



# Testing for Cytomegalovirus in Pregnancy

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**ABSTRACT** Congenital cytomegalovirus (CMV) infection represents a relevant cause of deafness and neurological damage in newborns. Intrauterine CMV transmission might result after primary or nonprimary infections, though at different rates (30% versus 0.2%, respectively). At present, a prenatal diagnosis of CMV infection is based mainly on maternal serology, the detection of CMV-DNA in amniotic fluid and fetal blood, and ultrasound (US) and magnetic resonance imaging (MRI). Recent evidences suggest that congenital CMV infection may be an immune-mediated disease and that evaluation of humoral and especially T-cell immunities may improve the overall prenatal diagnosis. This review summarizes the most recent advancements in the diagnosis of maternal and prenatal CMV infections.

**KEYWORDS** human cytomegalovirus, laboratory assays, congenital infections, IgG avidity, CMV-ELISPOT, CMV-QuantIFERON, immunoserology, imaging

The human cytomegalovirus (CMV) is a ubiquitous betaherpesvirus causing morbidity and mortality in immunocompromised patients and congenitally infected fetuses and newborns, resulting in a broad range of disabilities, including sensorineural hearing loss (SNHL), visual impairment, and motor and cognitive deficits. Other transient symptoms of congenital CMV infection (cCMV) may include hepatosplenomegaly, thrombocytopenia, and jaundice. The global prevalence of cCMV has been estimated at 0.7% (1), and fetal CMV transmission may arise from a maternal primary or nonprimary infection. The highest rate of cCMV occurs after primary infections in seronegative mothers (30 to 40%), while nonprimary infections, including CMV reactivations or reinfections, result in cCMV in 0.2 to 2% of cases, suggesting that preconceptional immunity may play a role in preventing intrauterine transmission (2). If the main burden of congenital infections in Europe and North America results from primary infections, then nonprimary infections represent the main cause of cCMV in developing countries or in poor socioeconomical contexts, since the seroprevalences among the resident populations are much higher (3). The severities of infections in neonates and infants are highly variable. It has been estimated that 10 to 15% of congenitally infected neonates are symptomatic at birth, and 40 to 58% of them will experience permanent long-term sequelae. Moreover, 13.5% of children with asymptomatic infections will develop late-onset sequelae, mainly consisting of hearing impairments and neurologic deficits. The effectiveness of antiviral treatment during pregnancy is still debated. Most of the concerns are related to the potential genotoxicity and teratogenicity of the drugs. The clinical benefits of CMV-specific hyperimmune globulin treatment are also disputed, due to discordant results obtained from different studies (4, 5).

In the last decade, major efforts have been made to improve the early laboratory diagnosis of maternal and fetal infections. Despite these efforts, at present, many countries in the world have not yet adopted a consolidated CMV screening program for pregnant women. This review presents the currently available tools and strategies for

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**TABLE 1** Methods for diagnosing cCMV

