

STRENGTHENING THE FIGHT AGAINST CONGENITAL CYTOMEGALOVIRUS INFECTION: THE CENTRAL ROLE OF LABORATORY DIAGNOSTICS

Lee Bee Her¹, Kamal Haikal **Mat Rabi**², Khayri Azizi **Kamel**², Tan Chin Liong^{2*}, Rozainanee
Mohd Zain²

¹ Department of Pathology, Hospital Sultan Ismail Petra, 18000 Kuala Krai, Kelantan, Malaysia.

² Virology Unit, Infectious Diseases Research Centre (IDRC), Institute for Medical Research (IMR), National Institutes of Health (NIH), Ministry of Health Malaysia, Setia Alam, 40170 Shah Alam, Selangor, Malaysia.

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***Corresponding author:**
Tan Chin Liong,
tancl@moh.gov.my,
Tel: +603-33628560

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ABSTRACT

Congenital cytomegalovirus (cCMV) infection is the leading infectious cause of permanent birth defects and neurodevelopmental disabilities worldwide, particularly non-genetic sensorineural hearing loss. In recent decades, increased global awareness of the risks of cCMV infection during pregnancy has led to improved screening initiatives and expanded research efforts. However, early detection remains a challenge, leading to a significant global health and economic burden. In view of the limitations of current diagnostic tools, particularly in low-resource settings, it is imperative to have a rapid, sensitive, suitable and affordable diagnostic tool, with systematic strategies that facilitate early diagnosis of cCMV infection to enable timely interventions and improve disease outcome. This review provides an overview of laboratory diagnostic methods and their roles in mitigating the burden of cCMV infection and the associated morbidity and mortality.

KEYWORDS: congenital infection, human cytomegalovirus, laboratory diagnosis, maternal screening, neonatal screening

INTRODUCTION

Human cytomegalovirus (CMV), also known as human betaherpesvirus 5, is a double-stranded DNA virus belonging to the genus *Cytomegalovirus* in the subfamily *Betaherpesvirinae* of the family *Orthoherpesviridae* (1). It has the largest genome among human herpesviruses, which are well known for their ability to establish lifelong latent infections in the human host (2). CMV infection is the most prevalent congenital infection globally and the leading non-genetic cause of sensorineural hearing loss (SNHL) in paediatric populations (3).

Congenital cytomegalovirus (cCMV) infection exhibits a broad spectrum of clinical presentations at birth, ranging from asymptomatic cases to severe disabilities or life-threatening conditions, although the majority are at the milder end. Only 10–15% of infected infants have symptomatic disease, with common clinical signs including jaundice, petechiae, hepatosplenomegaly, and microcephaly (4). Additional clinical findings may include pneumonia, gastrointestinal disorders, chorioretinitis with or without optic atrophy and cataracts, central nervous system abnormalities, prematurity or small-for-gestational-age status, and SNHL (5,6).

Approximately 10–15% of asymptomatic infants and 40–60% of symptomatic infants are at risk of developing SNHL, either present at birth or later in life (4,7). Late-onset SNHL occurs during the initial years of life, with a median age at onset of 33 months for symptomatic infants and 44 months for asymptomatic infants (7). Other long-term neurological sequelae include diminished motor skills, intellectual disability, and vision loss. Approximately 30% of infants with severe infection will experience a fatal outcome (4).

This review article will focus on the strengths and limitations of various laboratory techniques in diagnosing maternal, foetal, and neonatal CMV infections, thereby highlighting their role in mitigating the burden of cCMV infection and the associated morbidity and mortality.

EPIDEMIOLOGY

Global Prevalence of cCMV Infection

cCMV represents the most prevalent congenital infection worldwide; however, it remains markedly under-recognised by both healthcare professionals and the general public, despite its substantial disease burden.

A meta-analysis encompassing 77 neonatal screening studies from 36 countries has established a global prevalence of cCMV at 0.67% (95% confidence interval [CI], 0.54 to 0.83) (8). The meta-analysis revealed significant geographic variation in cCMV prevalence, with rates approximately three times higher in low- and middle-income countries (LMICs) compared to high-income countries (HICs). This disparity reflects the underlying epidemiology of maternal CMV infection, with pre-pregnancy CMV seroprevalence exceeding 90% in LMICs compared to 50–60% in HICs (8).

In another meta-analysis examining global CMV seroprevalence across different countries and World Health Organization (WHO) regions, the worldwide average seroprevalence among women of reproductive age is estimated at 86% (95% uncertainty interval [UI], 83 to 89), with the highest prevalence observed in the Eastern Mediterranean region (92%; 95% UI, 88 to 95) and the lowest in the European region (70%; 95% UI, 63 to 76) (9). The higher cCMV prevalence in LMICs, therefore, underscores the importance of non-primary maternal infection (including both reinfection with new CMV strains and

reactivation of latent virus) in the global burden of cCMV. In addition to a higher maternal CMV seroprevalence, other factors associated with increased cCMV rates include higher population-level HIV prevalence, lower socioeconomic status, and younger maternal age (8).

