OBSTETRICS

Contribution of fetal blood sampling to determining the prognosis of congenital cytomegalovirus infections: a case-cohort study in Switzerland

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BACKGROUND: Cytomegalovirus is responsible for the most common congenital infection, affecting 0.5% to 1.0% of live births in Europe. Congenital cytomegalovirus infection can be diagnosed during pregnancy by viral DNA amplification in the amniotic fluid, but the prognosis of fetuses without severe brain abnormalities remains difficult to establish on the basis of prenatal imaging alone.

OBJECTIVE: To identify predictors of moderate to severe symptomatic cytomegalovirus infection among fetal blood parameters and to propose an algorithm on the basis of these parameters and on prenatal imaging that would provide the best positive and negative predictive values.

STUDY DESIGN: Fetal blood sampling at 21−28 weeks gestation was performed in fetuses with congenital cytomegalovirus infection confirmed by amniocentesis after maternal infection in the first-trimester or periconceptional period. We compared the levels of hemoglobin, thrombocytes, γ-glutamyl transpeptidase, aspartate aminotransferase, alanine aminotransferase, β2-microglobulin, immunoglobulins G and M, and cytomegalovirus DNA viral loads in amniotic fluid and fetal blood between those with moderate to severe symptomatic infection and those with asymptomatic to mild infection (median follow-up of 36 months for live births).

RESULTS: Among 58 fetuses included, 25 (43%) had a moderate to severe symptomatic infection: 16 with severe cerebral abnormalities, 5 with multiple signs or symptoms at birth, 2 with bilateral sensorineural hearing loss, and 2 with neurodevelopmental delay. The values of thrombocytes, aspartate aminotransferase, β2 microglobulin, Immunoglobulin M, and cytomegalovirus viral loads differed significantly between fetuses with moderate to severe symptomatic infection and those with asymptomatic to mild infection. The optimal strategy to predict moderate to severe symptomatic infection was to first perform fetal brain imaging, followed by fetal blood sampling with the following cutoffs: thrombocytes $<$120,000/mL, viremia $\geq$5 $\log_{10}$/mL, and β2 microglobulin $\geq$12 mg/L). This recursive algorithm had a negative predictive value of 100% for moderately to severely symptomatic infection.

CONCLUSION: The combination of thrombocytes, β2-microglobulin, and cytomegalovirus viral load in fetal blood can be used for prognosis determination, particularly in cytomegalovirus-infected fetuses without severe brain abnormalities at the time of prenatal diagnosis. Future studies should evaluate whether these parameters remain useful in infected fetuses who have been treated with valacyclovir before fetal blood sampling.

Key words: β2-microglobulin, congenital infection, cytomegalovirus, fetal blood sampling, prenatal diagnosis, prognosis, thrombocytopenia, viral load, viremia

Introduction

Cytomegalovirus (CMV) is responsible for the most common congenital infection, affecting 0.5% to 1.0% of live births in Europe, and is the primary cause of nongenetic neurosensory impairment in children.[1] Among newborns with congenital CMV infection, 10% to 15% have symptoms identifiable immediately after birth (ie, cytomegalic inclusion disease), and 5% to 15% of "asymptomatic" neonates will present with late-onset hearing loss or neurodevelopmental impairments.[2] Both primary and nonprimary infected mothers can transmit CMV to their fetuses, but the risk of congenital infection in seronegative women is 4−10 times higher than in immune women (6% to 66% vs 1% to 10%).[3,4]

For many years, CMV systematic screening during pregnancy in Switzerland was not recommended, but in 2021, the guidelines of the Swiss Society of Obstetrics and Gynaecology were amended to allow systematic serologic screening in the first trimester, on the basis of immunoglobulins (Ig) M and G detection and IgG avidity, which would identify half of the congenital CMV infections, the other half resulting from secondary maternal infections that are often difficult to detect by this screening method.[3,5] In cases of maternal infection in the periconceptional period or the first trimester of pregnancy, during which the fetus is most vulnerable and at risk of presenting severe sequelae,[6] congenital CMV infection can be diagnosed by viral DNA amplification in the amniotic fluid obtained by amniocentesis from 17 weeks gestation (WG) and at least 8 weeks following seroconversion.[7] Among infected fetuses, the main prognostic factors are the timing of maternal infection and the presence of severe brain abnormalities, which can be assessed by neurosonography and magnetic resonance imaging (MRI): gyration anomalies (including lissencephaly and polymicrogyria), microcephaly, severe ventriculomegaly, cerebellar hypoplasia, and cystic or diffuse white matter

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AJOG at a Glance

Why was this study conducted?

To improve assessment of the prognosis of fetuses infected with cytomegalovirus (CMV) following maternal infection during the periconceptional period or the first trimester.

Key findings

By combining the results of fetal blood sampling (thrombocytes ≥120,000/mL, viremia <5 $\log_{10}$/mL, and β2 microglobulin <12 mg/L) with the absence of severe brain abnormalities in CMV-infected fetuses, there is a negative predictive value (NPV) of 100% for moderate to severe symptomatic infection in early childhood.

What does this add to what is known?

Fetal blood sampling should be discussed with pregnant women whose fetus is infected with CMV to improve the NPV of prenatal imaging, given that some severe cerebral anomalies may not appear until late in pregnancy. Each patient should be well informed about the benefits and risks of this procedure.

abnormalities (WMA).[6,8–11] It is still debated whether the CMV DNA load in amniotic fluid and fetal blood sampling (FBS) is a useful parameter to predict symptomatic congenital infections.[12–14] Overall, FBS is associated with a risk of 1% to 2% of fetal loss,[15,16] but could be particularly interesting in detecting fetuses that will develop symptomatic forms or long-term disabilities among those whose infection has been diagnosed prenatally but do not show severe brain abnormalities at midpregnancy prenatal imaging (but might develop anomalies later, such as polymicrogyria and WMA).[17,18] Thrombocytopenia was found to be a reliable prognostic factor in several studies, but discordant results were found for other parameters (viral load, liver enzymes, β2-microglobulin [β2m]).[12,17,19–23] Tertiary prevention using valacyclovir in mildly affected fetuses could reduce viral replication in the fetoplacental compartment, leading to an improvement in their perinatal and long-term outcomes.[24,25] Thus, it remains crucial for parental counseling and criteria for initiation of tertiary prevention to determine early and reliable prognostic markers of congenital CMV infection at the time of prenatal diagnosis. The primary objective of this study was to assess the prognostic value of CMV DNA load in amniotic fluid and fetal blood parameters for moderately to severely symptomatic infections in fetuses with congenital CMV infection after periconceptional or first-trimester maternal infection. The secondary objective of this study was to propose an algorithm on the basis of prenatal imaging and FBS criteria that would provide the best predictive values for moderately to severely symptomatic congenital CMV infections.