Method	Use	Limitations	cCMV transmission [reference(s)]	
			PPV (%)	NPV (%)
Maternal serology <sup>a,b</sup>				
Anti-CMV IgM	Diagnosis of maternal CMV infection	Aspecific and crossreactive results described; CMV IgM may persist long after (re-)infection	9.8–28.5 [51]	100.0 [51]
Seroconversion	Diagnosis of maternal CMV infection	Baseline and follow-up samples are required	25.0 [51]	100.0 [51]
Anti-CMV IgG avidity	Discrimination between primary and nonprimary maternal CMV infection	Less informative after >18 week of gestation, cutoff thresholds vary among different assays	18.8–26.3 [52]	90.9–100.0 [52]
CMV DNA in maternal blood <sup>a,b</sup>	Diagnosis of active maternal CMV infection	Decreasing sensitivity after the first month postinfection	56.3 [53]	64.7 [53]
Amniocentesis <sup>b</sup>				
CMV culture	cCMV diagnosis	High turnaround time	100.0 [6, 50]	84.6–92.6 [6, 50]
CMV DNA	cCMV diagnosis	False-positive results described	93.8–100.0 [6, 50]	92.7–93.1 [6, 50]
Cordocentesis <sup>c</sup>				
CMV culture	cCMV diagnosis	High turnaround time	100.0 [6]	64.6 [6]
CMV DNA	cCMV diagnosis	None described	100.0 [6, 50]	84.4–94.4 [6, 50]
Anti-CMV IgM	cCMV diagnosis	Low sensitivity; only ≥20 weeks of gestation	100.0 [6, 50]	70.6–88.0 [6, 50]
Imaging examinations				
Ultrasound	Predict cCMV outcome and severity	Sensitivity dependent on the timing of examination; not specific for CMV; complex interpretation requiring highly specialized medical personnel/neuroradiology consulting	22.2–70.6 <sup>d</sup> [42, 54]	88.3–95.7 <sup>d</sup> [42, 54]
Magnetic resonance imaging	Predict cCMV outcome and severity	Sensitivity dependent on the timing of examination; not specific for CMV; complex interpretation requiring highly specialized medical personnel/neuroradiology consulting	19.2–66.7 <sup>d</sup> [42, 54]	79.5–100.0 <sup>d</sup> [42, 54]

<sup>a</sup>Limited correlations with cCMV.

<sup>b</sup>Not associated with cCMV severity.

<sup>c</sup>Higher risk of fetal damage/loss compared to amniocentesis.

<sup>d</sup>Percentages for symptomatic cCMV in newborns.

diagnosing CMV infection in pregnancy and discusses the current drawbacks and future perspectives of prenatal cCMV diagnosis.

## DIAGNOSIS OF MATERNAL CMV INFECTION

**Clinical signs and symptoms in pregnant women.** In pregnant women, CMV infection is often pauci- or asymptomatic, and for this reason, it may be unnoticed clinically. Whenever present, the symptoms are generally nonspecific and resemble a mononucleosis- or flu-like syndrome, with fever, cervical lymphadenopathy, sore throat, fatigue, and myalgia. Laboratory findings may include lymphocytosis and elevated liver enzymes. Clinical symptoms are more likely to be present in primary-infected pregnant women than in women with recurrent infections or reactivations (6). Thereby, the diagnosis of maternal infection must take into account anamnestic data, including known or accidental CMV exposures, and clinical and laboratory data.

## SEROLOGY

**CMV IgG detection.** Serologic assays are the primary tools for assessing primary CMV infections during pregnancy. Table 1 summarizes the current uses, limitations, positive predictive values (PPVs), and negative predictive values (NPVs) in regard to fetal infections. At present, the gold standard for determining primary infections is CMV seroconversion, which is the detection of CMV IgG in a previously known nonimmune pregnant woman. However, since preconceptional serologic screening for CMV is not

routinely performed in several countries, seroconversion data may not always be available. As a surrogate method, the detections of CMV IgM and low-avidity IgG have been proven to be effective for the serologic diagnosis of primary infections (7).

**CMV IgM detection.** CMV IgM antibodies are present during primary and nonprimary infections, and thus, are not really informative for determining seroconversion. CMV IgM might persist up to 6 to 9 months after infection, and false-positive results may be due to aberrant and nonspecific crossreactive IgM (mostly from herpes simplex virus [HSV], varicella-zoster virus [VZV], and Epstein-Barr virus [EBV] infections) or to the interference from rheumatoid factor or other autoimmune disorders. The majority of the commercial serologic tests are based on enzyme-linked immunosorbent assays (ELISAs) that detect antibody responses to viral lysates covering a large repertoire of CMV epitopes. Studies comparing the performances of CMV IgM immunoenzymatic tests using native versus recombinant antigens revealed that the latter had lower sensitivities and specificities, probably due to antigen misfolding by a prokaryotic expression system (8). Interestingly, Busse et al. observed that the highest specificity was achieved with the Genzyme Virotech CMV ELISA, which combines crude viral lysates and the recombinant immediate-early protein 1 (IE1) (9).