Local Prevalence of cCMV Infection in Malaysia

The prevalence of cCMV infection in Malaysia remains insufficiently documented. A study involving 648 infants suspected of having CMV infection was conducted at a Malaysian tertiary hospital and reported a prevalence of cCMV at 0.3% (10). Nonetheless, CMV serology was employed as the screening modality, and only 2 of 39 samples sent for CMV viral load testing were received within the first three weeks of life. Consequently, these findings are likely to underestimate the true prevalence of cCMV in the population.

A more recent study conducted at the same tertiary hospital screened 219 infants with suspected congenital toxoplasmosis and cCMV using serological assays on paired sera and found serological evidence of acute cCMV infection in 1.8% of cases (11). However, no molecular tests were performed to confirm the diagnosis.

Local investigations into maternal CMV seroprevalence are likewise limited. In a 2011 study conducted in Malaysia, the researchers reported an immunoglobulin G (IgG) seroprevalence of 84% and an immunoglobulin M (IgM) seroprevalence of 7.2% among 125 healthy pregnant women (12).

PATHOGENESIS OF CONGENITAL CMV INFECTION

Maternal Infection and Viral Persistence

The natural history of CMV infection may be understood through four overlapping states: primary infection, latency, reactivation, and reinfection (13,14). Primary infection occurs when a previously seronegative individual acquires CMV for the first time. Following primary infection, the virus is not eradicated but persists in a non-productive latent state, particularly within peripheral blood monocytes and CD34+ haematopoietic stem and progenitor cells. Reactivation may subsequently occur from this latent reservoir, while reinfection refers to the acquisition of a new CMV strain despite pre-existing immunity (i.e., in a seropositive individual). These biological states are fundamental to cCMV pathogenesis, as congenital infection may occur following either maternal primary infection (MPI) or maternal non-primary infection (MNPI), with the latter including reactivation of latent virus or reinfection with a different strain (14).

CMV infection may also be considered in terms of productive (lytic) infection and non-productive (latent) infection. Productive infection involves viral gene expression, DNA replication, and production of infectious virions, whereas latent infection is characterised by persistence of replication-competent viral DNA without active virion production (15). Whether infection proceeds to productive replication or establishes latency is influenced by both the infected cell type and the host's immunological environment (15,16). Fibroblasts commonly support productive replication, while myeloid progenitor cells and monocytes may initially express viral genes before transcriptional silencing occurs, allowing the establishment of latency (17–19). Latently infected monocytes may contribute to systemic dissemination. When infected myeloid cells differentiate into macrophages or dendritic cells, particularly under inflammatory or immunologically permissive conditions, viral reactivation may occur with resumption of productive replication (17,20).

In pregnancy, maternal CMV infection marks the first step in the pathway leading to potential transplacental transmission to the foetus. CMV is transmitted through direct contact with infected body fluids, including saliva, urine, genital secretions, and blood (21). Among women of reproductive age, close contact with young children is an important risk factor because infected children, particularly those younger than two years, may shed CMV in saliva and urine for up to 24 months (22). Maternal infection is frequently asymptomatic or associated with non-specific symptoms; therefore, clinical presentation alone is insufficient for accurate diagnosis. MPI carries a higher risk of vertical transmission and adverse foetal outcomes (23); however, MNPI, including reactivation or reinfection, is also epidemiologically important, particularly in populations with high CMV seroprevalence (24). Although pre-existing maternal immunity reduces the risk of foetal infection, it does not eliminate it; therefore, both MPI and MNPI remain relevant to cCMV pathogenesis (24).

Placental Transmission

Vertical transmission occurs when CMV crosses the placenta and enters the foetal circulation. The placenta is an active foetal-derived organ that mediates maternal-foetal exchange. Its trophoblast layer differentiates into the outer syncytiotrophoblast, which is directly exposed to maternal blood, and the inner cytotrophoblast, which lies adjacent to placental fibroblasts, macrophages, and the developing foetal vasculature (25,26). Although the precise mechanisms of transplacental CMV transmission are not fully understood, placental infection, chronic villitis, trophoblast involvement, and infection of placental pericytes have been implicated (27,28). These processes may promote placental inflammation and vascular dysfunction, resulting in impaired perfusion, abnormal angiogenesis, and microcirculatory changes (27,29). Together, they may facilitate viral spread to the foetal circulation and contribute to foetal injury through placental dysfunction.

Foetal Dissemination and Tissue Injury

Once CMV enters the foetal circulation, it may disseminate haematogenously to susceptible foetal tissues. CMV has broad cell and tissue tropism (30). Although fibroblasts are highly permissive and commonly support productive replication, CMV may also infect endothelial cells, epithelial cells, smooth muscle cells, myeloid-lineage cells, and cells of the developing central nervous system (31–34). Foetal injury may result from direct viral replication in foetal tissues as well as indirect effects mediated by placental dysfunction and inflammation (28,31,35). This broad tropism helps explain the clinical spectrum of cCMV, ranging from asymptomatic infection to multisystem disease involving the brain, retina, inner ear, liver, kidney, and haematological system (36–38). Common foetal or neonatal manifestations include foetal growth restriction, microcephaly, ventriculomegaly, periventricular calcifications, chorioretinitis, hepatosplenomegaly, thrombocytopenia, petechiae, jaundice, and SNHL (4,39,40).

