



Congenital Cytomegalovirus Infection: A Narrative Review of the Issues in Screening and Management From a Panel of European Experts

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Maternal primary and non-primary cytomegalovirus (CMV) infection during pregnancy can result in *in utero* transmission to the developing fetus. Congenital CMV (cCMV) can result in significant morbidity, mortality or long-term sequelae, including sensorineural hearing loss, the most common sequela. As a leading cause of congenital infections worldwide, cCMV infection meets many of the criteria for screening. However, currently there are no universal programs that offer maternal or neonatal screening to identify infected mothers and infants, no vaccines to prevent infection, and no efficacious and safe therapies available for the treatment of maternal or fetal CMV infection. Data has shown that there are several maternal and neonatal screening strategies, and diagnostic methodologies, that allow the identification of those at risk of developing sequelae and adequately detect cCMV. Nevertheless, many questions remain unanswered in this field. Well-designed clinical trials to address several facets of CMV treatment (in pregnant women, CMV-infected fetuses and both symptomatic and asymptomatic neonates and children) are required. Prevention (vaccines), biology and transmission factors associated with non-primary CMV, and the cost-effectiveness of universal screening, all demand further exploration to fully realize the ultimate goal of preventing cCMV. In the meantime, prevention of primary infection during pregnancy should be championed to all by means of hygiene education.

Keywords: cytomegalovirus, neonatal screening, prenatal diagnosis, clinical laboratory techniques, congenital CMV, pregnancy, maternal screening

INTRODUCTION

Maternal primary and non-primary infection (exogenous reinfection with a different strain or endogenous viral reactivation) of cytomegalovirus (CMV) during pregnancy can result in *in utero* transmission to the fetus (1). Infants can be categorized as symptomatic or asymptomatic based on clinical symptoms/signs (**Table 1**) (2). Approximately 11% of live-born infants born with congenital CMV (cCMV) have abnormal clinical findings at birth (symptomatic) (3). Infants can experience

TABLE 1 | Possible signs and symptoms in children with congenital CMV (reproduced from Luck et al.).

CLINICALLY DETECTABLE SYMPTOMS/SIGNS
Physical examination
Small for gestational age (birth weight < -2 standard deviations for gestational age)
Microcephaly (head circumference < -2 standard deviations for gestational age)
Petechiae or purpura (usually found within hours of birth and persist for several weeks)
Blueberry muffin rash (intra dermal hematopoiesis)
Jaundice ^a
Hepatomegaly
Splenomegaly
Neurologic physical examination
Microcephaly (head circumference < -2 standard deviations for gestational age)
Neurologic signs (lethargy, hypotonia, seizures, poor sucking reflex)
ABNORMALITIES DETECTED INCIDENTALLY OR THROUGH SUBSEQUENT INVESTIGATION/SPECIALIST EXAMINATION
Laboratory results
Anemia
Thrombocytopenia (occurs in the first week but platelets often increase spontaneously after the second week)
Leukopenia, isolated neutropenia
Elevated liver enzymes (alanine aminotransferase/aspartate aminotransferase)
Conjugated hyperbilirubinemia
Cerebrospinal fluid
Abnormal cerebral fluid indices, positive CMV DNA
Neuroimaging
Calcifications, periventricular cysts, ventricular dilatation, subependymal pseudocysts, germinolytic cysts, white matter abnormalities, cortical atrophy, migration disorders, cerebellar hypoplasia, lenticuloatrial vasculopathy
Hearing test
Sensorineural hearing loss uni- or bilaterally
Visual examination
Chorioretinitis, retinal hemorrhage, optic atrophy, strabismus, cataracts

CMV, cytomegalovirus.

^aCMV-associated jaundice can be present at the first day after birth and usually persists longer than physiologic jaundice.

substantial morbidity, mortality, and long-term sequelae, including sensorineural hearing loss (SNHL), the most common sequela (4, 5). Infants without symptoms at birth are also reported to be at risk of developing long-term hearing loss (6). As a leading cause of congenital infections worldwide (7), cCMV infection meets many of the criteria for screening: it is clinically important, well defined and prevalent (4). Nevertheless, neither universal antenatal screening for CMV during pregnancy nor universal neonatal screening is routinely recommended (8) and there remain several challenges that impede their implementation. Roche Centralised and Point of Care Solutions and Roche Molecular Diagnostics convened a group of CMV experts (microbiologists, virologists, and clinicians) to discuss and offer strategies to address these barriers and knowledge gaps. This paper provides an overview of those discussions and is a

Abbreviations: cCMV, Congenital cytomegalovirus; CMV, Cytomegalovirus; DBS, dried blood spots; IgG, Immunoglobulin G; IgM, Immunoglobulin M; MRI, magnetic resonance imaging; PCR, polymerase chain reaction; SNHL, Sensorineural hearing loss.

narrative review of serologic and viral nucleic acid screening and diagnostics in the context of maternal, fetal and neonatal CMV infection.