Several semi- or fully automated commercial platforms assessing both CMV IgM and IgG are available and enable the processing and analyses of large batches of samples, while reducing potential operator-induced errors. The main methods include ELISAs, microparticle enzyme immunoassays (MEIAs), and chemiluminescent microparticle immunoassays (CMIAAs). Comparisons among these systems highlighted excellent agreement rates ( $\geq 93\%$ ), but suboptimal correlations for IgM detection (55 to 79%, depending on the platform) (10). These discrepancies may be due to the extraordinary high sensitivities of some systems, which may detect extremely low levels of IgM that were either produced during CMV reactivation or persisted long after the acute infection (6). For the above considerations, it is recommended that any positive CMV IgM result be confirmed by different tests and interpreted only in the context of a complete serology panel for CMV (IgM, IgG, and IgG avidity index) (6, 10).

**CMV IgG avidity determination.** The CMV IgG avidity assay is considered a primary tool to date the timing of an infection. This test is based on the notion that IgG avidity increases with time; low-avidity IgGs are associated with recent infections, while a high avidity index indicates past infections. The avidity is easily determined by coincubating the patient's serum with and without 6 M urea as a dissociating agent in an immunoenzymatic assay. The most important limitations of the CMV IgG avidity assays are the following. (i) There is variability in the ranges of low- and high-avidity thresholds among the different commercial kits available (11). (ii) The timing of the assay execution may critically affect the NPV. In fact, while a high IgG avidity index detected in the first trimester properly identifies past infections (NPV, 100%), intermediate-to-high values obtained after 21 weeks of pregnancy cannot rule out a primary infection (NPV, 90.9%) (6). (iii) The reported unusually long persistence ( $>18$  weeks) of low-avidity CMV IgGs may potentially result in the misdiagnosis of a primary CMV infection, particularly when CMV IgMs are also detectable in serum (12). The reason for the latter phenomenon is still unclear. Studies evaluating the kinetics of CMV IgG avidity maturation in primary-infected pregnant women found different patterns within the same cohort analyzed; patients who displayed a rapid increase in the IgG avidity index had a higher risk of vertical CMV transmission (13). Thus, there is evidence that the duration and intensity of CMV viremia may directly affect the kinetics of IgG avidity maturation. This hypothesis was corroborated by Fornara and colleagues (14) who compared IgG titers and neutralizing antibodies from symptomatic, paucisymptomatic, and asymptomatic pregnant and nonpregnant patients. Remarkably, the authors also observed that anti-gB IgGs were the first-produced antibodies after a primary infection, followed by neutralizing antibodies and lastly by gH/gL/pUL128/130/131 and gH/gL IgG antibodies.

## EPITOPE-SPECIFIC IgG IMMUNOBLOTS TO ASSESS PRIMARY VERSUS NONPRIMARY INFECTIONS

Epitope-specific IgG immunoblotting was investigated by Eggers et al. (15) to explore the potentially different patterns in primary versus nonprimary infections. The authors compared an in-house-developed anti-gB/gH epitope immunoblot assay to a standard microneutralization method. The authors showed that the CMV neutralizing and anti-gB/gH antibodies appeared 10 to 17 weeks after an infection, suggesting that gB/gH-specific immunoblotting may represent a useful bioassay to distinguish primary from nonprimary infections. This in-house-built Western blot presented a 93.6% sensitivity and 100% specificity for nonprimary infections. Moreover, Eggers et al. reported that a delayed anti-gB IgG appearance in primary infections was associated with a lower risk of intrauterine CMV transmission. Enders and colleagues (16) corroborated the immunoblot assay performances, suggesting its validity in determining the timing of CMV infection. Despite the diagnostic usefulness shown in these two reports, epitope-specific immunoblot assays are not commonly employed for CMV serologic diagnosis, since the assay is not standardized, is time-consuming, and requires complex data interpretation.