The timing of maternal infection influences both the likelihood of foetal transmission and the severity of foetal disease. Transmission risk generally increases with advancing gestational age, whereas foetal consequences are often more severe when infection occurs earlier in pregnancy, particularly during the first trimester or first half of pregnancy (41). This pattern may reflect the greater vulnerability of early placental development, organogenesis, and foetal neurodevelopment (26,28,31,42). Early foetal infection may therefore disrupt placental function and critical developmental processes, while later infection is more frequently transmitted but may be less likely to result in severe structural or neurodevelopmental injury.

Despite CMV's broad tropism, central nervous system involvement is particularly important in cCMV because infection during foetal development may disrupt critical neurodevelopmental processes, resulting in long-term sequelae (31). CMV may affect the developing brain through direct infection,

inflammation and immune-mediated damage, and hypoxic brain damage due to infection-induced placental insufficiency (36,43). The clinical importance of central nervous system involvement is reflected by the association of cCMV with microcephaly, ventriculomegaly, intracranial calcifications, neurodevelopmental impairment, cognitive deficits, SNHL, visual impairment, and seizures (38,44). Importantly, some infants who appear clinically asymptomatic at birth may later develop SNHL, supporting the need for early recognition and longitudinal follow-up of affected infants (4,39).

High-level, prolonged viral shedding in congenitally infected infants, particularly in urine and saliva, reflects active viral replication and dissemination following foetal infection (22). Similarly, foetal shedding of virus into amniotic fluid occurs only after transplacental passage and establishment of foetal infection, which explains why foetal assessment is generally performed after an appropriate interval following suspected maternal infection (42). These biological processes provide the basis for understanding the timing of foetal and neonatal testing, the selection of appropriate specimens, and the interpretation of laboratory findings in suspected cCMV infection.

LABORATORY DIAGNOSIS

Routine laboratory findings in cCMV infection may include elevated liver enzymes, thrombocytopenia, haemolytic anaemia, conjugated hyperbilirubinaemia, and elevated cerebrospinal fluid proteins (5,6,45). Specific laboratory techniques for diagnosing cCMV encompass microscopy and histopathology, viral culture and shell vial assays, nucleic acid amplification tests (NAATs), serology, and pp65 antigenaemia assay.

cCMV infection can be diagnosed either prenatally or postnatally. Prenatal diagnosis refers to assessments conducted from conception through birth, whereas postnatal diagnosis encompasses evaluations performed from delivery through three weeks of age.

PRENATAL LABORATORY DIAGNOSTIC TOOL

Diagnosis of Maternal CMV Infection

The primary goal of CMV testing in pregnant women is early diagnosis of MPI, ideally during the first trimester, when the risk of severe foetal sequelae is greatest, even though vertical transmission rates increase with advancing gestation (41).

MPI can be detected using serology that includes CMV IgM, IgG, and IgG avidity testing. A positive IgM with low IgG avidity confirms a recent primary infection within the preceding 3–4 months (46). CMV IgM alone is considered an unreliable indicator of primary infection owing to its variable sensitivity and poor specificity (46,47). If only IgM is detected, documentation of IgG seroconversion in paired sera taken 2–4 weeks apart provides definitive evidence of MPI (46,48). Current guidelines recommend that pregnant women undergo baseline serological testing, and those who are seronegative should repeat testing every four weeks until 14–16 weeks of gestation (49). CMV serology beyond 16 weeks is not recommended unless ultrasound findings are compatible with suspected foetal cCMV infection (49).

In contrast, MNPI, which includes reactivation of latent virus or reinfection with a different strain, cannot be reliably diagnosed using currently available standard serological or molecular methods (49). Many clinicians may attempt to diagnose MNPI by detecting positive CMV IgM, rising CMV IgG titres, or CMV DNA in blood; however, none of these is a reliable marker of active infection in a woman known to be seropositive before pregnancy. In a retrospective study of 53 pregnant women with confirmed MNPI leading to a congenitally infected infant, more than half (55%) had stable IgG titres, negative IgM, and

negative CMV-polymerase chain reaction (PCR) in serum (50). In MNPI, a positive CMV IgM may result from persisting IgM from a past infection or cross-reacting antibodies from other infections, leading to false-positive or misleading results (45).

Although both primary and non-primary CMV infections can be identified by detecting CMV DNA in whole blood, a negative PCR does not exclude infection. CMV DNAemia detection may be more useful in MPI than in MNPI. One study found CMV DNAemia in 75.7% of pregnant women with serologic evidence of primary infections, but in only 0.5% of those without such evidence (51). Viraemia in MNPI appears to be transient, with viral loads often being extremely low, thereby rendering detection challenging (51). Differentiating reactivation from reinfection in MNPI remains difficult using standard molecular or serological assays (52). Detection of anti-CMV strain-specific antibodies against polymorphic CMV epitopes using a strain-specific enzyme-linked immunosorbent assay (ELISA) may be useful for diagnosing reinfection (53). Nevertheless, distinguishing reactivation from reinfection in MNPI is often irrelevant to patient management, as both carry the same low risk of vertical transmission (4).

