

# Prevention of maternal cytomegalovirus infection: current status and future prospects

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**Abstract:** Human cytomegalovirus (CMV) infection is the most common cause of perinatal viral infection in the developed world, resulting in approximately 40,000 congenitally infected infants in the United States each year. Congenital CMV infection can produce varying degrees of neurodevelopmental disabilities. The significant impact of congenital CMV has led the Institute of Medicine to rank development of a CMV vaccine as a top priority. Vaccine development has been ongoing; however no licensed CMV vaccine is currently available. Treatment of pregnant women with CMV hyperimmune globulin has shown promising results, but has not been studied in randomized controlled trials. Education on methods to prevent CMV transmission, particularly among young women of child-bearing age, should continue until a CMV vaccine becomes available. The epidemiology, clinical manifestations, prevention strategies, and treatment of CMV infections are reviewed.

**Keywords:** cytomegalovirus, CMV vaccines, congenital CMV, CMV infection, immunoglobulin

## Introduction

Human cytomegalovirus (CMV) is a ubiquitous beta-herpes virus that leads to congenital infection in 0.4% to 2.3% of all newborns.<sup>1</sup> The risk of intrauterine transmission after primary CMV infection during pregnancy approaches 40%, with an increased risk of adverse fetal effects if infection occurs during the first half of pregnancy.<sup>2</sup> Of congenitally infected infants, approximately 10% are symptomatic at birth. Of the remaining 90% of infants who are asymptomatic at birth, 10%–15% will subsequently manifest evidence of permanent sequelae.<sup>3</sup> Congenital CMV is a significant cause of neurodevelopmental disability, including sensorineural hearing loss (SNHL) and intellectual disability (previously referred to as “mental retardation”). More children suffer from long-term sequelae as a result of congenital CMV infection than Down syndrome or fetal alcohol syndrome.<sup>4</sup> In this review, current concepts regarding the epidemiology, pathogenesis, and prevention of CMV infection are summarized, with an emphasis on strategies designed to improve awareness of the risk of CMV among women of childbearing age.

## Epidemiology

CMV is found worldwide, with the rate of seropositivity affected by geographic, socioeconomic, and ethnic background.<sup>2,3</sup> In developed countries, the prevalence of CMV seropositivity is 40% to 60% in individuals of middle to upper socioeconomic status and ~80% among those of lower socioeconomic status.<sup>3</sup> By comparison, virtually

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individuals in developing countries have been infected by CMV in early childhood.<sup>3</sup> In the United States, the seroprevalence of CMV is higher among non-Hispanic blacks and Mexican Americans than among non-Hispanic whites.<sup>5</sup>

Congenital CMV infection can occur as the result of a primary CMV infection, reinfection with a new strain of CMV, or reactivation of a latent infection.<sup>6,7</sup> Maternal immunity to CMV provides some protection against vertical transmission of the virus. If a primary CMV infection occurs in the period just prior to conception, the risk of transmission is 8.7%.<sup>8</sup> Primary maternal CMV infection occurring in the first, second, and third trimester results in congenital infection in approximately 25%, 50%, and 75% of fetuses, respectively.<sup>9</sup> In contrast, the risk of CMV transmission to the fetus after a recurrent maternal infection is only 0.15 to 2%.<sup>10,11</sup> Fowler et al demonstrated a 69% reduction in the risk of congenital CMV infection in future pregnancies in women who were seropositive for CMV when compared to seronegative women.<sup>12</sup> However, despite the risk reduction preconception immunity affords, over 60% of infants with congenital CMV infection are born to mothers with immunity to CMV prior to pregnancy, reflecting the high rate of CMV seropositivity in the population.<sup>13</sup> In populations with high maternal CMV seropositivity, the incidence of congenital CMV infection is greater than in populations of lower maternal seroprevalence.<sup>1</sup>