MATERNAL CMV SCREENING

CMV screening is offered to some pregnant women in parts of Europe, Israel, Australia and the USA in the setting of population-based studies, and independently of nationally endorsed screening programs (9). However, universal antenatal screening for CMV is not routinely recommended (8). Reasons not to screen include the absence of medication to prevent transmission and the difficulty of predicting sequelae (10).

The introduction of routine testing for CMV in pregnant women has several implications. Despite the difficulties mentioned above, the most important benefit of screening would be to identify fetuses at risk of developing sequelae.

Maternal screening, ideally early in the first trimester, would also identify those who were CMV-seronegative and thus allow information to be provided regarding hygiene and behavioral measures to prevent CMV infection. Evidence has shown that intervention based on the identification and hygiene counseling of CMV-seronegative pregnant women significantly prevents maternal infection (11). Hygiene counseling may also provide (as yet unproven) benefits for those who are seropositive.

During early pregnancy, repeat serologic screening with CMV-specific immunoglobulin G (IgG) and -M (IgM) antibodies of previously seronegative pregnant women at the end of the first trimester (or until week 20) would identify maternal primary CMV infection. Although there are no universally accepted guidelines, testing before 18–20 weeks of pregnancy is reasonable in order to identify late seroconversion at the end of the first trimester and implement fetal investigations. In the event of seroconversion, parents should be informed of the risk of vertical transmission [32% (3)] and the possible consequences.

Other strategies that could be put in place include maternal screening at the first prenatal visit and at birth, and neonatal screening at birth for those whose mothers tested seropositive, with diagnosis of the neonate by saliva or urine CMV DNA detection. Previously, this approach to targeted testing detected 82% of all cCMV infections (12). Notably, this study utilized culture for diagnosis; the detection rate is likely to be improved with polymerase chain reaction (PCR). This approach may be more cost-effective than screening all children.

However, screening tests do not identify which mothers will transmit the virus (13). Moreover, there are no surrogate markers to predict whether infection in the infant will lead to long-term sequelae.

Lastly, there are also risks for the mother associated with maternal screening for CMV that include the stress of having extra tests, the potential for unnecessary terminations (14), the potential risk of miscarriage or stillbirth from confirmatory amniocentesis [inversely correlated to skill/experience of operator (15)], and the cost. The challenge is in providing women with choice and information in the context of population-based economics.

Serologic and Molecular Testing for Maternal CMV Infection

Primary infections can be identified by serologic testing. During pregnancy, IgG and IgM serology is the preferred option; IgG avidity testing should be used only if CMV-specific IgM antibodies are positive. Many laboratories consider IgM-positive results, in combination with IgG avidity results, to discriminate between primary and non-primary CMV infections (16). Low CMV IgG avidity indicates primary infection within the preceding 3–4 months, with an increased risk of intrauterine transmission to the fetus (17).

CMV-specific serology testing is most useful in the first trimester due to the increased severity of disease when primary infection occurs during the embryonic or early fetal period (18). Quantitative IgG testing is helpful to detect seroconversion and the stage of infection. Low levels of CMV-IgG antibodies in maternal serum samples present challenges for the clinician: low IgG levels can be associated with both a true positive or a false-positive result (19), so clear guidelines are needed for the appropriate interpretation of serology results.

All serologic kits vary considerably in their accuracy of the “low” range of IgG values; a very low value in one test may be negative using a different test. CMV-IgG avidity testing should not be performed on serum samples with low IgG levels as these can give inappropriate IgG avidity results (20). An incorrect classification of primary CMV infection can lead to inappropriate management. Finally, maternal serology screening can be falsely reassuring as non-primary maternal infections will not be recognized: in Europe, this represents around 50% of all cCMV cases (21, 22).

The new WHO standard (23) was established for the calibration of anti-CMV IgG diagnostic kits with quantitative test interpretation and as an aid in the interpretation of serologic results in the framework of different assays, platforms, and clinical settings. The CMV standard could be of value, although in other settings such as rubella or toxoplasmosis screening, the use of a standard for calibrating IgG assays has proven to be suboptimal (24, 25). An algorithm for dealing with low positive IgG samples may be more useful than a WHO standard. In particular, in the absence of a gold standard method, an equivocal IgG serologic assay result in a pregnant woman should be considered negative. This strategy will ensure that these women are assigned to the highest CMV risk group for pregnancy outcome.

