

OBSTETRICS

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



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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 $\leq 114,000/\text{mm}^3$, 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 births¹ 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 (<5 th 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
		Intrahepatic 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 μ L of AF with the MagNaPure LC using the total nucleic acid extraction kit (Roche Diagnostic, Meylan, France). DNA extraction was performed from 200 μ L 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

nonsevere US abnormalities when showing at least 1 extracerebral abnormality or 1 mild brain abnormality (Table 1). TOP was discussed for fetuses with cerebral abnormalities on US or on MRI.

Antenatal treatment

Some women with an infected fetus presenting with nonsevere US anomalies, fetal viremia >3000 copies/mL, or fetal platelets $<100,000/\text{mm}^3$ were given valacyclovir orally (8 g per day) from the time of prenatal diagnosis up until delivery as part of an ongoing clinical trial (CYMEVAL II: NCT 01651585).

Classification in symptomatic and asymptomatic infection at birth

Symptomatic or asymptomatic status at birth relied on a combination of clinical examination, laboratory assessment (platelet count, liver enzymes, and serum bilirubin levels), audiometric assessment by automated auditory brainstem response, and cerebral imaging following the classification recently used in the study from Kimberlin et al.¹⁷ Babies with normal clinical examination findings, normal laboratory assessment, normal hearing, and normal imaging were classified into the asymptomatic group. Babies with abnormal clinical examination findings (intrauterine growth restriction [IUGR], petechiae, purpura, microcephaly, seizures, lethargy/hypotonia, poor suck, hepatosplenomegaly, hearing loss); severe abnormal cerebral imaging (multiple intracranial calcifications, periventricular hyperechogenicity, or severe ventriculomegaly [>15 mm]); and/or abnormal laboratory assessment (thrombocytopenia) were classified into the symptomatic group. Neonates presenting with normal clinical examination and only mild abnormalities at cerebral imaging including mild ventriculomegaly (<15 mm), pseudocysts in the germinal matrix, or echogenic lenticulostriated vessels were not considered as symptomatic.

An autopsy was performed in all cases of TOP, and fetuses were classified as symptomatic when overt

cerebral abnormalities such as microcephaly, ventriculomegaly, necrosis of the white matter in association with diffuse lesions of vasculitis, and encephalitis were seen at fetal brain histopathology.

Statistical analysis

Univariate logistic regression models were used to investigate the association between outcome (symptomatic or asymptomatic at birth) and each one of the following parameters as obtained within 1-2 weeks of prenatal diagnosis: US features, viral load in AF and in fetal blood, and fetal platelet count. Bivariate logistic regression models were based on combinations of US features and laboratory parameters obtained in AF (viral load) and in fetal blood (viral load and platelet count).

To better assess the prognostic value of fetal blood parameters, the combined value of fetal blood viral load and platelet count was studied using a recursive partitioning model.

CMV DNA load in AF has been shown to increase with gestational age¹⁸; therefore viral load in AF was adjusted to the time interval between seroconversion and amniocentesis by linear regression and presented in multiples of the median.

All tests were 2-tailed and a P value $<.05$ was considered statistically significant.

Ethics

All women gave written consent for the results of their screening tests to be used anonymously for research purposes. All women who underwent amniocentesis gave written consent for CMV prenatal diagnosis. According to French laws, institutional review board was obtained for offering prenatal treatment using valacyclovir for fetal infection (2011-001610-34).

Results

Population (Figure 1)

In all, 92 fetuses were diagnosed with CMV infection from 2008 through 2013 with at least 1 antenatal AF and/or fetal blood sample analyzed in our laboratory. Of these, 10 were not considered for

analysis because the status of the neonate was incompletely documented (5 cases) or because autopsy was not performed following TOP (5 cases). Among the 82 cases included for analysis, quantitative CMV PCR was done in our laboratory in AF, fetal blood, and in both AF and fetal blood in 65, 65, and 49 cases, respectively. Some cases had only an AF sample studied in Hôpital Necker-Enfants Malades laboratory because cordocentesis was declined. Other cases had only a fetal blood sample analyzed in our laboratory because amniocentesis and CMV PCR were performed in another center, and the patient with an infected fetus was referred to Hôpital Necker-Enfants Malades for further counseling and management.