It has generally been believed that the fetuses of pregnant women with preconception immunity to CMV are somewhat protected against the most significant neurodevelopmental sequelae of congenital CMV infection. A study comparing women with preconception immunity to CMV to those who acquired primary CMV infection in pregnancy showed that the women with preconception immunity have a significant reduction in transmission of CMV to the fetus, as well as decreased severity of disease in infected infants.<sup>11</sup> In that study, 25% of the infants whose mother had a primary infection had at least one sequela, compared with 8% when the infection was recurrent. However, subsequent studies have not demonstrated the same degree of protection conferred by preconception immunity in congenitally infected infants. Boppana et al studied infants with symptomatic congenital CMV infection as the result of both primary and recurrent maternal infection and found no difference in the severity of clinical findings between the two groups.<sup>14</sup>

## Pathogenesis

CMV is a linear double-stranded DNA virus, and its genome is comprised of over 250 kilobase pairs. The three

distinct regions of the CMV virus include the capsid containing the viral genome; the tegument layer containing phosphoproteins; and an outer lipid envelope containing glycoproteins. The capsid, which comprises 162 capsomere subunits arranged in icosahedral symmetry, houses the viral genome.<sup>15</sup> The capsid is surrounded by the tegument of the CMV virion. A large number of proteins are located in the tegument. These tegument proteins, including phosphoprotein 65 (pp65), are some of the most immunogenic proteins in the virion, and are the immunodominant targets of T-lymphocyte responses to CMV.<sup>16,17</sup> Surrounding the tegument is the envelope, which contains an as yet incompletely defined number of virally encoded glycoproteins. The most abundant glycoproteins include gB complex, gM/gN complex, and gH/gL/gO complex. Seropositive individuals typically mount a neutralizing antibody response directed against these glycoproteins making these protein products potentially useful subunit vaccine candidates.<sup>18-20</sup>

CMV is a complex virus that appears to employ multiple strategies to evade the host immune system.<sup>21</sup> A healthy, immunocompetent individual can control CMV infection, but only at great expense to the host immune system: remarkably, approximately 10% of both the CD4<sup>+</sup> and CD8<sup>+</sup> memory compartments in blood are specific for CMV-encoded proteins.<sup>22</sup> The chronic, persistent nature of CMV, characterized by frequent episodes of asymptomatic reactivation and shedding, probably contributes to the ability of the virus to cause congenital infection even in women with long-standing preconception immunity.

## Congenital and postnatal transmission

### Children as source of CMV

Daycare centers are a significant source of CMV infection. Children less than three years of age with postnatally acquired CMV infection have been demonstrated to excrete CMV in their urine and saliva for 6 to 42 months.<sup>23</sup> Children enrolled in daycare become infected with CMV between 15 and 70% of the time.<sup>24</sup> Seronegative mothers with children in group daycare are at significant risk of acquiring CMV infection, with at least 50% of them seroconverting within 1 year of their child's CMV infection.<sup>25</sup>

### Breast-feeding

CMV is excreted in the breast milk of seropositive women.<sup>26</sup> The risk of CMV transmission in infants breast-fed by seropositive women shedding virus in their breast milk has been reported to be 58% to 69%.<sup>27,28</sup> CMV infection acquired

in the postnatal period in healthy term infants typically is asymptomatic, only rarely producing any morbidity. There is no evidence that acquisition of CMV via breast milk leads to any adverse neurodevelopmental sequelae. In a study of CMV transmission through breast-feeding, all of the infants who acquired CMV infection had normal neurodevelopment at a mean follow-up of 51 months.<sup>28</sup>

While the safety of breast-feeding has been established in term infants in women shedding CMV virus, controversy exists on the safety of breast-feeding low birth weight, premature infants. Studies in low birth weight and very low birth weight preterm infants yield conflicting results with respect to the risk of developing symptomatic infection following breast milk acquisition of CMV.<sup>29</sup> A recent study of early postnatal CMV infection in preterm infants in an highly immune population demonstrated that symptomatic CMV infection was rare.<sup>30</sup> Efforts to reduce the infectivity of breast milk from seropositive mothers has included freezing breast milk at  $-20^{\circ}\text{C}$ , Holder pasteurization, and short-term pasteurization.<sup>31</sup> Of these methods, freeze-thaw is the best studied approach, and the technique most likely to retain the salutary immunological properties of breast milk. While freezing breast milk does lower the incidence of postnatally acquired CMV infection, it does not entirely eliminate the risk.<sup>32</sup> It remains unclear whether interventions designed to interrupt breast milk transmission of CMV to low birth weight, premature babies improve either short-term or long-term outcomes for these infants.