**Nonprimary infections: reinfection or reactivation?** Boppana and colleagues hypothesized that reinfections with a different CMV strain may lead to a higher risk of cCMV compared with that from viral reactivations (17). In this scenario, the serologic evaluation of the anti-gB/gH IgG polymorphic regions response was proposed as a potential system to distinguish whether the maternal infection arose from different CMV strains or from CMV reactivation (18). In that study, the authors showed that 18/96 serum samples tested, which were obtained from CMV-seropositive women, had positive results for at least 2 CMV antigenic epitopes, suggesting that serologic analysis may distinguish CMV reactivation from coinfection and reinfection. One important caveat of this system is the requirement for a prior patient serology specimen to use as a reference. The evaluation of the serologic response to polymorphic CMV epitopes would have promising applications in the assessment of cCMV risk, particularly in the populations characterized by a high CMV seroprevalence, where recurrent infections are more likely to occur.

## DETECTION OF CMV IN BIOLOGICAL FLUIDS

Molecular methods comprise the most common and standardized systems for detecting CMV nucleic acids to diagnose active CMV replication. Alternatively, direct viral isolation in cell culture provides the biological evidence for actively replicating virus.

**Biological specimens required for CMV diagnosis.** CMV can be isolated from several biological samples, including blood, urine, saliva, semen, vaginal secretions, and amniotic fluid. After collecting, the samples should be stored at 4°C and immediately sent to the laboratory for testing, especially when requesting CMV isolation from cell culture. Even though CMV is excreted in multiple biological fluids, blood and urine are the most commonly used specimens for testing. CMV shedding in urine is known to persist intermittently for a long time after a primary infection; thus, urine testing increases the chances of detecting an ongoing infection. The detection of virus in blood is mostly found in primary-infected pregnant women (19). Viremia usually persists for one month after a primary infection; however, it has also been reported in seropositive women with recurrent infections, though at a lower rate (20, 21).

**Direct isolation of CMV.** Viral isolation in cell culture is still considered the gold standard for diagnosing CMV infection, since it provides evidence of actively replicating virus. Urine specimens are very often used for isolating CMV in cell culture, whereas CMV isolation from peripheral blood is rarely performed, given the low sensitivity of the assay (6, 21). Primary human fibroblasts, mink lung epithelial cells (Mv1Lu), MRC-5 fibroblasts, and more recently, R-mix cells are permissive for CMV entry and replication and can be used for CMV diagnosis (22). The first and most relevant limitation of the viral culture method is the turnaround time required for viral replication. In the

presence of a high viral load, CMV foci may be evident 24 h after inoculating, but whenever the viral load is low, the CMV cytopathic effect may be visible only after 10 to 30 days. The second most important limitation related with viral culture is that contaminant microbial flora in the biological specimens may lead to cell culture contamination and toxicity. Other limitations of the culture assay are the standardization and interpretation of microscopic morphologies.

Rapid isolation of CMV by the shell vial method has been proposed as an alternative to standard cell culture and enables the detection of CMV within 24 h. The method requires direct and indirect immunofluorescence assays detecting the CMV immediate early IE1/IE2 proteins, which correlate with the presence of actively replicating virus. Studies performed over 21 to 30 days showed a good correlation between rapid and standard cell culture isolations, although sample cytotoxicity, defective and aberrant CMV particles, and low viral titers may affect the assay performances (23, 24). Despite the fact that the shell vial method is considered more sensitive than standard culturing, one report suggests that both assays should be used to maximize CMV detection, especially when blood specimens are used (23).