The value of viral DNA detection and quantification in blood, saliva, or urine to help determine the timing of maternal infection, or to estimate the risk of fetal transmission, is not yet established. Notably, two studies have demonstrated that persisting levels of maternal DNAemia during primary CMV infection at the moment of amniocentesis correlate with a high risk of CMV transmission to the fetus (26, 27), whilst one other study has shown that the presence of CMV DNA in maternal urine and maternal blood correlated with transmission of CMV to offspring (28).

Prenatal Diagnosis of Fetal CMV Infection

Ultrasound imaging has poor sensitivity in diagnosing fetal CMV infection (29) but is a useful tool to predict the prognosis of

fetal infection. Diagnosis of fetal CMV infection by CMV PCR in the amniotic fluid can be made with high sensitivity and specificity by amniocentesis after 20–21 weeks' gestation (30) [and >8 weeks after estimated maternal seroconversion (31)] and is the best available prenatal diagnostic tool (32). When the diagnosis of fetal infection is by way of amniocentesis, the prognostic evaluation of fetal infection relies on imaging using a combination of ultrasound and cerebral magnetic resonance imaging (MRI). Several studies have identified a residual risk of hearing loss at birth when imaging (ultrasound and/or MRI) examination was considered to be normal (33–38).

NEONATAL CMV SCREENING

Neonatal CMV screening would enable early detection of cCMV (following primary and non-primary maternal infection), but universal neonatal screening for CMV is currently not recommended by any public health body. Data from Uematsu and colleagues emphasize that without neonatal screening some infected neonates that develop neurological sequelae may go unrecognized (39).

Although universal screening is not performed, targeted screening of newborns who fail the neonatal hearing test has been implemented in some hospitals and states in the USA (40, 41). In the UK, Belgium and Australia, targeted testing of infants who were referred for further audiological testing (after failing the routine hearing screening) has also been trialed with some success (42–44). This combination of targeted newborn screening and early detection and interventions is likely to benefit children with cCMV (45).

Additionally, the costs associated with targeted neonatal screening look favorable compared with other screening programs (46, 47). However, this targeted approach would miss those CMV-positive infants who pass the newborn hearing test but are still at risk for late-onset SNHL (40, 48). In one study, 43% of infants with CMV-related SNHL in the neonatal period and cCMV infants who are at risk for late-onset SNHL were not identified by newborn hearing screening (49).

There are risks associated with neonatal screening, such as the potential for parental anxiety while waiting for confirmatory testing results. In addition, there may be anxiety related to the extended period of audiological monitoring that a cCMV-positive infant must undergo [up to 6 years (2, 50, 51)]. Most cCMV infections are asymptomatic and do not present a risk for the onset of late sequelae (5). Recent data demonstrated that primary maternal infections before the 14th week of pregnancy, the presence of a disseminated infection at birth, and imaging abnormalities in the neonate were risk factors for SNHL (52). This is a step toward the development of neonatal predictive markers that can be used to identify those at high risk of developing sequelae.

Diagnosis and Screening of Neonatal Infection

Testing is recommended for those who have any condition that might be indicative of intrauterine CMV infection (2). Traditionally, viral isolation and culture from urine or saliva was

the standard for diagnosing cCMV infection (53). Since PCR exhibits high sensitivity (54, 55), this is now the preferred option. False-positive tests have been reported for saliva, and therefore any positive saliva result should be confirmed by CMV detection in urine (21).

It should be noted that if the diagnosis is made after the first 2–3 weeks of life (2, 9), infection may have been postnatally acquired and attributable to infected breast milk from a seropositive mother (56), rather than cCMV. In this instance, congenital infection must be confirmed by detection of CMV from a sample taken at birth.

Saliva can be used to screen for cCMV; however, as this specimen type is not routinely collected from neonates, a change in infrastructure would be required before this could be rolled out on a large scale. As such, alternative technologies for universal screening are currently under evaluation. Due to widespread utilization in neonatal screening for other conditions, there has been much interest in using dried blood spots (DBS) taken at birth for CMV screening. However, screening DBS is less sensitive than PCR testing of saliva, with sensitivity ranging between 28 and 100% (57), and is contingent upon the method of extraction and DNA amplification and the patient group selected. The recent standardization of viral DNA extraction and innovative PCR techniques has led to improved sensitivity of DBS screening to around 80% (58). A potential limitation in the use of DBS is that only 80–90% of congenitally infected infants have detectable CMV in their blood soon after birth (59, 60). Despite this, the sensitivity of DBS screening has been shown to adequately detect those most at risk of developing SNHL (61).