Materials and Methods

Study settings

The Lausanne University Hospital is the only center in French-speaking Switzerland that offers FBS, and patients are referred for this examination and the prenatal follow-up of congenital CMV infection by the internal obstetrics policlinic, or by external hospitals, ultrasound and prenatal diagnosis centers and obstetrics and gynecology practices from French-speaking Switzerland. At enrolment, patients are counseled by maternal-fetal medicine specialists about the various follow-up options and the benefits and risks of invasive procedures. Patients undergoing follow-up for CMV infection are systematically offered the possibility of having their data collected for research purposes in the CMV-pregnancy registry without any additional procedure or change in their choice of follow-up.

Thus, this study was based on a cohort of fetuses infected with CMV after maternal infection in the periconceptional period (between 4 weeks before and 6 weeks after the last menstrual period) or the first trimester of pregnancy and who underwent FBS between 2007 and 2022, at the Lausanne University Hospital. The timing of maternal primary infection was estimated using symptom onset if present and/or results of CMV IgG, CMV IgM, and CMV IgG avidity in available sera. Prenatal diagnosis of congenital infection was achieved by amniocentesis performed after 17 WG and at least 8 weeks after maternal seroconversion. In cases with fetal infection, a prognosis assessment was offered, including FBS after 20 WG, serial ultrasound examinations with neurosonography (performed with transvaginal or transabdominal high-frequency probes) every 2–4 weeks, and fetal brain MRI between 30 and 34 WG.[26] After evaluation of the results of prenatal imaging and FBS, in-utero treatment with valacyclovir 8 g/d was proposed in cases with moderate signs on imaging or FBS (fetal viremia >3000 copies/mL or fetal platelets <100,000/mm^3) after 2016.[24] For terminations of pregnancy (TOP) and stillbirths, autopsies with neuropathologic and histologic examination were performed. For live births, the follow-up included laboratory tests (quantitative polymerase chain reaction [qPCR] in saliva and urine samples, blood cell counts, hepatic transaminases), transfontanellar ultrasound, MRI at term equivalent age, fundoscopy and ophthalmologic follow-up, serial clinical and neurodevelopmental examinations (at 6, 18, 42, and 60 months), and serial oto-acoustic emissions screening confirmed by auditory brainstem responses testing for cases of suspected sensorineural hearing loss (SNHL). Moderately to severely symptomatic congenital CMV diseases were treated with ganciclovir (6 mg/kg twice a day) for 6 weeks until 2015 and with valganciclovir (16 mg/kg twice a day) for 6 months from 2015.[27,28]

Study design

A case-cohort study was conducted to compare CMV DNA load in amniotic fluid and fetal blood parameters

between fetuses with moderate to severe symptomatic infection and those with asymptomatic to mild infection.

Participants

We included all pregnancies followed for congenital CMV infection diagnosed by amniocentesis, who underwent FBS, with documented fetal and neonatal outcomes and pediatric evaluation up to at least 6 months of life (for live births). Exclusion criteria were patients who declined to participate in clinical studies, the absence of data on fetal, neonatal, or childhood outcomes, or the absence of autopsy, including a neuropathologic examination after TOP. In addition, fetuses who had received valacyclovir before amniocentesis or FBS were excluded from this study.

Outcomes

Outcome definitions were based on a consensus issued in 2017,[29] and adapted to more recent data.[18,30,31] Moderately to severely symptomatic CMV infections were defined either by severe cerebral abnormalities confirmed on postnatal MRI or autopsy, or by multiple ($\geq$3) manifestations at birth or autopsy, or by moderate to severe disability during follow-up of the child (Table 1[17,18,29–31]). Mildly symptomatic CMV infections were defined by 1 or 2 isolated and transient manifestations (eg, mild hepatomegaly) or by isolated unilateral and mild SNHL (threshold >30dB and <70dB). Asymptomatic CMV infections were defined by the absence of clinical, biologic, neuroradiologic, neurologic, and neurosensory abnormalities.

Two independent examiners (maternal-fetal medicine specialist and neonatologist) reviewed the outcomes of the fetuses and children and classified them into "moderately to severely symptomatic" or "asymptomatic to mildly symptomatic." Discrepant cases were discussed with the rest of the team to determine the most appropriate classification.

Variables

Fetal imaging (ultrasound and MRI) was reviewed, and the findings were classified as extracerebral abnormalities, nonsevere cerebral abnormalities, or severe cerebral abnormalities (ie, corresponding to postnatal severe cerebral abnormalities presented in Table 1,[17,18,29–31] except for diffuse WMA, which is difficult to identify prenatally).

Viral load in amniotic fluid at the time of amniocentesis was collected. Fetal blood parameters assessed included hemoglobin, thrombocytes, γ-glutamyl transpeptidase (GGT), aspartate aminotransferase (AST), alanine aminotransferase (ALT), β2m, IgG and IgM, and the viral load at the time of FBS. CMV DNA viral loads were assessed by amplification of the viral genome in amniotic fluid and fetal blood by qPCR with a threshold of 250 copies/mL to define positivity.