Diagnosis of Foetal CMV Infection

Following the confirmation of maternal CMV infection, the definitive diagnostic test for foetal infection is NAAT of amniotic fluid for CMV DNA (4,45). While viral culture can detect active CMV infection in foetal samples, it is less sensitive and slower than NAATs, often taking days to weeks for results, compared to hours with PCR (4). Consequently, NAATs have become the preferred method for prenatal diagnosis of foetal CMV infection.

Based on early studies, most guidelines recommend that amniocentesis be performed at least six weeks after MPI and at or after 21 weeks of gestation for the diagnosis of foetal CMV infection (54,55). This timing reflects the 6–8 week interval required for transplacental passage of CMV from the mother to the foetus, followed by viral replication in the foetal kidneys and subsequent excretion into foetal urine. Additionally, foetal urination is well established by approximately 20 weeks of gestation, at which point urine becomes the predominant component of the amniotic fluid (42).

However, recent studies have demonstrated that, as long as more than eight weeks have elapsed since MPI, the sensitivity of prenatal diagnosis does not differ significantly when amniocentesis is performed between 17+0 and 19+6 weeks of gestation compared to at or beyond 20 weeks (56,57). For instance, in a study of 214 pregnant women with CMV MPI before 14 weeks, Faure-Bardon et al. (57) reported that early amniocentesis (mean 18 ± 1 weeks) in 67 mothers diagnosed cCMV with 95.8% sensitivity, 100% specificity, 100% positive predictive value (PPV), and 97.7% negative predictive value (NPV) via PCR of the amniotic fluid. In contrast, late amniocentesis (mean 23.1 ± 3.9 weeks) in another 147 mothers yielded 91.5% sensitivity, 100% specificity, 100% PPV, and 82% NPV, with no significant difference in diagnostic performance between the two groups ($P = 0.47$). Consequently, the recent European Congenital Cytomegalovirus Initiative (ECCI) expert group guidelines recommend performing CMV-PCR on amniotic fluid collected from 17+0 weeks of gestation for the diagnosis of foetal CMV infection, provided that maternal infection occurred at least eight weeks earlier (49).

Compared with second-trimester amniocentesis, chorionic villus sampling (CVS) provides an alternative method for diagnosing foetal CMV infection as early as 11–14 weeks of gestation (57). The risks of procedure-related miscarriage for both techniques are significantly lower than those traditionally reported. A comprehensive meta-analysis indicated that the pooled procedure-related risks of miscarriage for amniocentesis and CVS were 0.11% (95% CI, –0.04 to 0.26) and 0.22% (95% CI, –0.71 to 1.16), respectively (58). Although the complication risks are similarly low, CVS is generally not the

preferred diagnostic tool due to its lower sensitivity for detecting foetal CMV. In a study involving 37 pregnancies undergoing CVS at a median gestational age of 12+5 weeks (range, 11+2 to 14+3 weeks), CMV-PCR analysis of trophoblast tissue demonstrated a sensitivity of 50%, specificity of 100%, PPV of 100%, and NPV of 91% for the diagnosis of cCMV, with amniotic fluid PCR serving as the reference standard (57).

Cordocentesis, also known as percutaneous umbilical cord blood sampling, is a potential method for diagnosing cCMV. In one study using foetal blood for CMV antigen testing, culture, PCR, and serology, the sensitivity of each method was as follows: 57.9% for antigenaemia, 55.5% for viraemia, 82.3% for leucoDNAemia, and 57.9% for IgM; specificity was 100% for all assays (59). This invasive procedure is more technically demanding than amniocentesis, as it requires puncturing the umbilical cord, which carries risks of cord haematoma, bleeding, foetal bradycardia, and a slightly higher risk of foetal loss. With an experienced operator, cordocentesis carries approximately a 1.4% risk of foetal loss before 28 weeks of gestation and a 1.4% risk of perinatal death after 28 weeks (60). Similarly, Liao et al. (61) reported that cordocentesis-related foetal loss rates within two weeks of the procedure were 1.0% before 24 weeks and 0.8% after 24 weeks. Given the risks to the foetus, cordocentesis is not generally recommended for the diagnosis of foetal CMV infection (62). However, it may provide prognostic value for predicting foetal outcome by assessing a combination of markers in foetal blood, including β -2 microglobulin, platelet count, and CMV viral load (63).