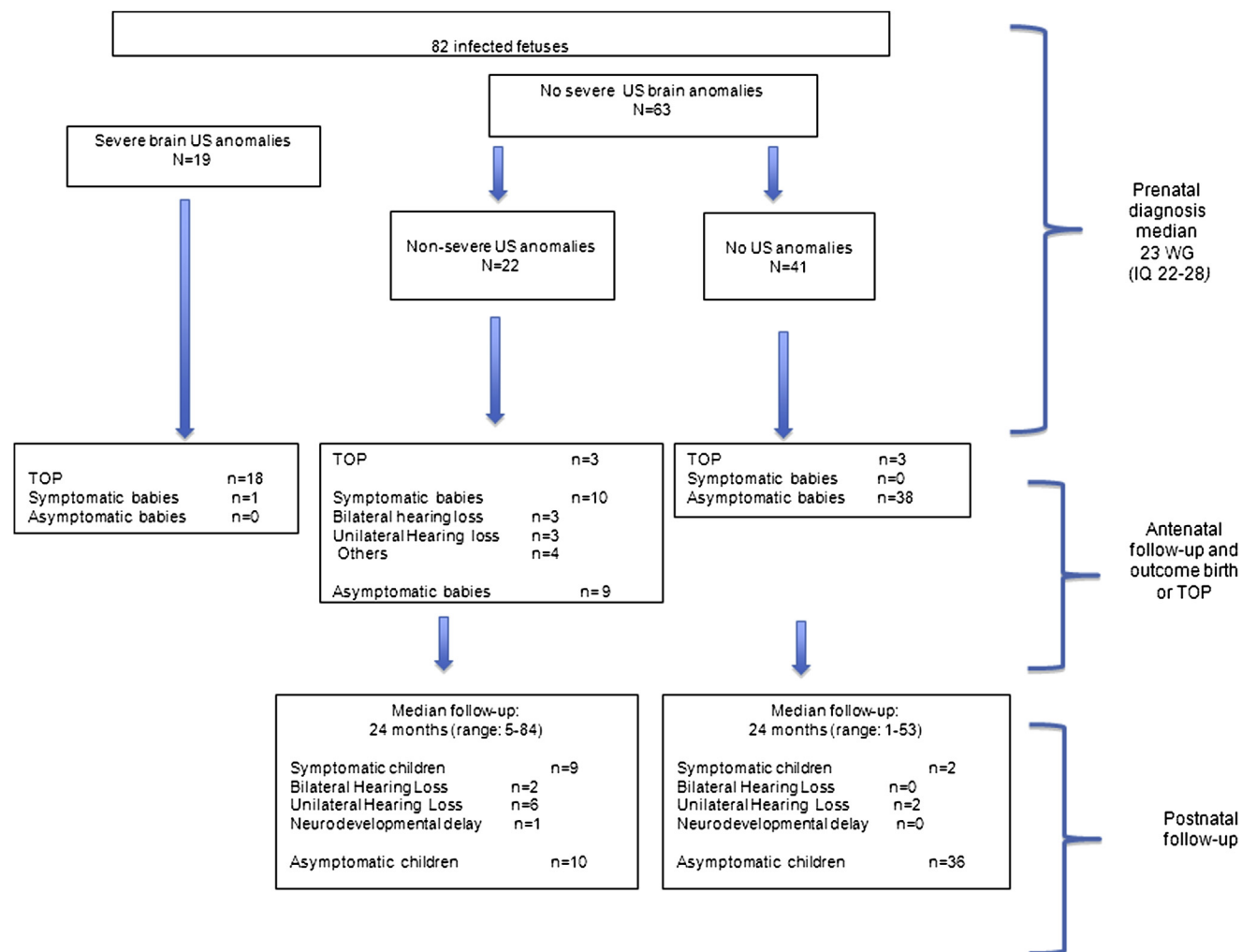
In all, 81 of these 82 cases of fetal infection followed maternal primary infection in the first, second, and third trimester in 62, 13, and 4 cases, respectively. The time of the primary maternal infection was unknown in 2 cases and 1 case arose from nonprimary maternal infection.