Statistical analysis

Differences between the groups involving qualitative variables were analyzed using the chi-square or Fisher exact tests when appropriate. Quantitative variables were described by their mean and standard deviation or by the median and extremes for variables whose distribution was not normal. The normality of the distribution of quantitative variables was assessed using the Skewness and Kurtosis test. The comparison between quantitative variables was performed using Student *t* or rank sum (Mann-Whitney) tests. Multivariate analyses were performed using logistic regressions to incorporate potential confounders or interactions identified from the univariate analyses. The cutoffs used for quantitative variables were obtained by receiver operating characteristic (ROC) analysis. Finally, a recursive partitioning analysis assessed the best algorithm to use to predict moderately to severely symptomatic congenital CMV

TABLE 1
Definition of moderately to severely symptomatic CMV infection

Severe cerebral abnormalities on postnatal MRI or autopsy	Manifestations at birth[a] or on autopsy[b]	Moderate to severe disabilities
Severe ventriculomegaly (>15 mm)	FGR (less than third percentile)	Cerebral palsy
Microcephaly ≤2 SD	Seizures[a]	Bilateral or severe (>70 dB) SNHL
Cerebellar hypoplasia or dysplasia	Petechiae[a]	Chorioretinitis or visual impairment (acuity <20/40)
Abnormal gyration (polymicrogyria, lissencephaly)	Thrombocytopenia[a]	Neurodevelopmental delay (Bayley-III overall, cognitive or motor score ≤2 SD)
Corpus callosum dysplasia	Hypotonia/lethargy[a]	
Cystic or diffuse WMA	Hepatomegaly/splenomegaly	
	Intracellular inclusions[b]	

Adapted from Rawlinson et al[29] with the removal of periventricular hyperechogenicity and intracerebral calcifications because of recent data on the absence of a poor prognosis associated with these signs if they are isolated.[17,30,31] Moderate to severe disabilities were completed by longer-term signs.[18]

FGR, Fetal growth restriction; *MRI*, magnetic resonance imaging; *SD*, standard deviation; *WMA*, white matter abnormalities.

[a] Seizures, petechiae, thrombocytopenia, hypotonia, and lethargy were recorded only for live births; [b] Intracellular inclusions were recorded only on autopsies.

Pomar. Fetal blood sampling in congenital cytomegalovirus infection. Am J Obstet Gynecol 2024.

infections on the basis of the likelihood ratios of the variables and thresholds identified in the previous analyses. Statistical significance was considered for a $P<.05$. The analyses were performed using STATA 16 software.

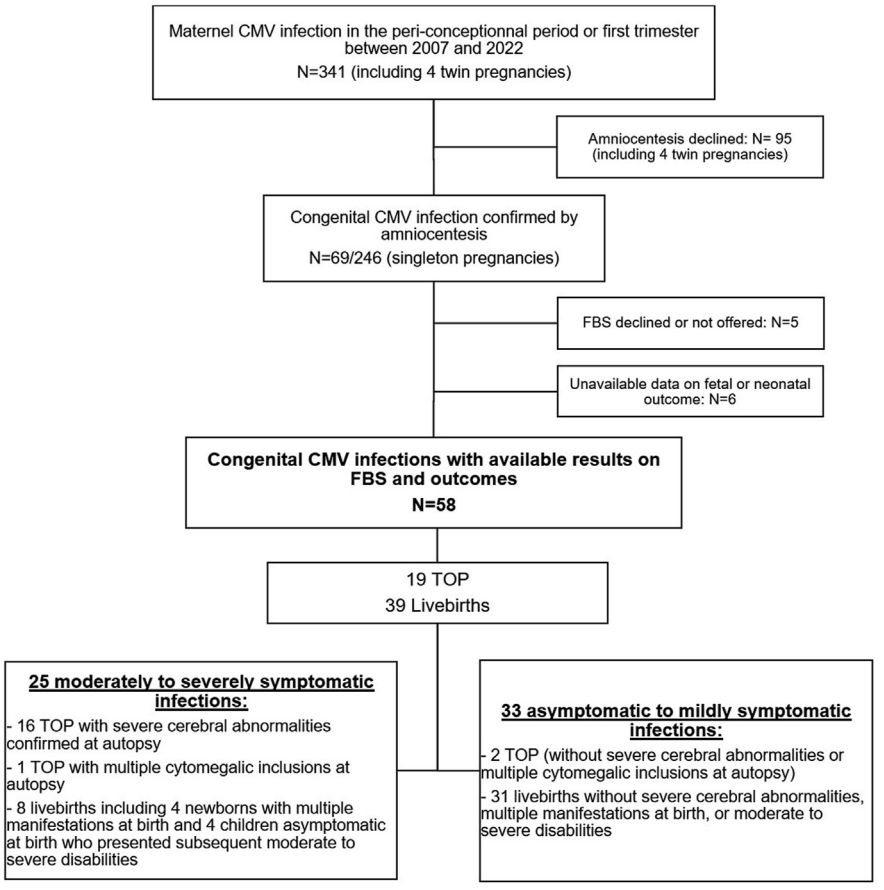
Ethics

This study was approved by the Vaud Cantonal Commission on Research Ethics (CER-VD) for research involving humans on October 7, 2015 (protocol 295/15) for cases included from 2007 to 2021 and on May 4, 2022 (protocol 2022−00066) for cases included from 2022. All participants gave written informed consent.

Results

Between 2007 and 2022, 341 pregnant women were followed in our center for CMV infection in the periconceptional period or the first trimester. Among those who agreed to undergo amniocentesis, 69/246 (28%) had a fetus with confirmed congenital CMV infection. One case was excluded from this study because FBS was not offered because of a short cervix and difficult access to the umbilical cord, and 4 other patients declined FBS. Six other cases were excluded from the analysis because of insufficient data on fetal or neonatal outcomes, preventing classification as either "moderately to severely symptomatic" or "asymptomatic to mildly symptomatic" according to our criteria (no autopsy after TOP in 2 cases and no data on neonatal outcome in 4 cases). Fifty-eight fetuses (84%) met the inclusion criteria and were retained for this case-cohort study. Of the 58 included pregnancies, 19 (33%) were terminated because of severe abnormalities on prenatal imaging (16 cases), severe thrombocytopenia at FBS without severe brain abnormalities on imaging (1 case), and in the presence of moderate thrombocytopenia without ultrasound signs (2 cases). Overall, 25 (43%) included fetuses met the criteria of "moderately to severely symptomatic infection" and were considered as cases in the analysis, and the remaining 33 fetuses were "asymptomatic to mildly symptomatic" and considered as "controls" (Figure 1).

FIGURE 1
Flowchart



CMV, cytomegalovirus; *FBS*, fetal blood sampling; *TOP*, termination of pregnancy

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Median postnatal follow-up for live births was 36 months (6−68 months). Details of fetal, neonatal, and childhood outcomes are presented in Supplementary Table 1.

Maternal characteristics and infection

Among maternal comorbidities, one patient had *Toxoplasma gondii* coinfection in the first trimester, for which a congenital infection was ruled out by negative amniocentesis. Maternal baseline characteristics did not differ between "moderately to severely symptomatic" and "asymptomatic to mildly symptomatic" fetuses, except for the presumed timing of maternal infection: it was significantly earlier in the group with "moderately to severely symptomatic" congenital infections, with a higher rate of infections occurring between 6 and 10 WG (Table 2).