Stored DBS can be used to diagnose cCMV retrospectively (2). In some countries (e.g., Germany), the use of DBS for retrospective diagnosis or screening of newborns is hampered by the destruction of samples after 3 months (62) due to data protection requirements. Thus, for certain countries, regulatory changes may be necessary to allow long-term storage and use in this context.

WHAT ARE THE GAPS IN OUR UNDERSTANDING?

Although significant advancements have been made, many questions remain unanswered in this field (Table 2). Well-designed clinical trials to address several facets of CMV treatment (in pregnant women, CMV-infected fetuses and both symptomatic and asymptomatic neonates and children) are required. Prevention (vaccines), biology, and transmission factors associated with non-primary CMV, and the cost-effectiveness of universal screening, all demand further exploration to fully realize the ultimate goal of preventing cCMV.

Currently, treatment with immunoglobulins or antiviral therapy to prevent intrauterine transmission of CMV in pregnant women with primary CMV infection is not recommended as studies have not yet conclusively shown a benefit (63–66). Data from a non-randomized study showed that biweekly administration of hyperimmunoglobulin until 20 weeks' gestation successfully prevented maternal-fetal transmission of

TABLE 2 | Studies required to improve the understanding of congenital CMV.

DIAGNOSIS	
Non-primary infection in pregnant women	Identify virologic and immunological markers predictive of cCMV in women seropositive before pregnancy Identify virologic and immunological CMV-specific tests to properly diagnose maternal non-primary infection in pregnancy
Universal neonatal screening	Evaluate the performance of CMV PCR in DBS to identify neonates with sequelae and determine cost-effectiveness
PREVENTION	
Prevention of fetal transmission in maternal primary infection	Randomized controlled studies with new antiviral drugs
Prevention of maternal infection	Randomized controlled studies of optimal education methods and efficacy of hygiene measures in general population
TREATMENT	
Treatment of fetal infection	Randomized controlled studies with new available antiviral drugs
Treatment of infection in the neonate	Randomized controlled studies: <ul style="list-style-type: none"> – with new antiviral drugs; – with known antiviral drugs to confirm the effectiveness of known therapies; – in asymptomatic babies with no clinical examination abnormalities at birth (including those developing later hearing loss); – to determine the optimal duration and dosage of treatment Registries of long-term treatment sequelae

(c)CMV, (congenital) cytomegalovirus; DBS, dried blood spot; PCR, polymerase chain reaction.

primary infections (65). These data need to be confirmed by a randomized clinical trial (67); if the results are confirmed, two-weekly intervals for testing seronegative women would be necessary. It has been suggested that a study that demonstrates treatment efficacy resulting in at least a 47% reduction in cCMV disease would make universal screening and treating for primary CMV in pregnancy cost-effective (10).

Whilst recent improvements in screening and diagnosis allow detection of primary CMV infection in pregnancy, unfortunately treatment options for CMV-infected fetuses and neonates are limited due to insufficient evidence for safety and effectiveness. Consequently, routine antiviral therapy to treat fetal CMV during pregnancy is not recommended (9). In infants with clinical disease at birth, early intervention with ganciclovir or valganciclovir can prevent hearing deterioration and improve developmental outcomes, although both treatments are associated with neutropenia (68, 69) and other possible long-term effects (70). Currently, valganciclovir treatment is recommended based on severity or number of symptoms. A European expert consensus statement recommends that treatment with oral valganciclovir (intravenous ganciclovir under certain circumstances) is only for those with: evidence of central nervous system disease; evidence of life-threatening disease, severe single-organ disease or multi-organ involvement; “moderate” cCMV disease once discussed on a case-by-case basis

TABLE 3 | Expert panel recommendations for the diagnosis, screening, and prevention of CMV infection.

RECOMMENDATIONS FOR DIAGNOSIS	
Primary infection in pregnant women	IgG, IgM, IgG avidity if positive IgM and IgG
Non-primary infection in pregnant women	No tools validated
Fetal infection	CMV PCR in amniotic fluid after 20 weeks and more than 8 weeks after presumed onset of maternal primary infection
Neonatal infection	CMV PCR in saliva or urine collected in the first 3 weeks of life
Retrospective diagnosis in toddlers with compatible symptoms	CMV PCR in neonatal DBS
RECOMMENDATIONS FOR SCREENING AND PREVENTION	
Primary prevention of maternal primary infection	Information for pregnant women on cCMV and application of hygienic measures to prevent maternal infection
Infection in pregnant women	No recommendation for screening Information for pregnant women on cCMV and application of hygienic measures to prevent maternal infection
Universal neonatal screening	No recommendation
Targeted testing in neonates who failed universal hearing screening	CMV PCR in saliva (if positive, confirm in urine or by DBS PCR if the infant is > 3 weeks of age)

(c)CMV, (congenital) cytomegalovirus; DBS, dried blood spot; Ig, immunoglobulin; PCR, polymerase chain reaction.

with a clinician with experience of managing infants with cCMV (2). The informal International Congenital Cytomegalovirus Recommendations Group recommend oral valganciclovir treatment for those neonates with “moderately” to “severely” symptomatic cCMV disease (9). Further development of efficacious antivirals with an acceptable safety profile is required.

