OBSTETRICS

Feasibility of predicting the outcome of fetal infection with cytomegalovirus at the time of prenatal diagnosis

Marianne Leruez-Ville, MD; Julien Stirnemann, MD; Yann Sellier, MSc; Tiffany Guilleminot, BA; Anne Dejean, MSc; Jean-François Magny, MD; Sophie Couderc, MD; François Jacquemard, MD; Yves Ville, MD

BACKGROUND: Congenital cytomegalovirus infection occurs in 0.7% of live births with 15-20% of infected children developing long-term disability including hearing loss and cognitive deficit. Fetal cytomegalovirus infection is established by viral DNA amplification by polymerase chain reaction in amniotic fluid obtained by amniocentesis following maternal seroconversion or after the diagnosis of ultrasound features suggestive of fetal infection. Severe brain ultrasound anomalies are associated with a poor prognosis. The prognosis of an infected fetus showing either no ultrasound features or nonsevere ultrasound anomalies is difficult to establish up until late in the second or third trimester of pregnancy.

OBJECTIVE: We sought to evaluate the prognostic value of fetal ultrasound, amniotic fluid, and fetal blood analysis at the time of prenatal diagnosis of fetal infection.

STUDY DESIGN: We reviewed all cases of fetal cytomegalovirus infection with a sample of amniotic fluid positive for viral DNA and/or fetal blood analyzed in our laboratory from 2008 through 2013. Prenatal ultrasound features along with cytomegalovirus DNA loads in amniotic fluid and in fetal blood and fetal platelet counts were reviewed in relation to gestational age at maternal infection, neonatal examination, and postnatal follow-up or postmortem examination.

RESULTS: In all, 82 fetuses were infected following maternal infection mainly in the first trimester. At the time of prenatal diagnosis at a median of 23 weeks, 19, 22, and 41 fetuses showed severe brain ultrasound abnormalities, nonsevere ultrasound features, and normal ultrasound examination, respectively. Nonsevere ultrasound features, higher DNA load in amniotic fluid, fetal platelet count $\leq$114,000/mm^3, and DNA load $\geq$4.93 $\log_{10}$ IU/mL in fetal blood were associated with a symptomatic status at birth in univariate analysis ($P < .001$, $P = .001$, and $P = .018$, respectively). Bivariate analysis combining ultrasound results and either adjusted viral load in amniotic fluid or fetal blood profile showed that these were independent prognostic factors of a symptomatic status at birth. Both fetal blood parameters were better predictors than amniotic fluid viral load. At the time of prenatal diagnosis, the ultrasound negative predictive value for symptoms at birth or at termination of pregnancy was 93%. The combined negative predictive values of ultrasound and viral load in amniotic fluid and that of ultrasound and fetal blood parameters were 95% and 100%, respectively. In fetuses presenting with nonsevere ultrasound features, the positive predictive values of ultrasound alone and in combination with amniotic fluid viral load or with fetal blood parameters were 60%, 78%, and 79%, respectively.

CONCLUSION: Risk assessment of infected fetuses for being symptomatic at birth is possible as early as the time of diagnosis by using a combination of targeted ultrasound examination along with viral load in amniotic fluid and in fetal blood together with platelet count. The advantage of using amniotic fluid is that it is available at prenatal diagnosis. One may wonder if increasing the negative predictive value of the overall assessment of an infected fetus from 95-100% is worth the additional risk of cordocentesis for fetal blood sampling. This can only be an individual decision made by well-informed women and it seems therefore appropriate to use the figures presented here and their confidence intervals for counseling.

Key words: cytomegalovirus, fetal DNA, fetal platelet count, prenatal diagnosis, ultrasound

Introduction

Congenital cytomegalovirus (CMV) infection occurs in 0.7% of live births[1] and 15-20% of infected children develop long-term disability including hearing loss (HL) and cognitive deficit.[2] Fetal CMV infection can be proven by viral DNA amplification in the amniotic fluid (AF) obtained by amniocentesis following documented maternal seroconversion or suggestive ultrasound (US) features.[3-10] When severe brain US anomalies are present, the prognosis is poor.[11,12] The prognosis of an infected fetus showing either no US features or nonsevere US anomalies is difficult to establish up until late in the second or third trimester of pregnancy. However, prognostic assessment at 20-24 weeks can be crucial, particularly in countries where termination of pregnancy (TOP) is not allowed at a later stage in pregnancy, but also to consider fetal treatment.[13]

We aimed to evaluate the prognostic value of fetal US together with that of AF and fetal blood analysis at the time of diagnosis in a series of proven fetal CMV infections.