POSTNATAL LABORATORY DIAGNOSTIC TOOL

Diagnosis of Congenital CMV Infection in Newborn

Prior to the widespread adoption of molecular diagnostic techniques, the gold standard for diagnosing cCMV was viral culture of urine specimens collected from newborns (64). The limitations of viral culture include its labour-intensive nature, extensive processing time, and lengthy turnaround duration. Given that CMV is a slow-growing virus, it may take four to six weeks for the virus to proliferate in conventional cell culture and for cytopathic effects to be observable (65–67). The turnaround time has been considerably reduced with the implementation of the shell vial culture technique, which obviates the need to observe cytopathic effects and enables detection of immediate-early antigens by immunofluorescence within two to three days (65–67).

Notwithstanding this, shell vial culture has been predominantly replaced by NAATs in contemporary practice, owing to their enhanced sensitivity, specificity, and expedited turnaround times (64). As viral culture necessitates the presence of viable viral particles, suboptimal specimen storage and delayed transport to the laboratory diminish its sensitivity. Conversely, real-time PCR is less susceptible to these factors, as it detects the nucleic acid of both viable and non-viable viruses. Furthermore, the availability of quantitative PCR facilitates measurement of viral load in the specimen, thereby enabling monitoring of disease progression and therapeutic response (68). The principal drawback of PCR is its heightened susceptibility to contamination owing to its extreme sensitivity. Furthermore, its higher cost and technical complexity may hinder deployment in resource-constrained nations, where serological assays are the primary diagnostic modality (69).

Although serological testing continues to be utilised in several countries, numerous guidelines recommend refraining from its use in the diagnosis of cCMV (49,70). While CMV IgM demonstrates a relatively high specificity for diagnosing cCMV, ranging from 99.3% to 100%, its sensitivity has been reported to vary significantly, from 25% to 84.4% (59,69,71). A negative CMV IgG can effectively exclude the diagnosis of cCMV in an infant with a normal immune system; however, a positive CMV IgG merely indicates the presence of maternal antibodies and is not helpful for diagnosing cCMV (4).

Establishing a diagnosis of cCMV requires detecting the virus in body fluids such as urine, saliva, or blood, with the critical caveat that the sample must be obtained within the first three weeks of life (4,5). Testing performed after three weeks of age cannot distinguish between congenital and postnatal CMV infections, as postnatal infection, acquired through vaginal secretions during delivery, breast milk, close contact, or blood transfusion, typically does not produce detectable viral shedding in urine or saliva within the first three weeks of life (49). Distinguishing between congenital and postnatal infection is clinically important because cCMV can lead to serious sequelae (e.g., SNHL, neurodevelopmental delay), whereas postnatal infection in otherwise healthy term infants is generally benign (72).

Among the available sample types, urine has been regarded as the gold-standard specimen for diagnosing cCMV, primarily because the virus predominantly replicates in the kidneys, resulting in significant viral shedding in urine (22,73). However, urine collection can be challenging in newborns. Collection using a urine bag may be hampered by several factors, including contamination, sample loss, and insufficient diuresis (74). One study suggested using sterile cotton balls as an alternative collection strategy, which worked well for PCR but not for viral culture (75). Additionally, a large-scale multicentre study demonstrated that urine sample collection using filter cards is a robust method for detecting cCMV infection, with a PPV of 94% (76).

Given these collection challenges, saliva has been increasingly recommended for cCMV screening, owing to its ease of collection and higher acceptance among parents (49,55). Numerous studies have documented excellent sensitivity and specificity of saliva for diagnosing cCMV (77,78). In a universal screening study of 34,989 infants, real-time PCR assays of liquid saliva and dried saliva specimens were compared with rapid culture of saliva specimens obtained at birth (77). The authors concluded that both liquid and dried saliva samples had high sensitivity and specificity. The liquid-saliva PCR assay demonstrated 100% sensitivity and 99.9% specificity, while the dried-saliva PCR assay demonstrated 97.4% sensitivity and 99.9% specificity. Another study evaluated the performance of saliva real-time PCR by comparing it with reference-standard urine detection (rapid culture or real-time PCR) in newborns undergoing targeted cCMV screening over a three-year period (78). Among 856 concurrently tested newborns, the authors reported a sensitivity of 98.3%, specificity of 91.5%, PPV of 45.6%, and NPV of 99.9% for saliva real-time PCR.

Despite the high sensitivity of saliva PCR, the potential for false-positive results due to peripartum contamination with breast milk or genital secretions means that a positive saliva result should be confirmed by a subsequent specimen, preferably urine (4,51). To avoid CMV contamination from breast milk, collecting saliva at least one hour after breastfeeding has also been recommended (70). Nevertheless, the CMV and Hearing Multicentre Screening (CHIMES) study reported false-positive rates, adjusted for breastfeeding, ranging from 0.03% in white Hispanic individuals to 0.14% in white non-Hispanic individuals, suggesting that saliva PCR results are unlikely to be significantly influenced by breastfeeding or other perinatal exposures (79). These findings support the reliability of saliva as a screening tool without strict pre-collection feeding restrictions. While urine does not present the same specificity concerns, collection challenges render it unsuitable for large-scale screening, unlike saliva (80).

