]. The aim of our study was to ascertain whether DNAemia detected in the peripheral blood of pregnant women with primary HCMV infection at the time of amniocentesis has a prognostic value in terms of congenital infection and neonatal symptomatic disease.
METHODS

Study Design
This is a prospective observational study of all consecutive pregnant women referred to our maternal-fetal medicine division with primary HCMV infection from January 2007 to December 2014. In all cases, HCMV-specific immunoglobulin (Ig) M and IgG were redetermined by means of automated chemiluminescence immunoassay, followed by IgM confirmation by immunoblot combined with determination of anti-HCMV IgG avidity [21–23]. Primary infection was diagnosed and precisely dated based on seroconversion for HCMV and/or HCMV IgM-positive and low or moderate HCMV IgG avidity results and clinical and laboratory history. We excluded from the study patients <18 years old, those whose pregnancies ended in miscarriage or abortions during the first trimester, those refusing prenatal invasive diagnosis and/or enrolled in the Congenital HCMV Infection Prevention (CHIP) trial [24], those who had undergone hyperimmune globulin administration at other hospitals, and those lost to follow-up. Detailed counseling was provided on fetal risks, the advisability of US monitoring, and the possibility of prenatal invasive diagnosis.

Experimental Procedures
After informed written consent, maternal whole-blood samples were collected at the time of amniocentesis in all pregnant women >18 years of age with a diagnosis of primary HCMV infection. Amniocentesis was performed in all patients after 20 weeks of gestation and ≥6 weeks after maternal infection. Amniotic fluids were subjected to a direct search for HCMV in culture and for the viral genome by real-time polymerase chain reaction (PCR). Maternal whole-blood samples were analyzed with real-time PCR to determine viral DNAemia [25].

Outcome Measurement
With respect to neonatal outcome, newborns were classified as uninfected or infected on the basis of virus isolation and/or real-time PCR using urine samples obtained within the first 2 weeks of life. Aborted fetuses were classified as infected in the presence of positive immunohistochemical staining for HCMV early antigen (ppUL44) performed on placenta and fetal tissue [26].

Symptomatic or asymptomatic status at birth or at neonatal follow-up relied on a combination of clinical examination, laboratory assessment, audiometric assessment by automated auditory brain-stem response, and cerebral imaging following the classification used in the study by Lanari et al [27]. Second trimester aborted and intrauterine dead fetuses were analyzed by means of macroscopic, histological, and immunohistochemical characterization. Fetuses were defined as symptomatic following the classification reported elsewhere by Gabrielli et al [26]. Final results were analyzed according to the number of weeks elapsed between maternal infection and prenatal invasive diagnosis.

Ethics
The study was carried out following the ethical rules of St Orsola-Malpighi Hospital, Bologna, Italy. Informed written consent was requested from all enrolled pregnant women, and the study protocol was approved by the Ethics Committee of St Orsola Malpighi Hospital. The study was also conducted according to the principles of the Declaration of Helsinki.

Statistical Analysis
Median, range, and absolute and relative frequencies were used as descriptive statistics. The Fisher exact test was applied to analyze dichotomous data, and odds ratios (ORs) were computed together with their 95% confidence intervals (CIs). Data were managed and analyzed using the IBM SPSS Statistics software package (version 23), and differences were considered statistically significant at 2-tailed P < .05.

RESULTS
Primary HCMV infection was diagnosed in 606 pregnant women between January 2007 and December 2014. After 365 patients were excluded (Figure 1), 239 (39.4%) pregnant women were enrolled in the study (median age, 32 years; range, 18–44 years). The onset of primary HCMV infection was precisely dated in all but 3 of these patients (1.3%). Of the 236 dated infections, maternal primary infection occurred in the periconceptional period (from 6 weeks before to 3 weeks after the date of last menstrual period) in 28 women (11.9%), in the first 13 weeks of gestation in 182 (77.1%), and between 14 and 23 weeks of gestation in 26 (11.0%) [28]. In the 236 pregnant women with dated infection, the invasive procedure was performed 6–8 weeks after maternal infection in 42 patients (17.8%) and later than 8 weeks in 194 (82.2%). Enrolled pregnancies resulted in

![Figure 1. Study population.](https://academic.oup.com/cid/article-abstract/64/2/207/2811180)
222 newborns (92.9%) and 17 terminations of pregnancy (7.1%) in the second trimester.