Amniocentesis and fetal imaging

Amniocenteses confirming congenital CMV infections were performed at a median age of 21 years old and 22 WG in the "moderately to severely symptomatic" and the "asymptomatic to mildly symptomatic" groups, respectively ($P=.887$). Viral loads in amniotic fluid differed significantly between these groups (median viral loads of 7.0 $\log_{10}$ IU/mL for symptomatic vs 5.7 $\log_{10}$ IU/mL for asymptomatic), even when considering gestational age at amniocentesis and the timing of maternal infection in a multivariable analysis ($P=.002$) (Figure 2). Nineteen fetuses

TABLE 2
Maternal characteristics

Characteristics	Moderately to severely symptomatic congenital infections, n=25 (43%)	Asymptomatic to mildly symptomatic congenital infections, n=33 (57%)	*P* value
Age, y	30 (20–41)	31 (19–43)	.567
Gravidity	2 (1–5)	2 (1–3)	.187
Parity	1 (0–2)	1 (0–2)	.593
Comorbidities			
Toxoplasmosis coinfection	0 (0)	1 (3)	.380
DVT and PE	1 (4)	0 (0)	.246
History of Turner syndrome	1 (4)	0 (0)	.246
History of IUFD	1 (4)	1 (3)	.841
Maternal symptomatic CMV infection	2 (8)	4 (12)	.610
Type of maternal infections			
Primoinfection	24 (96)	32 (97)	.841
Reinfection or reactivation	1 (4)	1 (3)	
Timing of maternal infection			
<6 WG	10 (38)	11 (38)	.036
6–10 WG	14 (58)	12 (34)	
10–14 WG	1 (4)	10 (28)	
Valacyclovir 8 g/d after FBS	4 (16)	6 (18)	.828

Values are presented as median (minimum to maximum) or n (%).

CMV, cytomegalovirus; *DVT*, deep vein thrombosis; *FBS*, fetal blood sampling; *IUFD*, Intrauterine fetal demise; *PE*, pulmonary embolism; *TOP*, termination of pregnancy; *WG*, weeks of gestation.

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(76%) of the "moderately to severely symptomatic" group and 8 (24%) of the "asymptomatic to mildly symptomatic" group had ≥1 ultrasonographic abnormality suggestive of congenital CMV infection. Sixteen (64%) and 1 (3%) fetuses, respectively, had severe cerebral abnormalities suspected on neurosonography or fetal MRI (Table 3). In the "moderately to severely symptomatic" group, the 16 fetuses exhibiting severe cerebral abnormalities underwent TOP, and these lesions were confirmed at autopsy. The fetus with severe brain abnormalities in the "asymptomatic to mildly symptomatic" group was suspected of having isolated occipital polymicrogyria on fetal MRI at 32 WG, which was refuted on postnatal imaging. This child had no severe signs or symptoms at 18 months of life and was therefore classified as asymptomatic.

Fetal blood parameters

FBS was performed at a median age of 22 WG in the "moderately to severely symptomatic" group and at 23 WG in the "asymptomatic to mildly symptomatic" group (*P*=.433). Distribution of viral loads in fetal blood (median values of 5.2 $\log_{10}$ IU/mL vs 4.0 $\log_{10}$ IU/mL; *P*=.004), thrombocytes (105,000 vs 208,000/mm^3; *P*<.001), AST (21 vs 15 IU/L; *P*=.005), β2m (13.1 vs 6.0 mg/mL; *P*=.012), and IgM titers (5.8 vs 0.0; *P*=.007) differed significantly between these 2 groups, even when considering gestational age at FBS and the presumed timing of maternal infection in multivariable analysis (Figure 2).

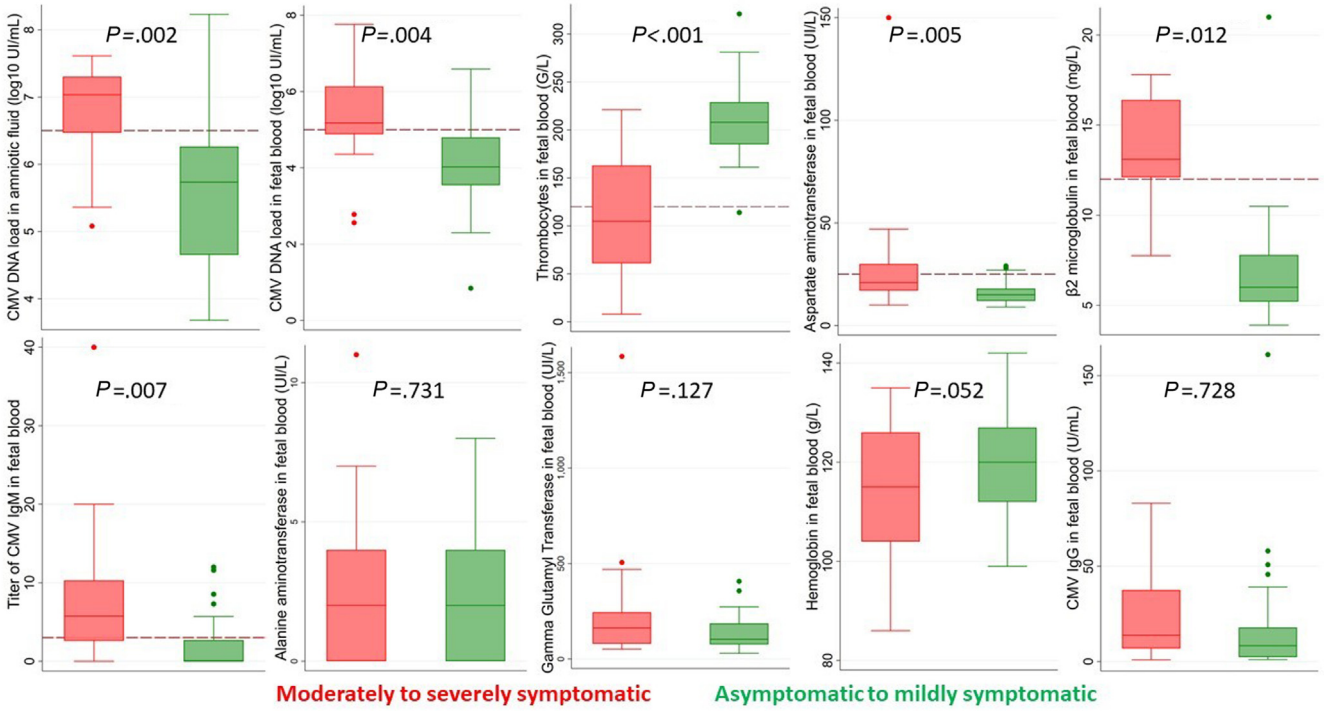
Strategies to predict moderately to severely symptomatic infections