**Molecular methods for CMV diagnosis.** Molecular assays have revolutionized the diagnosis of CMV infection, overcoming almost completely the previous cited methods. Several systems are available, including quantitative assays (nucleic acid sequence-based amplification [NASBA], the hybrid capture assay, and PCR) and qualitative PCR, nowadays rarely employed (25). Real-time PCR is the most commonly used method for the molecular diagnosis of CMV infections, due to its excellent sensitivity and specificity and to the availability of commercial kits and automated platforms. Moreover, PCR performance is less affected by specimen transport and storage (19). An important limitation of quantitative PCR lies in the interassay and interlaboratory variability of copy number determination, which might impact patient management and clinical decisions. Recently, the WHO released a CMV international standard to overcome the interassay and interlaboratory issues with the aim of reaching an interlaboratory variability of  $<0.5 \log_{10}$  UI/ml (26). However, the multicentric study conducted with the WHO CMV standard showed interlaboratory variability higher than the expected target of  $0.5 \log_{10}$  UI/ml, so the route to CMV harmonization and standardization still requires further improvements.

### **MATERNAL CMV-SPECIFIC T-CELL IMMUNITY AS A MARKER FOR INTRAUTERINE INFECTION**

Interferon gamma release assays (IGRA) are widely used to detect patients' cell-mediated immunities (CMI). Enzyme-linked immunosorbent spot (ELISPOT) and QuantiFERON (QFT) assays are the most standardized and employed platforms and were recently used to detect CMV-specific CMI in solid organ and allogeneic hematopoietic stem cell transplant recipients (27, 28). Although their performances for CMV CMI were very similar, interassay differences were found (29, 30). Recently, several studies assessed the role of CMV CMI in pregnant women. Bialas and colleagues showed that  $CD4^+$  T-cell depletion in infected pregnant rhesus macaques led to dismal and more severe outcomes compared with those of immunocompetent pregnant primates, thus suggesting a protective role of  $CD4^+$  T cells with respect to cCMV (31). A previous study of primary-infected pregnant women had already identified CMV-specific  $CD4^+$  T cells as key players in maternal CMV infection, and a low lymphoproliferative response associated significantly with an increased risk of congenital transmission (32). In a study of a cohort of 80 pregnant women with active CMV infections, our group observed that pregnant women with primary infections had significantly higher CMV T-cell immune responses compared with those with nonprimary infections. Furthermore, in primary-infected cases, the maternal CMV-specific T-cell immunity positively correlated with congenital transmission (33). Although the results from interferon (IFN)- $\gamma$  ELISPOT and CMV-QFT assays were correlated, only the ELISPOT assay discriminated between transmitter and nontransmitter mothers (34, 35). In particular, a higher risk of cCMV was observed when maternal CMV T-cell responses were  $>185 \text{ spots}/2 \times 10^5$  peripheral

blood mononuclear cells (PBMCs). An association of CMI with cCMV was also reported in a recent study employing CMV-QFT. Eldar-Yedidia and colleagues showed that a CMV-QFT response normalized to the positive control was associated with an increased risk of congenital infection (36). Although the above-mentioned studies of Saldan and colleagues and that of Eldar-Yedidia and colleagues find that the maternal CMI is a predictor of vertical CMV transmission, these studies differ in the performances of CMV-QFT for assessing cCMV. Larger studies should be performed to assess whether CMV-QFT predicts vertical CMV infection.