US abnormalities at the time of prenatal diagnosis (Figure 1)

The median gestational age at amniocentesis was 23 (interquartile range 22-28) weeks. At the time of prenatal diagnosis, brain abnormalities were seen on US in 19 of 82 cases. Among the 63 other cases, 41 presented without any US features but 22 cases presented with at least 1 nonsevere US abnormality (Table 2). In 12 of 22 cases there was only 1 isolated US abnormality including hyperechogenic bowel, IUGR, and hepatomegaly and splenomegaly in 8, 3, and 1 case, respectively. In the other 10 cases there was a combination of 2-3 nonsevere US abnormalities.

Antenatal treatment

In the group of 63 cases without severe brain US abnormalities, 37 women received valacyclovir orally, including 18 (44%) of 41 cases without any US features and 19 (86%) of 22 cases with nonsevere US abnormalities. In this population, prenatal valacyclovir treatment was not associated with a better prognosis ($P = 1.0$).

FIGURE 1
Description of population

IQ, interquartile range; *TOP*, termination of pregnancy; *US*, ultrasound; *WG*, weeks of gestation.

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Prenatal and postnatal follow-up (Figure 1)

TOP was performed in 18 of the 19 cases with severe brain abnormalities diagnosed by US at the time of prenatal diagnosis. All 18 terminated fetuses were classified as symptomatic at autopsy; the only live-born baby in this group was severely symptomatic and is now severely handicapped.

Among the 41 cases without any US features at the time of prenatal diagnosis, 38 had an uneventful prenatal follow-up leading to the birth of 38 asymptomatic neonates, while 3

subsequently developed severe brain US abnormalities in the late second or third trimester of pregnancy leading to TOP. Of these 38 babies, 36 remained asymptomatic at follow-up and 2 developed delayed unilateral HL at the age of 18 and 24 months, respectively.

In the group of 22 cases with non-severe US abnormalities at the time of prenatal diagnosis, 3 developed severe brain lesions leading to TOP. Nineteen babies were born alive; 9 of them were asymptomatic and remained so at ages up to 6-84 months except for 1 who

developed mild unilateral HL after the age of 1 year. Ten babies were symptomatic at birth with unilateral hearing loss (3 cases), bilateral hearing loss (3 cases), isolated IUGR (1 case), isolated thrombocytopenia (1 case) and an association of thrombocytopenia and IUGR (2 cases). Two of the 4 neonates with normal audiometry at birth developed unilateral HL in the second year of life. One child was born with isolated thrombocytopenia and showed both motor and cognitive developmental delay at the age of 26 months.

Contribution of fetal laboratory parameters in the group of 63 cases without severe brain US abnormalities at the time of prenatal diagnosis

Figure 2, A, shows that CMV DNA levels in AF increased with the time interval from seroconversion to amniocentesis and those were therefore adjusted accordingly and expressed in multiples of the median in subsequent statistical analyses. Distribution of CMV DNA loads in fetal blood as well as that of fetal platelet count according to symptomatic or asymptomatic status at birth or at TOP are shown in Figure 2, B.