The optimal cutoffs to predict "moderately to severely symptomatic" congenital CMV infections according to ROC analysis (Supplementary Figure 1) were (1) CMV DNA load ≥6.5 $\log_{10}$ IU/mL in amniotic fluid, (2) CMV DNA load ≥5 $\log_{10}$ IU/mL in fetal blood, (3) thrombocytes <120,000 /mm^3, (4) AST ≥25 IU/L, (5) IgM titer ≥3, and (6) β2m ≥12mg/L.

The prognostic values of prenatal imaging findings, CMV DNA in amniotic fluid and fetal blood parameters are presented in Table 4 and the prognostic values of different strategies combining these parameters. Overall, the best strategy seems to combine severe cerebral abnormalities at prenatal imaging with any abnormal blood parameter using the cutoffs defined above to reach a sensitivity of 100% and a negative predictive value (NPV) of 100%.

A recursive analysis was conducted on fetal imaging and blood parameters to propose an algorithm for the prediction of "moderately to severely symptomatic" CMV infections (Figure 3). In this analysis, the presence of moderate thrombocytopenia <120,000 thrombocytes/mm^3, CMV DNA load >5 $\log_{10}$ IU/mL in fetal blood, or β2m >12 mg/L in cases with severe brain abnormalities suspected at prenatal imaging achieved a positive predictive value (PPV) of 100%, and the absence of these markers made it possible to distinguish a false-positive case in which MRI suspected isolated polymicrogyria which was refuted after birth. In cases without severe brain abnormalities at prenatal imaging, moderate thrombocytopenia had a PPV of 83% for symptoms at birth, and the presence of CMV DNA load >5 $\log_{10}$ IU/mL or β2m >12 mg/L in fetal blood in cases without thrombocytopenia had a

FIGURE 2
Distribution of CMV DNA in amniotic fluid and fetal blood parameters by the outcome of congenital CMV infection



Boxes represent the median and interquartile range, whiskers represent the range excluding outliers >1.5 × interquartile range from the upper or lower quartile, and points represent outliers. *P* values are estimated using logistic regressions adjusted on gestational age at amniocentesis or fetal blood sampling and on the presumed timing of maternal infection. Dot lines represent the optimal cutoffs to predict moderately to severely symptomatic congenital CMV infection according to receiver operating characteristics) analysis for parameters whose distribution is significantly different between the groups.

CMV, cytomegalovirus; *IgG*, immunoglobulin G; *IgM*, immunoglobulin M.

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PPV of 44%. Four cases with high CMV DNA load or β2m without thrombocytopenia did not have multiple symptoms at birth but had severe bilateral SNHL (n=2) or delayed cognitive development (n=2) during follow-up. All cases without severe brain abnormalities, thrombocytopenia, and with values of CMV DNA load and β2m <5 $\log_{10}$ IU/mL and 12 mg/L in fetal blood were asymptomatic to mildly symptomatic (transient manifestations or mild and unilateral SNHL) during their follow-up, reaching a NPV of 100% for "moderately to severely symptomatic" infections. AST levels and IgM titers did not contribute to improving prognosis prediction in this analysis.

An additional analysis considering only ultrasound and FBS results up to 28 WG, without fetal brain MRI results, is presented in Supplementary Figure 2. This analysis shows results similar to those of the analysis, including MRI. The primary difference in analyses stems from a case that was more rapidly diagnosed with severe infection because of the identification of thrombocytopenia, a high viral load, and an increased β2m at 25 WG in the absence of ultrasound findings. This fetus demonstrated cystic WMA on MRI 6 weeks later, and the pregnancy was terminated.

Comment

Principal findings

This study highlighted 2 important findings. First, we showed that fetuses who will develop moderately to severely symptomatic congenital CMV infections have higher CMV DNA values in amniotic fluid and fetal blood, higher β2m, aspartate aminotransferase, and IgM titer, and lower thrombocytes in fetal blood than those remaining asymptomatic or mildly symptomatic. Secondly, combining the presence or absence of severe brain abnormalities on prenatal imaging (up to 32 WG) with moderate thrombocytopenia, high viremia, and high β2m could result in a PPV and NPV of 100% for moderately to severely symptomatic congenital infections with a PPV of 44% to 83% if any of these blood parameters is abnormal in cases without severe cerebral abnormalities. This latter category represents a gray area in terms of prognosis, as shown in our study with several cases that were interrupted on the basis of thrombocytopenia or a high viral load and did not demonstrate severe abnormalities at autopsy (Figure 3).

TABLE 3
Prenatal ultrasound and MRI abnormalities

	Moderately to severely symptomatic congenital infections, n=25 (43%)	Asymptomatic to mildly symptomatic congenital infections, n=33 (57%)	*P* value
Number of ultrasounds	5 (3–8)	5 (4–8)	.346
Fetal brain MRI	12 (48)	20 (61)	.339
GA at the onset of abnormalities	22 WG (16–33)	23 WG (17–34)	.748
Signs of congenital CMV infection	19 (76)	8 (24)	<.001
Extracerebral abnormalities:	18 (72)	5 (15)	<.001
Placentomegaly (>40 mm)	5 (20)	1 (3)	
IUGR (less than third percentile)	5 (20)	3 (9)	
Hyperechogenic bowels	9 (36)	5 (15)	
Oligohydramnios or polyhydramnios	4 (16)	4 (12)	
Hepatomegaly	3 (12)	1 (3)	
Intrahepatic calcifications	2 (8)	0 (0)	
Splenomegaly	1 (4)	0 (0)	
Ascites	2 (8)	0 (0)	
Hydrops	1 (4)	0 (0)	
Nonsevere cerebral abnormalities:	17 (68)	5 (15)	<.001
Ventriculomegaly <15 mm	5 (20)	2 (6)	
Intraventricular adhesions	2 (8)	0 (0)	
Intracerebral calcifications	7 (28)	1 (3)	
Subependymal cysts	3 (12)	1 (3)	
Choroid plexus cysts	4 (16)	3 (9)	
Lenticulostriatal vasculopathy	6 (24)	3 (9)	
Severe cerebral abnormalities:	16 (64)	1 (3)	<.001
Severe ventriculomegaly >15 mm	3 (12)	0 (0)	
Cystic WMA	8 (32)	0 (0)	
Microcephaly ≤2 SD	3 (12)	0 (0)	
Cerebellar hypoplasia/dysplasia	2 (8)	0 (0)	
Lissencephaly	3 (12)	0 (0)	