HCMV DNAemia was analyzed in 239 pregnant women with a diagnosis of primary HCMV infection who underwent amniocentesis; 32 samples (13.4%) were positive and 207 (86.6%) negative for HCMV DNA. The viral loads in maternal whole-blood samples were very low, ranging from <500 to 900 copies/mL. Viral DNA was detected in 39 amniotic fluid samples (16.3%); 12 (37.5%) were found in the 32 women with and 27 (13.0%) in 207 women without viremia ($P = .001$; OR, 4.00; 95% CI, 1.76–9.10).

Congenital infection was diagnosed after birth or abortion in 56 cases (39 newborns and 17 fetuses), with an overall HCMV transmission rate of 23.4%. Vertical transmission occurred in 14 of 32 pregnant women (43.8%) with viremia and in 42 of 207 (20.3%) without HCMV DNAemia ($P = .006$). HCMV DNA in maternal blood showed an OR of 3.06 (95% CI, 1.41–6.64) for vertical transmission; thus, sensitivity, specificity, and positive and negative predictive values were 25.0% (14 of 56), 90.2% (165 of 183), 43.8% (14 of 32), and 79.7% (165 of 207), respectively.

Congenital infection was confirmed in all 39 pregnancies with amniotic fluid positive for HCMV DNA, whereas of the 200 pregnancies (83.7%) with negative amniotic fluid, the absence of infection was confirmed at birth in 183 newborns (91.5%). Among 17 cases of discordant results between amniotic fluid and postnatal urine tests, only 2 pregnant women had HCMV DNAemia (11.8%). Thus, there was no significant difference ($P > .99$) in the proportion of false-negative prenatal diagnosis between the women with (2 of 32; 6.3%) and those without DNAemia (15 of 207; 7.2%).

Fifteen of the 56 infected aborted fetuses or newborns (26.8%; 8 fetuses and 7 newborns) were defined as symptomatic at autopsy or pediatric follow-up, and the remaining 41 (9 fetuses and 32 newborns) were asymptomatic (73.2%). The frequency of symptomatic disease was significantly higher in the aborted fetuses than in the newborns (47.1% vs 17.9%; $P = .046$; OR, 4.06; 95% CI, 1.16–14.3). Symptomatic infection occurred in 6 of 14 infected fetuses or newborns (42.9%) from women with viral DNAemia at the time of amniocentesis and in 9 of 42 (21.4%) from women without viral DNAemia at that time ($P = .16$; OR, 2.75; 95% CI, 0.76–9.99).

Regarding the time elapsed between maternal infection and amniocentesis, HCMV maternal viremia and vertical transmission were found in 9 and 12 of the 42 patients with an interval of 6–8 weeks (21.4% and 28.6%), respectively, and in 23 and 44 of the 194 with an interval of >8 weeks (11.9% and 22.7%) (OR for viremia, 2.03 [95% CI, 0.86–4.77; $P = .13$]; OR for vertical transmission, 1.36 [0.65–2.99; $P = .43$]). In particular, congenital infection was significantly higher in women with viremia (11 of 23; 47.8%) than in those without viremia (33 of 171; 19.3%) when amniocentesis was performed >8 weeks after maternal infection (OR, 3.83; 95% CI, 1.56–9.45; $P = .006$), whereas no significant difference was found when amniocentesis was performed earlier (3 of 9 [33.3%] vs 9 of 33 [27.3%]; OR, 1.33 [95% CI, 0.27–6.50; $P = .70$]). The sensitivity, specificity, and positive and negative predictive values for viremia in later invasive procedures were 25.0% (11 of 44), 92.0% (138 of 150), 47.8% (11 of 23), and 80.7% (138 of 171), respectively.