### CONGENITAL CMV INFECTION AS AN IMMUNE-MEDIATED DISEASE?

Serological and immunological studies found that cCMV occurred in the presence of high  $T_H1$  (IFN- $\gamma$  ELISPOT) and low and delayed  $T_H2$  (CMV IgG avidity) maternal responses. These findings suggest that an imbalanced maternal  $T_H1/T_H2$  immune response may lead to cCMV. An altered cytokines pattern was previously reported at the placental level in cCMV cases. In 2012, Hamilton et al. reported increased monocyte chemoattractant protein 1 (MCP-1) and tumor necrosis factor (TNF)- $\alpha$  levels in placentae from CMV-infected stillborn neonates and in placental histocultures following CMV infection (37). Recently, it was also reported that CMV infection of the maternal decidua led to higher expressions of IFN- $\gamma$  and IFN- $\gamma$ -induced protein 10 (IP-10) (38). Perturbation of the cytokine immune balance at the placental and decidual levels and the triggering of a strong  $T_H1$  immunity may, in fact, favor viral dissemination to the fetus and also reduce nutrient uptake and gas exchange across the placenta, an alteration that could ultimately lead to tissue hypoxia and explain the neurological abnormalities observed in symptomatic babies. We speculate that an altered placental cytokine pattern, along with an imbalanced T-cell response in peripheral blood, may lead to focal lesions at the placenta, allowing CMV crossing.