## Other

CMV can also be transmitted through close non-sexual contact, sexual activity, blood transfusions, and organ transplantation.<sup>4</sup> Two methods are currently employed to decrease the risks of CMV transmission through blood transfusion, including utilizing leukocyte-reduced and CMV-negative blood products. Although leukocyte reduction has dramatically reduced the risk of transfusion-associated CMV infection, reports in the literature are conflicting about whether this intervention completely eliminates any risk of transmission.<sup>33,34</sup> A recent survey of the American Association of Blood Bank (AABB) physician members showed that 65% of those responding felt both leukocyte-reduced and CMV-negative blood components were equivalent in their ability to prevent transfusion transmission of CMV.<sup>35</sup> However, despite the AABB survey results on attitudes toward leukocyte reduced blood products, fetuses and neonates are more likely to receive CMV-negative blood products compared to other groups receiving transfusions.<sup>35</sup>

## Clinical manifestations

### Maternal CMV

Most CMV infections are subclinical in healthy immunocompetent hosts.<sup>1</sup> When present, clinical symptoms of primary CMV infection include flu-like syndrome, fever, myalgias, pharyngitis, weakness, and fatigue. Laboratory abnormalities can include elevated liver transaminases and lymphocytosis. Most studies have shown that pregnancy does not appear to affect the clinical course of CMV infection. A more recent look at primary CMV infection in pregnancy by Nigro et al demonstrated substantially higher rate of symptoms associated with CMV infection than previously reported.<sup>36</sup> In a cohort study, 32 women (31%) diagnosed with primary CMV experienced either persistent fever or a flu-like illness.<sup>36</sup> Practitioners should have a high index of suspicion when caring for at risk pregnant women with undiagnosed illness or fever.

### Congenital CMV

Only 10% of congenitally infected fetuses are symptomatic at birth. Clinical symptoms include microcephaly, growth restriction, hepatosplenomegaly, chorioretinitis, jaundice, petechiae, hearing impairment, thrombocytopenia, hyperbilirubinemia, and anemia.<sup>13,37</sup> The risk for neurologic sequelae is increased when infection occurs in the first trimester.<sup>38</sup> The majority of infants with symptomatic congenital CMV infection at birth have evidence of central nervous system (CNS) impairment. In a study of 106 infants with symptomatic congenital CMV infection, 68% of infants had at least one clinical finding suggestive of neurologic impairment.<sup>37</sup> It was reported in the early 1980s that infants with symptomatic congenital CMV infection at birth have a 91% chance of developing long-term sequelae from the infection, and a 29% death rate, although advances in neonatal intensive care make mortality much less likely today.<sup>39</sup> Of infants with asymptomatic congenital CMV infection at birth, 10% to 15% will go on to develop symptoms, typically manifested as SNHL.

Congenital CMV infection is the most common non-genetic cause of SNHL in children. In the DECIBEL study, 23% of children with profound SNHL had congenital CMV infection.<sup>40</sup> Permanent hearing loss occurs in approximately 14% of children with congenital CMV infection.<sup>41</sup> SNHL from congenital CMV can present later in childhood. Approximately 6% to 23% of children with congenital CMV infection who are asymptomatic at birth will subsequently develop hearing loss.<sup>42</sup> However, symptomatic infection at

birth appears to be much more likely than asymptomatic infection to be associated with delayed SNHL, with a reported rate of delayed-onset hearing loss in symptomatic newborns of 33.7%.<sup>43</sup>