Pomar. Fetal blood sampling in congenital cytomegalovirus infection. Am J Obstet Gynecol 2024. (continued)

Results in the context of what is known

Moderate to severe thrombocytopenia was associated with adverse outcomes in CMV-infected fetuses in several previous studies.[17,21,22,32,33] Moderate thrombocytopenia defined as thrombocytes/mm^3 <120,000 is concordant with the cutoffs used by others (100,000 to 114,000 thrombocytes/mm^3), with a sensitivity >90% for symptomatic congenital CMV infections.[17,21,22]

In addition, higher viral loads in amniotic fluid and fetal blood were associated with symptomatic infections in other studies,[12,17,19,20,34] with better predictive values for viremia than CMV DNA load in amniotic fluid,[17] probably because the risk of overlapping results between symptomatic and asymptomatic fetuses is higher in amniotic fluid.[19,20,35] In our recursive algorithm, CMV DNA load in amniotic fluid was not retained as it failed to identify additional cases that were not already detected by thrombocytopenia or high viremia; on the contrary, it would have led to false-positive cases. High viremia was defined as CMV DNA load >5 $\log_{10}$ IU/mL, which is consistent with a cutoff of 4.93 $\log_{10}$ IU/mL previously suggested by Leruez-Ville et al[17] reaching an NPV of 97% for symptomatic infection in fetuses not presenting with thrombocytopenia nor severe brain abnormalities. Our recursive algorithm found an NPV of 100% using cutoffs close to theirs and by adding the value of β2m in fetal blood. In addition, the latter parameter was found to be associated with symptomatic cCMV infections in other studies,[12,22,36] and a cutoff of 11.5 mg/L had a PPV of 100% and an NPV of 95%, reaching 100% when combined with high viremia,[12] which is consistent with the NPV reported in our study. β2m analysis is used for the assessment of renal function, but because direct and indirect signs of CMV-related nephritis were rarely observed in our cohort, it seems that the β2m increase observed in symptomatic fetuses is more related to lymphoid cell stimulation during the acute phase of CMV infection.[36] The increase in IgM titer reported in our

TABLE 3
Prenatal ultrasound and MRI abnormalities *(continued)*

	Moderately to severely symptomatic congenital infections, n=25 (43%)	Asymptomatic to mildly symptomatic congenital infections, n=33 (57%)	*P* value
Polymicrogyria	5 (20)	1 (3)	
Corpus callosum dysplasia	2 (8)	0 (0)	

Values are presented as median (minimum to maximum) or n (%).

CMV, cytomegalovirus; *FBS*, fetal blood sampling; *GA*, gestational age; *IUGR*, intrauterine growth restriction; *MRI*, magnetic resonance imaging; *SD*, standard deviation; *TOP*, termination of pregnancy; *WMA*, white matter abnormality.

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study, and others may also indicate activation of circulating lymphocytes, particularly in fetuses with high viremia.[7,12,22]

An increase in liver enzymes was associated with symptomatic CMV infections in fetuses and neonates.[12,22,37] This increase is probably because of viral inclusion in hepatocytes causing viral hepatitis and moderate liver cytolysis. In our study, we only found a significant increase in AST in the moderately to severely symptomatic group. In addition to AST, Fabbri et al[12] reported an increase in GGT in symptomatic fetuses. Hawkins-Villarreal et al[22] observed that high levels of GGT were associated not only with liver cytolysis but also with brain damage in infected fetuses. High GGT, according to the cutoff suggested by Hawkins-Villarreal et al[22] (≥183 UI/L), was more common in fetuses with severe brain abnormalities in our cohort, but this difference did not reach statistical significance (6/16, 37.5% in symptomatic vs 10/42, 23.8% in asymptomatic fetuses; *P*=.297). Overall, liver enzymes were not retained in our recursive partitioning analysis, as in our settings, all fetuses with high AST or GGT also had at least one abnormal value of viremia, thrombocytes, or β2m.

Clinical implications

Prenatal diagnosis of congenital CMV infections is achieved by amniocentesis either after positive screening for maternal infection or after the discovery of suggestive fetal signs at ultrasound. After a primary maternal infection in the periconceptional period or during the first trimester, the risk of maternal-fetal transmission is estimated to be 21% and 37%, respectively.[4] Furthermore, 19% to 29% of fetuses infected at this stage are likely to have neurologic lesions, with an increased risk of late-onset sequelae (cognitive, motor, and sensory impairments).[4,6,38] Secondary and tertiary prevention with valacyclovir could reduce the risks of maternal-fetal transmission and subsequent sequelae in infected fetuses, but 11-12% of treated fetuses will still be infected,[39,40] and 18% of them could present with a symptomatic infection at birth.[24] Although fetuses with severe structural brain abnormalities are at high risk of symptomatic infection at birth and permanent neurologic sequelae,[1,41–43] the prognosis of fetuses with nonsevere abnormalities or no signs at ultrasound is more difficult to assess, especially at midgestation when it is too early to investigate gyration. If there is an isolated nonsevere sign or no sign, their risk to develop late-onset neurologic sequelae is estimated to be 10% to 15%,[44] and if they have multiple abnormalities, their risk might increase to 48%.[31] Our results and those of others concerning the contribution of FBS could be particularly useful for fetuses without severe brain abnormalities, as the absence of thrombocytopenia, high viremia, and high β2m in fetal blood could reach an NPV close to 100% for moderately to severely symptomatic infections.[12,17,22] Pregnant women whose fetus has no or moderate signs of CMV infection in midtrimester US should be informed about the possibility of increasing the NPV by FBS to decide whether it is worth the additional risk of FBS (1% to 2% of fetal loss[15,16]).