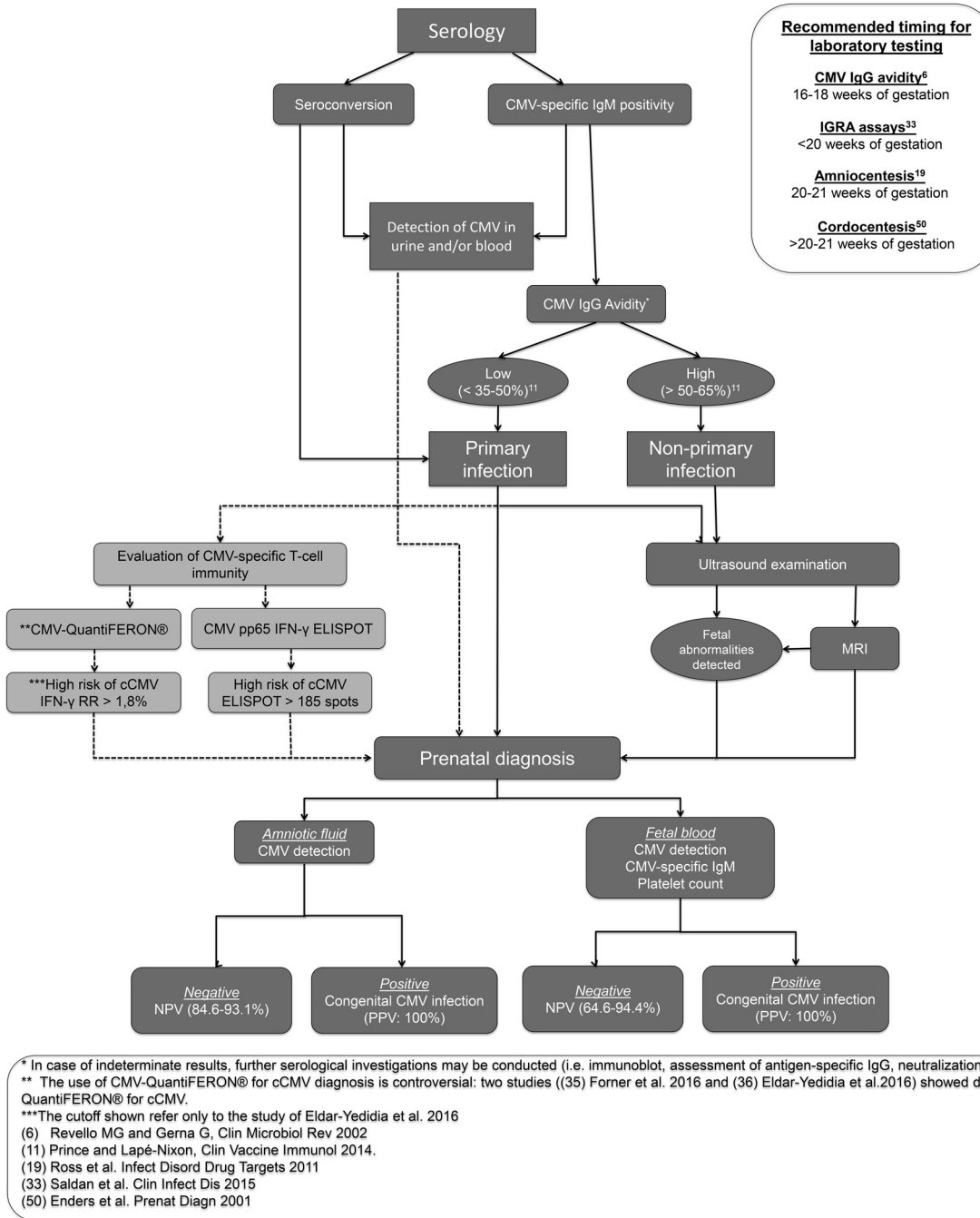
### PRENATAL DIAGNOSIS OF cCMV

At present, invasive and noninvasive diagnoses of fetal infections can be offered to pregnant women with active CMV infection, particularly to primary-infected women at high risk of cCMV (Fig. 1). Invasive methods are based on amniocentesis and cordocentesis, while noninvasive methods include ultrasound (US) and magnetic resonance imaging (MRI).

**Amniocentesis.** Prenatal diagnosis of cCMV can be accomplished either by viral culture or by detecting CMV DNA in an amniotic fluid specimen. Due to a higher sensitivity (90 to 100%), molecular diagnosis by PCR is presently preferred over CMV culture (19). As reported in Table 1, molecular methods show the highest PPVs (100%) for diagnosing fetal infections. However, the criteria for improving the NPV involve performing amniocentesis (i) 6 to 8 weeks after the estimated onset of maternal infection, and (ii) from 20 to 21 weeks of pregnancy onwards, when CMV excreted from fetal kidneys into the amniotic fluid becomes detectable. Even when following these indications, false-negative results were reported (6). Whether evaluating CMV viral loads in amniotic fluid predicts disease severity in newborns remains controversial. Some authors suggest that viral loads  $>10^5$  genome equivalents (GEq)/ml are highly indicative of symptomatic disease, whereas values  $<10^3$  GEq/ml exclude severe cCMV forms (19, 39, 40). Opposing results regarding the association between symptomatic disease and viral loads in amniotic fluid have been reported. Particularly, some authors found asymptomatic infants with viral loads  $>10^5$  GEq/ml in the amniotic fluid and symptomatic infants with viral loads  $<10^5$  GEq/ml (19). These discrepancies may result from differences in the timing for amniocentesis executions (19). For this reason, CMV DNA detection in amniotic fluid should consider not just the viral load by itself, but also the amniocentesis timing (40).

**Cordocentesis.** Cordocentesis enables the evaluations of fetal blood infections, CMV IgM, and other hematological and biochemical parameters. Given that this invasive procedure carries a higher risk of adverse events compared with that from amniocentesis, its utility in the prenatal diagnosis of cCMV is still controversial. In fact,





**FIG 1** cCMV diagnosis flowchart. The novel findings on cCMV diagnosis are indicated in light gray. Relevant references for cCMV diagnosis are also reported.

albeit showing 100% specificity, fetal CMV DNAemia and fetal serology have reduced sensitivities compared with that of amniocentesis (39). On the other hand, there is evidence that fetal CMV viral load and IgM titers tend to be higher in symptomatic fetuses (6), and more recently, thrombocytopenia was reported as a prognostic biomarker for cCMV severity (40). Given that cordocentesis does not improve the prenatal diagnosis of cCMV and it is potentially harmful to the fetus, the procedure should not be universally recommended, unless specific ethical and medical reasons suggest the execution of this approach.