## Diagnosis

### Diagnosis of maternal infection

Diagnosis of maternal primary CMV infection can be challenging. Maternal primary CMV infection is confirmed if there is documented IgG seroconversion; however, most women do not have a baseline pre-pregnancy serology for comparison. A second method to diagnose primary CMV infection is to test for CMV-specific IgM, which is an indicator of recent or active CMV infection. Several problems exist with CMV IgM as a screening test. First, CMV IgM can be present for up to 9 months after a primary infection.<sup>2</sup> Second, CMV IgM can be produced in both recurrent infections, as well as following reactivation of infection; thus, the finding of IgM antibodies does not allow discrimination of the timing of infection.<sup>44</sup> Third, there is discordance among commercially available kits for CMV IgM, as well as false positive results.<sup>45</sup> The AxSYM CMV IgM assay is very sensitive when compared to other commercial assays.<sup>46</sup> A highly sensitive IgM assay can identify more pregnancies at risk.

In pregnant women with CMV-specific IgM, anti-CMV IgG avidity testing can be utilized to differentiate primary CMV infection from reactivation of latent infection or reinfection.<sup>44</sup> Low avidity indices are indicative of an acute or recent primary CMV infection, and persist for approximately 18 to 20 weeks.<sup>44</sup> Lazzarotto et al demonstrated that if the CMV IgG avidity index is performed prior to 18 weeks gestation, it has 100% sensitivity for detecting pregnancies at risk for transmitting CMV to the fetus, however the sensitivity is decreased to only 62.5% if the test is performed after 20 weeks gestation.<sup>47</sup> Additionally, a recent CMV infection can be excluded if a high avidity is demonstrated in the first 12 to 16 weeks of pregnancy. If high avidity antibodies are present in the first trimester, a pregnancy is unlikely to result in symptomatic congenital transmission. In pregnancies where primary maternal infection is confirmed, further evaluation is directed toward determining if the fetus is infected (see Figure 1).

### Diagnosis of fetal infection

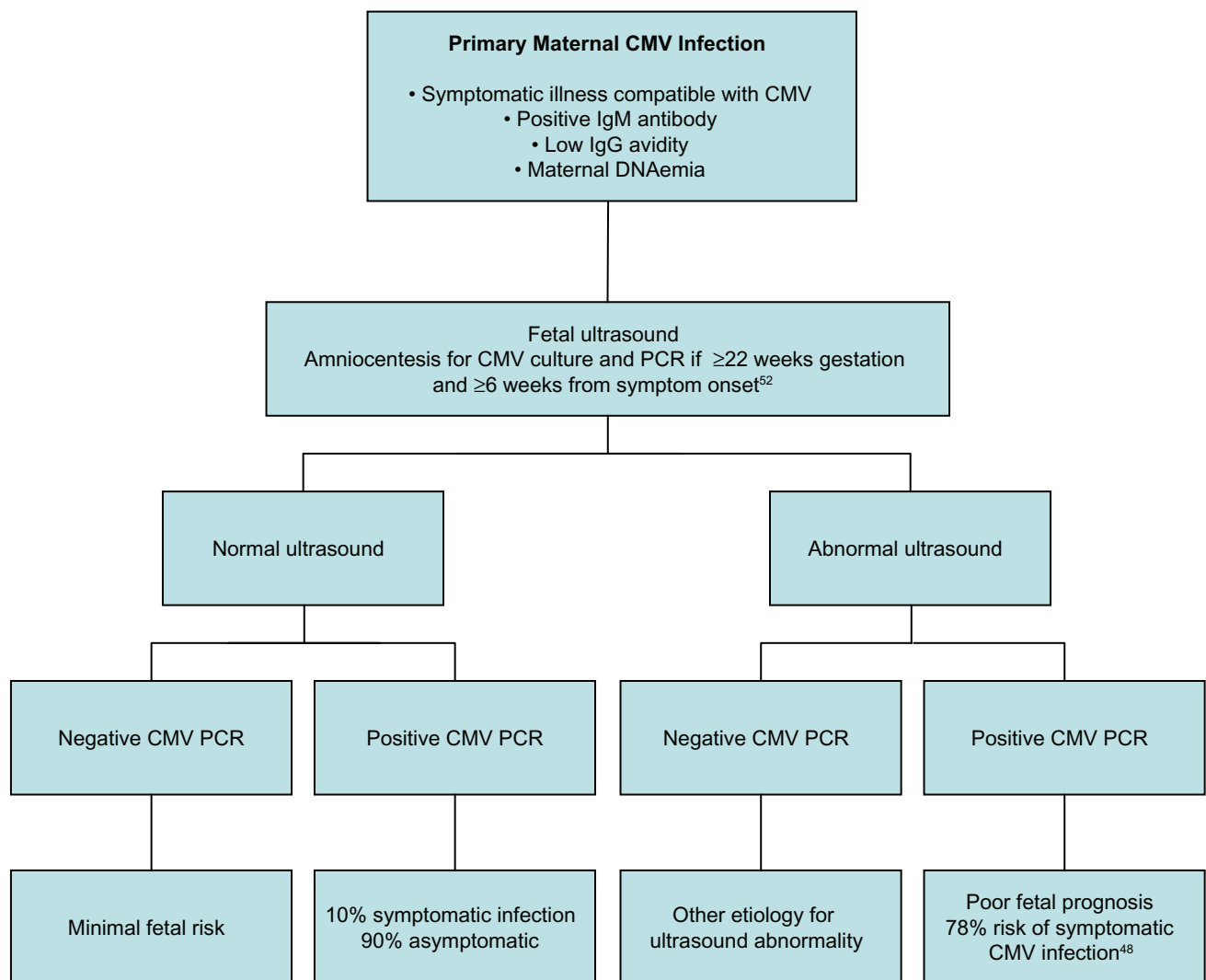
Ultrasound can detect anomalies associated with CMV congenital infection, although many of the findings are non-specific. Prenatal ultrasonographic findings of congenital CMV infection include intrauterine growth restriction