Research implications

In our cohort, tertiary prevention with valacyclovir was proposed after FBS and ultrasound results for fetuses with moderate signs of infection, but we did not propose secondary prevention as it was not recommended in Switzerland at the time of this study.[5] It would be interesting to evaluate whether our predictive algorithm is reproducible in other cohorts with similar settings. Moreover, it would be worth evaluating this algorithm in fetuses that have received secondary prevention with valacyclovir[39,40] as this treatment could improve blood parameters at the time of FBS, particularly decreasing their viremia.[17,25]

Strengths and limitations

The main strength of our study is the extensive prenatal and postnatal follow-up of fetuses included, reducing classification bias as much as possible. Another strength is the inclusion of potential cofounders and interactions in multivariable analysis to propose a fair comparison of fetal blood parameters between moderately to severely symptomatic and asymptomatic to mildly symptomatic infections. As CMV DNA in amniotic fluid and fetal blood increase with gestational age and with the time interval between maternal infection and amniocentesis or FBS,[19,45] our analyses were adjusted for these variables, particularly because maternal infection occurred earlier in the moderately to severely symptomatic group (which is the main risk factor for symptomatic infections[3,4,6]).

We used an extensive definition of symptomatic congenital CMV infections, including long-term neurosensory and neurodevelopmental impairments, but we cannot exclude that some asymptomatic or mildly symptomatic children will present with later sequelae (cognitive or behavioral abnormalities), underestimating the proportion of moderately to severely

TABLE 4
Prognostic values of fetal imaging, amniocentesis, and fetal blood parameters for the prediction of moderately to severely symptomatic congenital CMV infections

Variables	aOR (95% CI)	*P* value	Se	Sp	PPV	NPV
Any abnormality at prenatal imaging (US or MRI)	9.9 (2.6–40.3)	<.001	76	76	70	81
Severe prenatal cerebral abnormalities	56.9 (6.6–489.1)	<.001	64	97	94	78
Any abnormality on the prenatal US (MRI excluded)	9.6 (2.5–38.3)	<.001	72	79	72	79
Severe prenatal cerebral abnormalities	NA	<.001	60	100	100	77
CMV DNA in AF: for each $\log_{10}$ IU/mL increase	5.9 (2.2–15.6)	<.001				
For a CMV DNA load in AF ≥6.5$\log_{10}$ IU/mL	12.5 (3.1–50.0)	<.001	72	79	72	79
Any abnormality at FBS	NA	<.001	100	55	63	100
Viremia: for each $\log_{10}$ IU/mL increase	1.3 (1.0–1.6)	0.049				
For a viremia ≥5 $\log_{10}$ IU/mL	7.0 (2.0–24.5)	0.002	64	79	70	74
Thrombocytes: for each 50G/L decrease	4.1 (1.0–8.4)	<.001				
For moderate thrombocytopenia <120 G/L	54.0 (5.8–505.1)	<.001	56	97	93	74
AST (IU/L): per increase of 5 IU/L	1.9 (1.1–3.3)	0.021				
For a moderate increase in AST ≥25 IU/L	6.9 (1.9–24.7)	0.003	56	85	74	72
IgM (titer): per increase of 1	1.3 (1.1–1.5)	0.007				
For a titer ≥3	6.2 (1.9–20.5)	0.003	64	79	70	74
β2m: per increase of 1 mg/L	1.5 (1.1–2.1)	0.012				
For a β2m ≥12 mg/L	21.9 (2.5–194.9)	0.006	91	92	91	92
Any abnormality on US or MRI combined with						
DNA load in AF ≥6.5 $\log_{10}$ IU/mL	9.2 (2.3–43.8)	<.001	84	64	64	84
FBS	NA	<.001	100	48	60	100
FBS and DNA load in AF ≥6.5 $\log_{10}$ IU/mL	NA	<.001	100	42	57	100
Any abnormality on US combined with						
DNA load in AF ≥6.5 $\log_{10}$ IU/mL	10.5 (2.5–50.3)	<.001	84	67	66	85
FBS	NA	<.001	100	52	61	100
FBS and DNA load in AF ≥6.5 $\log_{10}$ IU/mL	NA	<.001	100	45	58	100
Severe cerebral abnormalities on US or MRI with						
DNA load in AF ≥6.5 $\log_{10}$ IU/mL	75.3 (8.5–669.0)	<.001	96	76	75	96
FBS	NA	<.001	100	79	78	100
FBS and DNA load in AF ≥6.5 $\log_{10}$ IU/mL	NA	<.001	100	48	60	100
Severe cerebral abnormalities on US with						
DNA load in AF ≥6.5 $\log_{10}$ IU/mL	89.1 (9.9–3799.0)	<.001	96	79	77	96
FBS	NA	<.001	100	82	81	100