In contrast to urine and saliva, blood is not the preferred specimen for diagnosing cCMV, as detectable viraemia at birth is present in only a minority of infected infants (4). Moreover, CMV viral loads in blood are typically much lower than those found in urine and saliva (22). Nonetheless, a positive CMV-PCR result from a blood sample obtained within the first three weeks of life is diagnostic of congenital infection.

Another specimen type, dried blood spots (DBS), has shown variable performance for cCMV screening. The CHIMES study, involving over 20,000 infants, reported a sensitivity of only 34.4% but an excellent specificity of 99.9% for DBS-PCR compared with saliva rapid culture (81). Recent studies have demonstrated improved analytical performance of DBS-PCR, attributed to factors such as the number of DBS punches used for extraction, the composition of elution buffers, extraction methods, and the PCR targets employed (82,83). A meta-analysis of 15 studies reported a pooled sensitivity of 84% and a specificity exceeding 99% (84). A positive DBS-PCR result is diagnostic of cCMV infection, but a negative result cannot rule out the diagnosis. Therefore, DBS testing is more suitable for retrospective diagnosis than for prospective screening.

Beyond qualitative detection, the clinical utility of quantitative CMV DNA viral load testing is limited by inconsistent results, a lack of standardised thresholds linked to disease risk or severity, and methodological variability (4,39). Despite the WHO international standard for analytical harmonisation (85), studies report mixed findings on the association between viral load and disease outcomes (86–90). Thus, the role of quantitative viral load in predicting severity, guiding treatment, and counselling parents remains uncertain and requires validation in large prospective studies (4). Currently, no established threshold exists for newborn testing across specimen types (blood, urine, saliva, and others) that clearly correlates with disease onset or progression risk (4).

Finally, detection of CMV in formalin-fixed, paraffin-embedded placental tissue via histopathologic examination, immunohistochemistry, PCR, and *in situ* hybridisation has previously been described as a feasible alternative for diagnosing cCMV (91,92). Placental analysis is particularly valuable in cases where congenital infection was not suspected at birth, as it provides a retrospective diagnosis of *in utero* exposure when clinical samples are obtained only after the three-week diagnostic window or in cases of foetal demise (92). Furthermore, the combination of placental pathology and PCR results offers prognostic value; for instance, the presence of specific histological features such as haemosiderin and villous calcification has been associated with the development of hearing loss, while a positive CMV-PCR in placental tissue correlates with a higher frequency of intrauterine growth restriction (93).

INNOVATIVE AND EVOLVING DIAGNOSTIC METHODS FOR CONGENITAL CMV INFECTION

Recently, an increasing number of emerging technologies have shown promising value for cCMV diagnostics. These include metagenomic next-generation sequencing (mNGS), rapid whole genome sequencing (rWGS), high-resolution melt (HRM) analysis for detection of CMV DNA at the single-nucleotide level, and clustered regularly interspaced short palindromic repeats (CRISPR)-based technologies. The mNGS and rWGS show high potential to be sensitive and specific tests for CMV, but further research is needed (94,95). Meanwhile, HRM is an easy-to-use, highly sensitive technology that enables the provision of antiviral genomic information and multiplexing for broad pathogen detection with distinct melting points (96–98). In addition, integrating CRISPR-based viral diagnostic techniques with paper-based biosensors, nucleic acid amplification-free electrochemical methods, or nucleic acid amplification technologies (including isothermal amplification) removes the need for laboratory thermal cyclers, a significant barrier to widespread clinical adoption due to cost and usability (99).

These approaches offer rapid turnaround times and can be performed outside traditional laboratory settings. Biosensors are devices that measure a biological or chemical response by producing a signal proportional to the analyte concentration (100). They typically comprise three main elements: (1) a bioreceptor (biomediator)—such as antibodies, nucleic acids, enzymes, organelles, or microorganisms—that provides selective recognition of the analyte; (2) a transducer (e.g., electrochemical, optical, or piezoelectric) that converts the biological interaction into a measurable signal; and (3) a signal processor

with associated electronics and a visualisation method for result monitoring (101,102). Biosensors can provide rapid turnaround times, making them suitable as point-of-care devices that enable quicker, easier, and more cost-effective diagnostics (39,100). Several studies have demonstrated their potential for rapid, sensitive detection of CMV infection, particularly in serum and urine samples (103,104). They represent a potential alternative or adjunct to nucleic acid testing (105). However, their use in real-world settings has yet to be evaluated.

Testing for CMV cell-mediated immunity includes interferon-gamma release assays (IGRAs) such as QuantiFERON-CMV and enzyme-linked immunosorbent spot (ELISpot), as well as intracellular cytokine flow cytometry. These are among the immunological methods that can be used to determine CMV infection in pregnancy using a whole-blood sample (106,107). However, the clinical value of maternal CMV-specific T-cell immunity remains under investigation, and it may be associated with a lower risk of vertical transmission (108).