Letermovir is a new agent approved for use in the prophylaxis of CMV infection in CMV-seropositive recipients of an allogeneic hematopoietic stem cell transplant over the age of 18 years (71). A recent case study revealed potential efficacy in pediatric allogeneic hematopoietic stem cell transplant patients (72). Further studies are required to determine whether it is safe and effective for treating those with cCMV.

More asymptomatic infants will be discovered if screening programs become widespread. Currently, antiviral therapy for asymptomatic infants is not recommended (2) but, like symptomatic infants, they are at risk of developing late-onset sequelae (53). Trials that investigate which interventions are effective and safe for asymptomatic infants or those with isolated hearing loss or subtle neuroimaging abnormalities, as well as older children that develop late-onset hearing loss, are necessary. Since clinical trials are ongoing (73–75), some of these points will hopefully be clarified in the next few years and the best management plan for this population determined.

As well as trials targeting treatment for specific populations, data on prevention are also required. It is known that natural infection confers some protection against both horizontal and vertical transmission (13, 76), therefore the development of an effective vaccine is feasible. Clinical trials of CMV vaccines

should evaluate protection against cCMV infection (77). Recent modeling data of a single fictional cohort of 390,000 adolescent women suggest that vaccination could be cost-effective (78).

In the absence of a vaccine to prevent infection, a greater focus on education and prevention strategies for cCMV infection are needed for women intending to become pregnant, those already pregnant, and healthcare professionals alike. Preconception screening in those attending a fertility clinic, with resultant counseling to improve personal hygiene in those who were not immune to CMV, has shown that hygiene counseling (albeit in this highly selected cohort) is effective in reducing CMV exposure (79). In pregnant women, hygiene counseling of CMV-seronegative pregnant women significantly prevents maternal infection (11). De Vries and colleagues showed that non-primary infections account for the majority of CMV-related hearing loss, suggesting prevention research should encompass all pregnant women, not just those who are seronegative (80). Therefore, the current method aimed at preventing transmission of CMV—education concerning hygiene measures to be taken around small children [such as avoiding kissing babies on the mouth, not sharing cutlery with young children, and hand hygiene after a diaper change (11)]—should be performed regardless of knowing the mother’s serostatus. However, in the general population, there is inadequate evidence to show that education translates into a decrease in maternal infection (4). Studies assessing the efficacy of hygiene measures on the prevention of CMV infection in pregnancy, and resultant cCMV, are necessary in women of reproductive age and are ongoing in the UK (81).

Clinicians do not know whether a non-primary infection is a reactivation or an infection with another strain of CMV. This distinction may be important in understanding the etiology of CMV disease, thus more research on the role of non-primary maternal CMV infections in congenital infection is necessary. At present, there are no tools validated to identify women at risk of transmitting the virus after a non-primary infection.

Finally, further evidence of cost-effectiveness is required. Whilst the cost-effectiveness of universal and targeted newborn cCMV screening programs has been assessed in the UK and USA (46, 47), and the economic burden of cCMV in the UK estimated (82), currently there are insufficient cost-benefit data, which hinders the implementation of screening.

CONCLUSIONS

cCMV infection results in significant consequences for the infected neonate. Despite this, universal maternal or neonatal screening for CMV and cCMV is not routinely recommended. A summary of our current recommendations for diagnosis, screening, and prevention is provided in **Table 3**. Presently there are significant gaps in understanding that prevent the implementation of universal screening, including insufficient data on cost-effectiveness and the lack of evidence for safe and efficacious treatments for those infected. Additionally, further data on non-primary maternal infection and the risk of cCMV infection are necessary. In the near future, we are confident that many aspects related to diagnosis, maternal and fetal therapy, and active prevention will surely present an improvement and our recommendations may change. Until then, and in the absence of

a vaccine, hygiene recommendations to prevent CMV infection should be made to all pregnant women.

AUTHOR CONTRIBUTIONS

TL and ML-V wrote the first draft of the manuscript. All authors (TL, DB-G, M-LD, IF, SL, SM, and ML-V) contributed to manuscript revision, read and approved the submitted version.

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