Recursive partitioning model applied to the combined interpretation of CMV DNA load and platelet count in fetal blood showed that if the platelet count was $\leq 114,000/\text{mm}^3$ there was a 62.5% risk of a symptomatic status at birth or at TOP and if the platelet count was $>114,000/\text{mm}^3$ the risk of a symptomatic status was 57% in cases with CMV DNA load $\geq 4.93 \log_{10}$ IU/mL in fetal blood but dropped to 3.1% if the viral load was $<4.93 \log_{10}$ IU/mL (Figure 3). Based on this model, the fetal blood profile was classified as abnormal when platelet count was $\leq 114,000/\text{mm}^3$ or CMV DNA level was $\geq 4.93 \log_{10}$ IU/mL.

Univariate analysis showed that any one of the following—presence of nonsevere US features, higher viral load in AF, higher viral load in fetal blood, lower fetal platelet count, or abnormal fetal blood profile—was significantly associated with a symptomatic status at birth or at TOP ($P < .001$, $P = .018$, $P = .001$, $P = .002$, and $P = .001$, respectively). Bivariate analysis of US assessment combined with either adjusted viral load in AF or fetal blood profile showed that the 3 factors were independently predictive of the status at birth or at TOP (Table 2).

Receiver operating characteristic curve analysis comparing adjusted CMV DNA load in AF with CMV DNA load in fetal blood and with fetal platelet count showed that both fetal blood parameters were better predictors of the status

TABLE 2

Prognostic factors of symptomatic status at birth or at termination of pregnancy in 63 fetuses presenting with no ultrasound features or nonsevere ultrasound features at time of prenatal diagnosis at 23 (interquartile range 22–28) weeks

	Logistic regression		
	OR	95% CI	Pvalue
Univariate model			
CMV DNA in fetal blood (for each 1 log of IU/mL increase) N = 54	5.77	2.02–16.53	.001
Platelet count (per each 10,000/ mm^3 increase) N = 49	0.74	0.60–0.89	.002
Presence of nonsevere US symptoms N = 63	18.29	4.29–78.04	<.001
Abnormal fetal blood results N = 50	40.44	4.64–352.72	.001
Adjusted CMV DNA in amniotic fluid (for each 1 log of IU/mL increase) N = 48	2.31	1.15–4.64	.018
Bivariate model			
Presence of nonsevere US symptoms	10.45	1.96–55.63	.006
Adjusted CMV DNA in amniotic fluid (for each 1 log of IU/mL increase) N = 48	2.35	1.09–5.08	.03
Abnormal fetal blood results	17.76	1.92–164.02	.011
Presence of nonsevere US symptoms N = 50	5.36	1.01–28.53	.049

Complete laboratory data were not available for all cases. Some amniocenteses were done in another center and therefore amniotic fluid sample was not tested in Hôpital Necker-Enfants Malades laboratory and some women declined cordocentesis.

Abnormal fetal blood results = platelet count $\leq 114,000/\text{mm}^3$ and/or CMV DNA load $\geq 4.93 \log_{10}$ IU/mL.

CI, confidence interval; CMV, cytomegalovirus; OR, odds ratio; US, ultrasound.

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at birth or TOP than AF viral load (Figure 4).

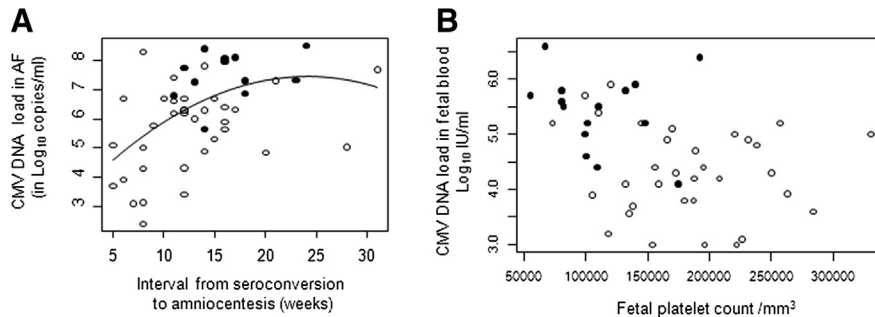
Positive predictive value (PPV) and negative predictive value (NPV) of nonsevere US features alone or combined with fetal laboratory parameters are reported in Table 3.