Materials and Methods

Study population

Fetuses with a prenatal diagnosis of CMV infection and at least 1 prenatal sample of AF and/or fetal blood analyzed for CMV quantitative polymerase chain reaction (PCR) in our laboratory from 2008 through 2013 were included in the study. All cases were from the Paris area and were followed up at the fetal medicine unit of Hôpital Necker-Enfants Malades either from the beginning of the pregnancy or after referral for a positive CMV PCR in AF diagnosed in

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TABLE 1
Classification of ultrasound abnormalities

Severe US brain abnormalities	Mild US brain abnormalities	Extracerebral US abnormalities
Ventriculomegaly ≥15 mm	Mild ventriculomegaly (>10–15 mm)	Hyperechogenic bowel[41]
Periventricular hyperechogenicity	Intraventricular adhesions	Hepatomegaly (left lobe ≥40 mm)[40]
Hydrocephalus	Intracerebral calcifications	Splenomegaly (longest diameter ≥40 mm)[42]
Microcephaly < −2SD	Subependymal cysts	Intrauterine growth retardation (<5th centile)
Increased cisterna magna ≥8 mm	Choroid plexus cysts	Oligoamnios (deepest vertical pool <2.5 cm)
Vermian hypoplasia	Calcifications of lenticulostriate vessels in basal ganglia	Polyhydramnios (deepest vertical pool >10 cm)
Porencephaly		Ascites
Lissencephaly		Pleural effusion
Periventricular cystic lesions of white matter		Fetal hydrops, subcutaneous edema
Agenesis of corpus callosum		Placentomegaly ≥40 mm[43]
		Intrahepatitic calcifications

US, ultrasound.
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another center. Prenatal data were reviewed including time of maternal primary infection, fetal serial US, magnetic resonance imaging (MRI) examination, and follow-up. Outcome was assessed by targeted neonatal examination or postmortem examination following TOP.

Evaluation of maternal infection type and date of maternal primary infection

All available sera were analyzed retrospectively or prospectively for CMV IgG, CMV IgM, and CMV IgG avidity. The LIAISON CMV IgG, CMV IgM, and CMV IgG avidity assays were used until October 2012 (DiaSorin, Antony, France). From October 2012, the LIAISON XL CMV IgG, CMV IgM, and CMV IgG avidity assays were used (DiaSorin). The assessment of the onset of primary infection was based on clinical symptoms. In their absence, the onset was set arbitrarily halfway between the date of the last negative serum sample and the first positive one. In addition, an IgG avidity test of <10% was considered consistent with an onset of infection within the previous 3 weeks.[14] Prenatal diagnosis following maternal primary infection was achieved by amniocentesis performed at least 6 weeks following seroconversion and not earlier than 20 weeks' gestation.[3]

Quantitative CMV PCR assay

DNA extraction was performed from 200 μL of AF with the MagNaPure LC using the total nucleic acid extraction kit (Roche Diagnostic, Meylan, France). DNA extraction was performed from 200 μL of fetal whole blood using the QiaAmp DNA mini blood kit (Qiagen, les Ulis, France). DNA extracts from fetal blood were amplified both undiluted and 1:10 diluted. DNA amplification used a real-time commercial quantitative CMV PCR assay (CMV-R Gene, Argene BioMerieux, Marcy l'Etoile, France). Results were expressed in copies/mL and in IU/mL in AF and in fetal blood samples, respectively. The results obtained in whole blood with the Argene commercial test were calibrated to an international reference standard made available since 2010.[15]

Prenatal follow-up of infected fetuses

All infected fetuses were followed up in our fetal medicine unit. Amniocentesis was performed following either maternal primary infection or the diagnosis of suggestive US features. Fetal blood sampling by cordocentesis to check for fetal platelets and fetal viremia was offered in cases with an infected fetus (positive CMV PCR in AF).[16]

Infected fetuses were followed up by US every 2-3 weeks. US features suggestive of fetal CMV infection were recorded as part of serial targeted fetal US examination. Ventriculomegaly was defined as increased measurement of lateral ventricles at level of glomus. Hydrocephalus was defined as triventricular or quadriventricular dilatation in relation with microencephaly in this case. The right lobe of liver was measured in parasagittal plane as described in Vintzileos et al.[40] Measurement >40 mm in second trimester is considered abnormal and as fetuses were examined at mean of 23 wk, this cut-off was chosen accordingly (see video 1). Hyperechogenic bowel was only considered when echogenicity of bowel was equal or more intense than that of fetal bones (see video 2).[41] Fetal cerebral MRI was performed at 32-34 weeks of gestation or earlier if US examination suspected brain lesions. Brain anomalies were subdivided into severe and mild (Table 1). Fetuses were classified as having severe US abnormalities when showing at least 1 severe brain abnormality. Fetuses were classified as having