**DISCUSSION**

This study aimed to ascertain if maternal viremia at the time of amniocentesis in women with primary HCMV infection has a prognostic value in terms of congenital infection and neonatal symptomatic disease. HCMV DNA in maternal blood has been investigated as an important—but not clarifying—element to diagnose primary infection, secondary to serological investigation [19]. Data from the literature indicate that viral DNA can be detected in maternal blood within 1 month after the onset of primary HCMV infection and could be present in about 50% and 25% of pregnant women 3 and 6 months after infection [11]. Other studies have evaluated the role of maternal viremia at the time of amniocentesis. A 1998 study by Revello and colleagues [18] did not find any prognostic value in a small cohort of pregnant women ($n = 23$) in which the percentages of infected and uninfected fetuses or newborns were similar for women with or without circulating virus. They subsequently failed to find any correlation between maternal viremia and fetal infection in 132 pregnant women who underwent amniocentesis after primary infection. DNAemia was detected in 27 (37%) of 73 of pregnant women with HCMV-negative amniotic fluid and in 22 (37%) of 59 of women with HCMV-positive amniotic fluid, suggesting that maternal DNAemia was unrelated to detection of virus in the amniotic fluid. This finding was also confirmed with regard to postnatal diagnosis [19].

Our data diverge from previous reports. In our sample, PCR DNA performed at the time of amniocentesis seems to be significantly correlated with congenital infection. Maternal viremia was associated with a 3-fold greater chance of vertical transmission. The exact reason for this correlation is still not known and could suggest that fetal infection was related to the invasive procedures, but we can rule out any iatrogenic effect for the following reasons. First, the unexpected number of infected newborns: only 2 (11.8%) of the 17 false-negative amniocentesis results were observed in women with viremia, compared with 15 infected newborns for whom HCMV PCR results were negative in both amniotic fluid and maternal blood. Second, during the study period, 189 women with primary infection declined amniocentesis; vertical transmission occurred in 61 (32.3%) of these newborns, with no significant difference from findings in pregnant women who underwent amniocentesis ($P = .06$). Finally, the vertical transmission rate in our cohort is similar to that reported in the literature.

We do not think viremia should delay amniocentesis. Viremia could persist even if amniocentesis were delayed. In our study, amniocentesis was performed after an interval of 6–8
weeks in 42 cases and after >8 weeks in 194 cases. Viremia was found in 9 patients (21.4%) in the first group and 23 (11.9%) in the second group \((P = .13)\). Vertical transmission was similar in both groups, occurring in 28.6% fetuses or newborns in the first group versus 22.7% in the second, but the rate was higher in women with viremia when amniocentesis was performed >8 weeks after maternal infection \((OR, 3.83)\). This result could appear surprising and indicates that viremia should not influence the timing of amniocentesis.

Our findings could have a practical impact on patient counseling. Viral DNAemia could help predict the risk of fetal infection to enable better counseling of women with primary infection and help them decide on the need for an invasive procedure. This strategy may reduce both the use of invasive procedures and the proportion of women faced with anxiety. The absence of maternal viremia around the time scheduled for amniocentesis could reassure pregnant women unwilling to undergo an invasive procedure, particularly when the time elapsed between maternal infection and planned amniocentesis is >8 weeks.

In conclusion, this is the largest study investigating the correlation between maternal viremia and congenital infection and the first to evaluate the implications of infection on symptomatic congenital disease. Maternal HCMV viremia at the time of amniocentesis is significantly correlated with congenital infection, but our results rule out an iatrogenic risk, confirming that 6 weeks is a sufficient interval between maternal infection and amniocentesis for accurate diagnosis of fetal HCMV infection. If our results are confirmed by future studies, the search for viral DNA in maternal blood could contribute to a noninvasive assessment of the risk of fetal infection. This would be particularly welcome for those women unwilling to undergo an invasive procedure.

Notes

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