Detection of CMV antigen (CMV lower matrix protein pp65) in peripheral blood leucocytes using monoclonal antibodies by immunofluorescence, immunoperoxidase, and other antigen-detection methods is not routinely practised for suspected CMV infection in pregnancy. This is due to a lack of standardisation across laboratories, operator dependence, the need for an adequate number of cells, and a short specimen processing time (109). While this technique is common in transplant monitoring, it is rarely employed for cCMV evaluation. Moreover, some experts argue that cell-free virus in plasma better reflects active viral replication than cell-associated detection (110).

Amniotic fluid can be used to evaluate host-response proteins associated with foetal CMV infection and its severity, such as retinoic acid receptor 2 (Chemerin) and galectin-3-binding protein (Gal-3BP) (111). Nonetheless, further research is needed to improve the prediction of severity in cCMV infection, as the negative and positive predictive values require validation in large prospective cohorts.

SCREENING & PREVENTION

The optimal approach to cCMV screening remains a subject of ongoing debate. Two primary strategies exist: universal screening, which tests all newborns regardless of risk factors, and targeted screening, which focuses on high-risk populations such as those who fail newborn hearing screening or have clinical findings suggestive of cCMV (112).

Targeted screening programmes typically identify approximately 75% of cCMV cases with hearing loss (112). Additional screening criteria include maternal CMV history, suspicious prenatal ultrasound findings, clinical signs at birth (petechiae, hepatosplenomegaly, jaundice, thrombocytopenia), and preterm birth or low birth weight (112). However, targeted screening inevitably misses asymptomatic infants who may subsequently develop late-onset sequelae, particularly hearing loss.

A cost-effectiveness analysis using data from large prospective cohorts reported that both universal and targeted newborn cytomegalovirus screening were cost-saving (113). Despite the favourable economic data, universal newborn cCMV screening remains uncommon globally. State-wide universal screening programmes are currently operational only in specific regions of Canada (e.g., Ontario) and the United States (e.g., New York and Minnesota) (114). Most countries, including Malaysia, have not yet adopted universal screening, citing concerns about evidence gaps, implementation challenges, and the need for standardised protocols (114).

To bridge the gap in universal cCMV screening, innovative diagnostic workflows such as pooled saliva PCR testing offer a viable public health solution. A landmark 2022–2023 study in Jerusalem demonstrated the efficacy of universal pooled saliva PCR, which was subsequently confirmed by urine PCR (115). By pooling approximately six samples per test on average, the laboratory workflow spared 83% of individual saliva assays. The pooling strategy resulted in only a marginal 3.05 PCR-cycle decrease in diagnostic sensitivity. Crucially, this high-throughput method identified a cCMV birth prevalence of 3.4 per 1,000, wherein 55.6% of the infected neonates were asymptomatic and would have evaded detection under standard targeted screening protocols. This underscores pooled molecular testing as a highly sensitive and economically scalable strategy for widespread cCMV screening.

In the Malaysian clinical landscape, standard prenatal monitoring includes basic infectious disease screening (e.g., syphilis, HIV, and malaria in endemic areas), whereas CMV screening is performed only on a targeted, risk-based basis (116). This targeted testing is typically conducted using CMV serology, as part of TORCH (Toxoplasmosis, Rubella, CMV, and Herpes simplex virus) panel investigations, for pregnant women presenting with poor obstetric histories, such as recurrent miscarriages, perinatal mortality, abnormal foetal ultrasonography (e.g., small for gestational age, malformations), or intrauterine foetal death (116,117). The diagnostic integration of CMV IgG/IgM screening with advanced IgG avidity testing, interpreted alongside maternal risk profiles, is crucial for distinguishing primary from non-primary maternal infection. However, laboratory data suggest that the diagnostic yield of CMV serology remains low when utilised solely for isolated, non-specific ultrasound abnormalities such as foetal growth restriction or polyhydramnios (118,119).

There are three tiers of prevention strategies in maternal, foetal, and neonatal cCMV infection: primary, secondary, and tertiary (52,114). Primary prevention aims to reduce the risk of maternal primary infection or reinfection during pregnancy. Currently, no vaccine is approved to prevent maternal CMV infection or subsequent foetal transmission, primarily because the virus elicits an incomplete and poorly protective natural immune response, allowing frequent reactivations and reinfections to occur (52). This poor immune response, together with poorly understood correlates of protection, poses significant challenges to developing a vaccine that elicits robust neutralising antibodies. Consequently, primary prevention relies heavily on behavioural interventions, such as hand hygiene and avoiding exposure to young children's saliva (14,52,120).

Secondary prevention requires robust, laboratory-guided screening and aims to reduce the risk of maternal-foetal transmission among pregnant mothers with laboratory evidence of seroconversion (52). In the event of seroconversion, comprehensive parental counselling regarding vertical transmission risks is mandated (121). Valaciclovir, a prodrug of acyclovir, has shown promise in the secondary prevention of foetal infection by reducing vertical transmission when administered to pregnant women with primary CMV infection. An individual patient data meta-analysis demonstrated that administration of oral valaciclovir at a dose of 8 g/day reduces vertical transmission of CMV by 70% when MPI occurs periconceptionally or in the first trimester (122). This evidence has prompted renewed interest in antenatal CMV screening in some HICs, though universal antenatal screening is not yet recommended in most guidelines (114).