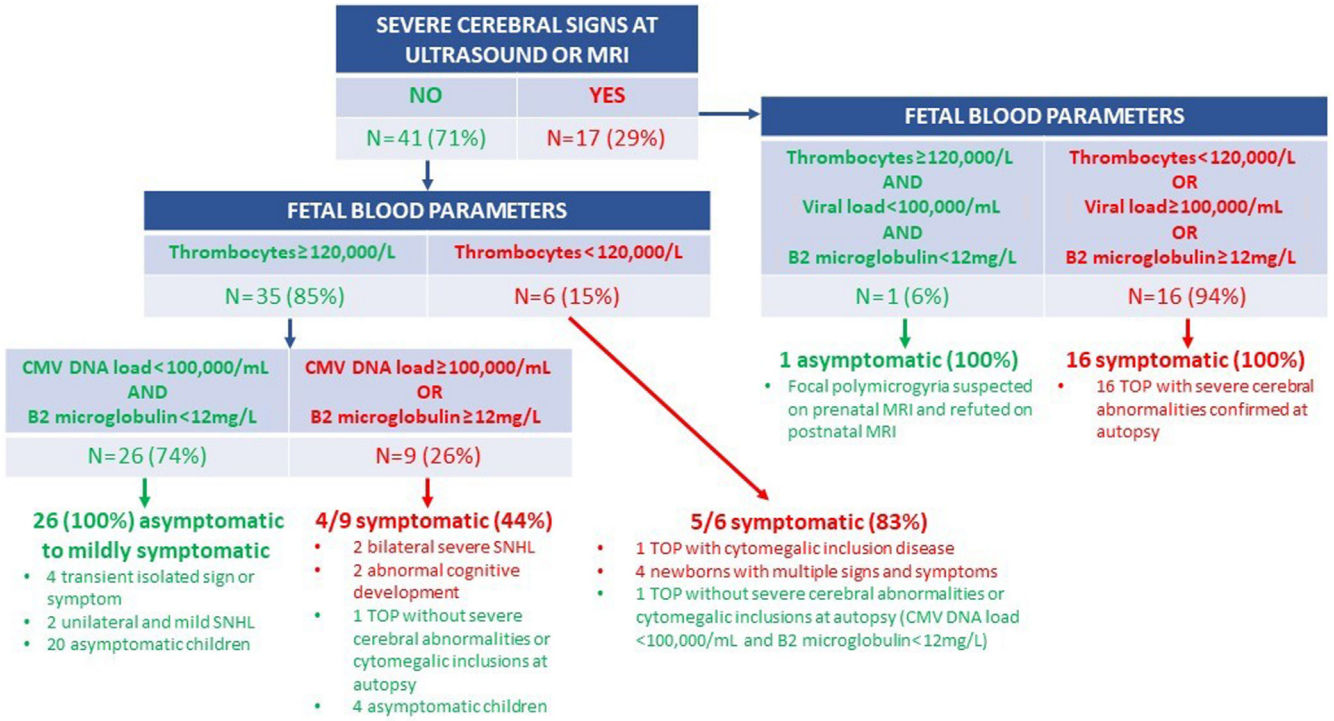
Values are presented as median (minimum to maximum) or %. aOR (95% CI) were based on the presumed timing of maternal infection and gestational age at amniocentesis for CMV DNA load in amniotic fluid or gestational age at FBS for fetal blood parameters using logistic regressions. aOR was unavailable for strategies with a sensitivity of 100%, and *P* values were estimated using univariable analysis for these strategies. Any abnormality on prenatal imaging included any of the signs described in Table 3. Any abnormality on FBS included CMV DNA load ≥5 $\log_{10}$ IU/mL in fetal blood, moderate thrombocytopenia <120,000 thrombocytes/mm^3, AST ≥25 UI/L, IgM titer ≥3, or β2m ≥12 mg/L.

AF, amniotic fluid; *aOR*, adjusted odds ratio; AST, aspartate aminotransferase; *β2m*, β2-microglobulin; *CI*, confidence interval; *CMV*, cytomegalovirus; *FBS*, fetal blood sampling; IgM, immunoglobin M; *MRI*, magnetic resonance imaging; *NA*, not available; *NPV*, negative predictive value; *PPV*, positive predictive value; *Se*, sensitivity; *Sp*, specificity; *US*, ultrasound.

Pomar. Fetal blood sampling in congenital cytomegalovirus infection. Am J Obstet Gynecol 2024.

symptomatic fetuses in our study and therefore overestimating the NPV reported. Similarly, although all included TOP underwent autopsy with neuropathologic examination, we cannot exclude the possibility that some fetuses without severe or multiple signs at autopsy might have shown neurosensory or cognitive abnormalities if the

FIGURE 3
Recursive algorithm for the prognosis of congenital CMV infections



SNHL, sensorineural hearing loss; *TOP*, termination of pregnancy

Pomar. Fetal blood sampling in congenital cytomegalovirus infection. Am J Obstet Gynecol 2024.

pregnancies had continued, which could also lead to an underestimation of the proportion of moderately to severely symptomatic infections. However, 29% and 43% of fetuses included in our study had severe prenatal abnormalities and moderate to severe symptomatic CMV infection, which is close to the expected proportions following maternal infection in the periconceptional period or first trimester.[4,6,44] These proportions might be overestimated in our study, as systematic screening for CMV was not recommended in Switzerland before 2020. Thus, we cannot exclude an overrepresentation of fetuses found to be CMV-infected after the discovery of suggestive fetal signs at ultrasound. This second scenario seems unlikely, as most of the included patients were followed at the University of Lausanne or in partner practices proposing systematic CMV screening (despite Swiss recommendations) for many years. No patients received valacyclovir before amniocentesis and FBS; thus, it is not expected that treatment with valacyclovir modified the values of CMV DNA in amniotic fluid or fetal blood at the time of FBS. However, even if the proportion of patients treated with valacyclovir as tertiary prevention (after the results of FBS) did not differ between the groups, we cannot exclude that this treatment decreased the expected proportion of symptomatic disease among fetuses having high viral loads or thrombocytopenia at FBS, as it is expected.[24,25] Similarly, children with symptomatic congenital infections at birth have been treated with valganciclovir, which may also reduce the rate of expected sequelae in these children. This could result in an underestimation of the PPV of high viremia, high β2m, or moderate thrombocytopenia for symptomatic infection in fetuses and children that have not received antiviral treatments. Finally, the use of thrombocytopenia as both a predictive factor and an outcome criterion could lead to a classification bias. However, the fact that isolated and transient thrombocytopenia alone does not constitute a criterion for being considered "moderately to severely symptomatic" mitigates this potential classification bias. In addition, the 2 severely symptomatic children with thrombocytopenia at birth in our study had other signs and symptoms from birth and cerebral palsy in childhood for one and bilateral SNHL for the other.

Conclusion

The prognosis of fetuses with congenital CMV infection can be reliably assessed by a combination of antenatal imaging and FBS. Fetuses who do not have severe brain abnormalities, thrombocytopenia, high viremia, and high β2m could have a very low risk of moderate to severe symptomatic CMV infection during childhood. The validity of this algorithm for fetuses who received secondary prevention with valacyclovir during pregnancy and for the detection of abnormalities in the longer term remains to be proven. Nevertheless, pregnant women whose fetus shows no or

moderate signs of congenital CMV infection should be informed of the possibility of reducing uncertainty about their prognosis through FBS after counseling about the additional risk associated with this procedure.

CRediT authorship contribution statement

Léo Pomar: Writing — original draft, Validation, Supervision, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Agathe Contier:** Writing — original draft, Methodology, Formal analysis. **Milos Stojanov:** Writing — review & editing, Investigation, Formal analysis, Conceptualization. **Cécile Guenot:** Writing — review & editing, Investigation. **Joanna Sichitiu:** Writing — review & editing, Investigation. **Anita C. Truttmann:** Writing — review & editing, Validation, Data curation. **Yvan Vial:** Writing — review & editing, Validation, Data curation, Conceptualization. **David Baud:** Writing — original draft, Supervision, Resources, Project administration, Methodology, Investigation, Conceptualization. ■

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