**CMV imaging in pregnancy.** Imaging tests during pregnancy can detect morphological fetal abnormalities compatible with cCMV, and may thus be useful both for the

diagnosis of and prognosis for cCMV severity. Due to concerns for potential teratogenicity, computed tomography (CT) scans are not an option for assessing fetal development. Targeted US and MRI are the most commonly used systems for detecting fetal abnormalities. CMV infection may cause ventriculomegaly, periventricular echogenicity with or without cysts, intracranial calcifications, microcephaly, and cortical migrational abnormalities. Extracranial abnormalities can also be observed, mainly bowel hyper-echogenicity, hepatosplenomegaly, and intrauterine growth retardation.

**(i) Ultrasound imaging.** In the absence of a serologic screening program for CMV infection in pregnancy, US findings during the second or third trimester of pregnancy may provide the first evidence of presumptive cCMV. If the diagnosis of maternal and fetal infection by amniocentesis has already been established, regular US assessment every 3 to 5 weeks is recommended to detect signs of symptomatic fetal infection. Discordant results from several studies on US predictivity were reported for clinical outcomes of infected fetuses (41, 42), suggesting that the timing for US execution and transient fetal morphological features might critically influence the diagnosis. In fact, at present, only severe cranial abnormalities, such as ventriculomegaly and microcephaly, have been unequivocally associated with poor prognoses (43). Neurosonographic tests may improve the diagnostic performance, reaching PPV and NPV values comparable to those reported for MRI (44).

Recently, the combination of US features and fetal thrombocytopenia at the time of prenatal diagnosis showed 79% PPV and 91% NPV in predicting any symptoms at birth or at the termination of pregnancy, while US alone reached 93% NPV, similar to those of US and MRI executed during the third trimester (40 and reported in Table 1). The combined use of imaging and laboratory testing specifically pinpoints cCMV cases, since other common congenital infections (mainly toxoplasmosis, rubella, HSV, and VZV infections) and chromosomal disorders share similar neurological alterations indistinguishable by US alone (43).

**(ii) MRI.** MRI is advised whenever fetal intracranial abnormalities are detected by US and should be performed during the third trimester (54). MRI was shown to be more sensitive than US (45), though results might be more difficult to interpret, and specialized neuroradiology consultation is required. It is generally accepted that negative MRI findings concomitant with negative US results reliably exclude severe outcomes for infected fetuses (46).

## TO SCREEN OR NOT TO SCREEN: THE HAMLETIC DILEMMA AND FUTURE PERSPECTIVES

As previously stated, at present, a universal screening program for CMV infection in pregnant women has not been implemented worldwide for several reasons, including: (i) because safe and effective therapies and vaccines for preventing intrauterine transmission do not exist, (ii) because symptomatic cCMV might occur after primary or nonprimary maternal infection, and (iii) because only a limited proportion of congenitally infected newborns are symptomatic at birth or develop late-onset CMV-related sequelae. Several European countries (Austria, Belgium, France, Germany, Italy, the Netherlands, Portugal, and Spain) and Israel *de facto* offer serologic CMV screening and counseling to advise pregnant women on preventive strategies and eventual laboratory reevaluation (47). In other countries, such as in the United States, CMV testing is recommended only (i) when there is clinical suspicion of CMV infection, or (ii) fetal abnormalities are detected by routine imaging (48).

At present, there is a large debate on the costs for CMV screening. The estimated costs reported from U.S. studies refer only to the postnatal diagnosis and treatment of cCMV (49). Undoubtedly, any cost for maternal screening should consider not just the direct costs, but also the indirect future burden for disability management and detrimental social impact. Based on long-term experience, we have reasons to believe that maternal screening provides superior and beneficial results in both the short and long terms. However, relevant challenges need to be overcome in the near future to optimize maternal CMV screening, with a need to develop (i) more sensitive and



specific assays to specifically pinpoint vertical transmission in nonprimary infections, (ii) novel noninvasive maternal biomarkers capable of assessing early cCMV transmission, and (iii) biomarkers predictive of the severity of fetal abnormalities.

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