Tertiary prevention aims to reduce the severity of long-term sequelae, such as SNHL and neurodevelopmental delay, in infants with confirmed cCMV infection through early postnatal diagnosis and timely initiation of antiviral therapy. Valganciclovir (an oral prodrug of ganciclovir) is the standard of care for neonates with symptomatic cCMV and has been shown to improve hearing and neurodevelopmental outcomes when initiated within the first month of life (114). Ongoing research continues to evaluate

optimal treatment duration, long-term efficacy, and the potential role of therapy in infants with isolated hearing loss or asymptomatic infection.

KEY DIAGNOSTIC GAPS IN CONGENITAL CMV INFECTION

Currently, there is no standardised newborn screening programme for cCMV worldwide. While universal screening has been implemented in select regions of Canada and the United States, the vast majority of countries still rely on a targeted, risk-based approach (114). This strategy often depends on a failed newborn hearing screen or overt clinical manifestations, thereby missing 85–90% of asymptomatic cases. Of these asymptomatic infants, approximately 10–15% will subsequently develop late-onset sequelae, such as SNHL or neurodevelopmental delay (4). Therefore, the principal limitation of hearing-targeted screening is its inability to detect infants who develop late-onset SNHL after passing the initial newborn hearing assessment.

In Malaysia, a national newborn hearing screening programme does not yet exist, although universal newborn hearing screening (UNHS) has been implemented through various hospital initiatives (123,124). Newborns are typically tested for cCMV only after failing the second hearing screen, which is often not completed until after the third week of life (123). This timing poses a significant diagnostic challenge, because CMV detection beyond three weeks of age cannot distinguish between congenital and postnatal infection—the latter being frequently asymptomatic and of little clinical consequence.

Additionally, a lack of clinician awareness may contribute to delayed cCMV testing (10). Many healthcare providers do not routinely suspect cCMV in newborns with subtle signs (e.g., jaundice, growth restriction, petechiae), leading to missed diagnoses in symptomatic infants who could benefit from early treatment.

As noted, CMV-PCR on urine or saliva collected within the first three weeks of life is the current gold standard for diagnosing cCMV. However, in many resource-limited settings, PCR testing is available only at central reference laboratories (116). In Malaysia, for example, many clinicians still rely on CMV serology to diagnose cCMV due to limited access to PCR testing and its long turnaround times (10,11). Consequently, many cases are likely to be missed due to the suboptimal sensitivity of CMV IgM testing.

Given the current limited availability of CMV PCR in Malaysia, further local studies are urgently needed to assess the prevalence of cCMV and the diagnostic utility of PCR. Such evidence would inform policymakers, raise clinicians' awareness, and facilitate increased nationwide uptake of CMV PCR. While European guidelines provide a useful framework, their recommendations cannot be directly extrapolated to Malaysia without local validation. Key epidemiological parameters, including maternal seroprevalence, birth prevalence of cCMV, and laboratory capacity, likely differ substantially from those in European settings, though local data remain limited (116). Therefore, any future screening strategies for Malaysia must be preceded by local prevalence studies and cost-effectiveness analyses tailored to the Malaysian healthcare context.

CONCLUSION

cCMV infection remains a major global health concern due to its often asymptomatic presentation at birth and its potential to cause long-term sequelae, including SNHL and neurodevelopmental delay. Despite advances in understanding CMV biology and transmission, timely diagnosis remains challenging, particularly in LMICs.

Molecular methods, especially PCR-based techniques, have become central to cCMV diagnosis. Current gold-standard approaches, such as PCR testing on amniotic fluid, saliva, and urine, offer high sensitivity and specificity. An integrated diagnostic strategy combining maternal serology, prenatal PCR on amniotic fluid for high-risk cases, and postnatal PCR on saliva and urine within the first three weeks of life enables early detection, risk stratification, and timely intervention. However, the implementation of this multi-tiered approach is limited by cost, infrastructure, and technical expertise, particularly in resource-limited settings. Affordable, rapid, and sensitive diagnostic tools are therefore urgently needed.

Beyond laboratory aspects, clinical and public health challenges persist, including the lack of uniform screening programmes and consensus on treating asymptomatic newborns with isolated hearing loss. Antiviral agents such as valaciclovir and valganciclovir show promise for prenatal and neonatal use, but concerns regarding safety, accessibility, and optimal timing remain unresolved.

Moving forward, global public health strategies should prioritise raising awareness, improving access to diagnostics, and investing in large-scale screening initiatives, particularly in high-prevalence countries such as Malaysia. Concurrently, research should continue on vaccine development and standardised viral load thresholds to guide treatment decisions.

Ultimately, a shift from reactive to proactive diagnostics, coupled with preventive strategies and timely treatment, is imperative to mitigate the impact of cCMV on neonatal and public health worldwide. Such approaches must be tailored to local prevalence, risk factors, geographic and socioeconomic contexts, and resource availability.

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