In our study, asymptomatic neonates have a good prognosis since none developed neurological impairment or bilateral HL. We could not make any statistical correlation between fetal parameters and long-term outcome because the length of postnatal follow-up was not homogeneous within the cohort with 7 asymptomatic neonates lost to follow-up: 3 were not seen after birth and 3 were seen for the last time at the 4-month visit with 21 children followed up for <24 months.

Comment

Congenital CMV infection is a burden affecting 0.7% of all neonates worldwide.² Prenatal diagnosis is feasible and usually performed either as a result of screening for maternal primary infection in pregnancy, mainly in the first and early second trimester of pregnancy,^{14,19–21} or upon the discovery of suggestive US features in the fetus.^{16,22–25} However, since only around 10% of infected fetuses will be symptomatic at birth, the prognosis remains difficult to establish early enough for considering TOP or prenatal treatment in those cases likely to become severely symptomatic in the third trimester of pregnancy or at birth. The originality of the present study is in the choice of assessing the prognostic value of US features together with laboratory

FIGURE 2
Distribution of fetal cytomegalovirus DNA loads and platelet counts



A, CMV DNA levels in copies/mL in relation to time interval between maternal infection and amniocentesis; curve represents mean value obtained by linear regression. **B**, Scatter plot showing CMV DNA loads in fetal blood as well as fetal platelet counts in relation to asymptomatic or symptomatic status at birth or at termination of pregnancy (TOP). Empty dots are cases asymptomatic at birth; full dots are cases symptomatic at birth or at TOP.

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parameters at the time of prenatal diagnosis in a large population of well-documented cases of fetal infection following maternal primary CMV infection that occurred mainly in the first trimester of pregnancy.

Prognostic value and limitations of fetal imaging

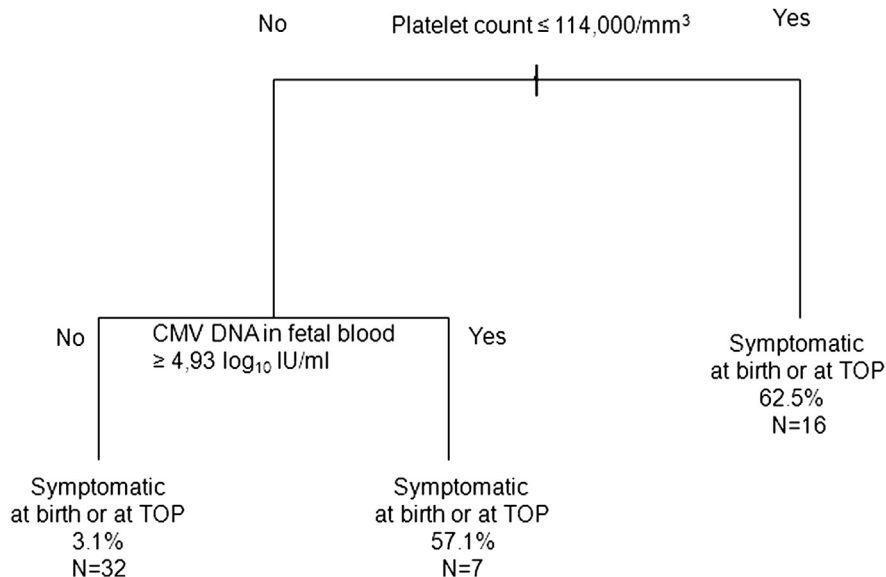
Severe brain involvement in the second trimester of pregnancy led to a dismal prognosis, as expected.^{16,26} However, the most valuable contribution of our

cohort is in its subgroup presenting either with no US features or with nonsevere US features (Table 1). Previous contributions identified a residual risk of severe infection at birth including deafness and more severe neurodevelopmental abnormalities of 1-5% and 0-5%, respectively, when US examination of an infected fetus was considered to be normal.^{25,27-29} These risk estimates were based on imaging, and mainly US alone, throughout the pregnancy.^{23,25-27} Indeed the severity of brain involvement might be delayed up until late in pregnancy and the prognostic value of US at diagnosis is expected to be lower than the prognostic value of imaging obtained later in pregnancy. However, we report a 93% NPV of US features gathered at the time of diagnosis for the presence of symptoms at birth or at TOP for severe brain abnormalities. This is therefore comparable to the reported overall performance of imaging, including fetal MRI, throughout pregnancy.^{16,22-25} However, a normal US examination or the presence of nonsevere symptoms missed 3 of 41 (7%) cases that eventually progressed to become severely symptomatic in the third trimester. This 7% uncertainty is therefore what pregnant women have to deal with when deciding to continue with the pregnancy following a positive amniocentesis for CMV at 23 weeks with either normal US examination or the presence of nonsevere abnormalities.

Added prognostic value of laboratory parameters

In our population, a fetal blood profile showing platelet count >114,000/mm³, CMV DNA fetal blood level <4.93 log₁₀ IU/mL, or a low adjusted AF viral load can reduce this uncertainty further down to 0% or 5% for any symptoms at birth (Table 3). We have previously reported that the PPV of isolated extracerebral US features at any time during the pregnancy being overall symptomatic at birth was 55%.¹⁴ This is compatible with the 60% PPV found for nonsevere US features in the current study although based on a different

FIGURE 3
Contribution of fetal laboratory parameters to the prognosis



Algorithm of contribution of cytomegalovirus (CMV) DNA load in fetal blood and of fetal platelet count in establishing prognosis of infected fetus (recursive partitioning model).

TOP, termination of pregnancy.

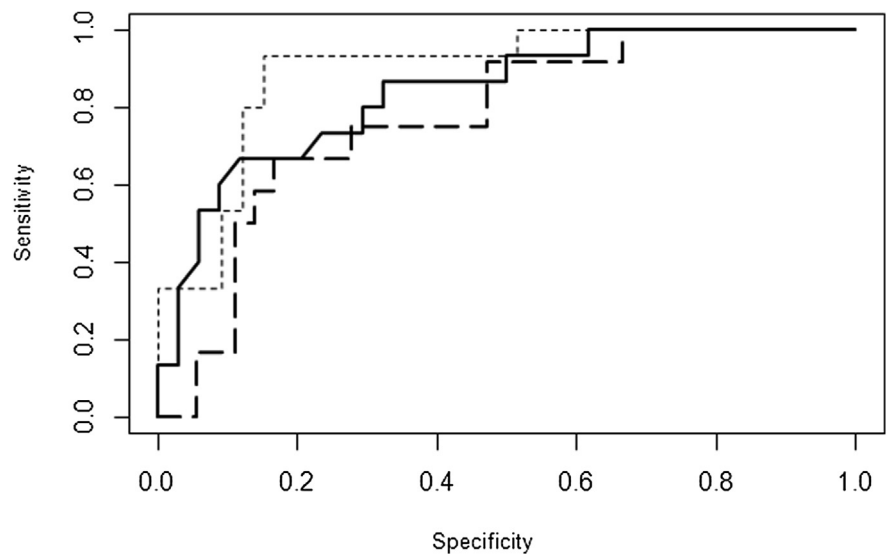
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cohort. In this population of fetuses with nonsevere US features at the time of diagnosis, an abnormal fetal blood profile or a high adjusted AF viral load reduced this uncertainty further with PPV of 79% and 78%, respectively, for any symptom at birth (Table 3).

The advantage of using AF for CMV quantitative PCR is that it can be done on the fluid sampled and used to establish prenatal diagnosis. However its interpretation has become controversial^{5,30-34} in part because of a significant positive correlation between gestational age at amniocentesis and the CMV DNA load in AF^{18,33} but also with the time interval between maternal primary infection and amniocentesis.¹⁸ This increase in CMV DNA loads in AF with gestation is likely to reflect the accumulation of CMV DNA excreted over time as well as the enhanced fetal urine production throughout pregnancy.³⁵ In our study, the time of maternal primary infection was known in almost all cases and CMV DNA load in AF was adjusted to the interval between amniocentesis and maternal primary infection. Higher adjusted CMV DNA loads in AF were significantly associated with a symptomatic status at birth (Figure 2). However, the level of CMV DNA in AF was not as good a predictor of being symptomatic at birth or leading to late development of severe brain lesions in utero as were fetal blood parameters including platelet count and viral load (Figure 4). Fetal blood sampling by cordocentesis for diagnostic purposes, particularly karyotyping, has fallen into disuse and has largely been replaced by amniocentesis, hence reducing its practice to a few centers that are involved in fetal blood transfusions. However, blood analysis in congenital CMV infection has proven useful for the assessment of infected neonates.³⁶⁻³⁸ Similarly, its application to the fetus has shown that both thrombocytopenia and high viral load in fetal blood were associated with a higher risk of giving birth to a symptomatic neonate or leading to TOP for severe brain abnormalities.^{16,39} The cut-off values reported here for platelet count and

FIGURE 4

Receiver operating curves for prediction of symptomatic status



Receiver operating characteristic curves comparing value of adjusted cytomegalovirus DNA load in amniotic fluid (large hatched line) and in fetal blood (small hatched line) and of fetal platelet count (bold line) for prediction of symptomatic status at birth or at termination of pregnancy.

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CMV DNA load may therefore be used in clinical decision-making algorithms. However, it should be stressed that even if standardized units have been used, the implementation of CMV DNA load cut-off values should be validated in different clinical and laboratory settings.

Strengths and limitations of this study

The additional strengths of our study are the extensive longitudinal prenatal assessment including serial imaging together with invasive fetal testing and the use of strict criteria of TOP only in cases with undisputable brain lesions all

TABLE 3

Positive and negative predictive values of nonsevere ultrasound features alone or combined with fetal laboratory parameters for any symptoms at birth or at termination of pregnancy

	PPV	NPV
Nonsevere US features alone N = 63	60%	93%
Nonsevere US features and adjusted CMV DNA in amniotic fluid >1 MoM N = 58	78%	90%
Nonsevere US features or adjusted CMV DNA in amniotic fluid >1 MoM N = 58	44%	95%
Nonsevere US features and abnormal fetal blood results N = 53	79%	91%
Nonsevere US features or abnormal fetal blood results N = 53	50%	100%

CMV, cytomegalovirus; MoM, multiples of median; NPV, negative predictive value; PPV, positive predictive value; US, ultrasound.

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confirmed at autopsy with significant macroscopic and microscopic lesions. This should help to overcome the main limitations of previous series in which autopsy findings of viral inclusions were used to justify severity.³⁹

One limitation of this study is that although the total number of infected fetuses was relatively large (N = 82), we focused on a smaller subgroup of 63 fetuses for which all 3 laboratory parameters were not always available. Another limitation is that the duration of postnatal follow-up, although a median of 18 months, varied, with some children lost for follow-up and others too recently born. Finally, valacyclovir given to some pregnant women to treat infected fetuses as part of an ongoing trial could have hampered the interpretation of our results. However and possibly because fetuses with nonsevere US anomalies were more often treated than fetuses without any US anomalies (86% vs 44%), treatment with valacyclovir was not associated with a better prognosis in this population, thus not interfering with the interpretation of fetal parameters to predict neonatal outcome.

Conclusion

Prognostic assessment of fetuses infected with CMV for the risk of being symptomatic at birth is possible as early as the time of diagnosis in the second trimester by using a combination of targeted US examination, viral load in the AF and in fetal blood, together with platelet count. One may wonder if increasing the NPV of the overall assessment of an infected fetus from 95-100% is relevant and 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 interval for counseling. ■

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