(IUGR), microcephaly, ventriculomegaly, periventricular calcifications, echogenic bowel, hydramnios, hydrops, pleural effusion, and placental enlargement.<sup>48</sup> The sensitivity of ultrasound to detect congenital CMV infection is poor given that the majority of congenitally infected infants are asymptomatic. In a study of 600 pregnant women with primary CMV infection, abnormal ultrasound findings were detected in 51/600 (8.5%) of those pregnancies and in 23/154 (14.9%) fetuses with documented congenital infection.<sup>48</sup> The positive predictive value of an abnormal ultrasound predicting symptomatic congenital infection in women with primary CMV infection was only 35.3% when fetal infection status was unknown, compared to 78.3% when congenital CMV infection was confirmed.<sup>48</sup>

Fetal infection can be diagnosed by documenting the presence of CMV in the amniotic fluid (AF), most typically demonstrated by PCR analysis (below). Amniocentesis should be offered to women with documented seroconversion, serologic studies suggestive of a primary CMV infection, or abnormal ultrasound findings consistent with congenital CMV infection. The drawback of definitive testing is the risk associated with invasive diagnostic testing. The risk of procedure-related pregnancy loss has been demonstrated to be approximately 1/300 to 1/500.<sup>49,50</sup> The risk of iatrogenic vertical CMV transmission through amniocentesis is minimal.<sup>51</sup>

The timing of diagnostic testing is important. Amniocentesis for fetal diagnosis of congenital CMV infection should be performed after 21 to 23 weeks gestation, and at least 6 weeks after documentation of primary maternal infection, as earlier testing can lead to false negative results.<sup>52,53</sup> In cases where maternal seroconversion occurs in the first trimester, the interval between seroconversion and testing of the AF may need to be increased.<sup>52</sup> The timing of diagnostic testing is due to the fact that CMV is excreted through the fetal urine at detectable levels 6 to 9 weeks after maternal infection, and fetal urination is increased after 20 to 21 weeks gestation. When testing AF  $\geq$  6 weeks after maternal infection and  $\geq$  23 weeks gestation the sensitivity of prenatal diagnosis of congenital CMV infection increased from 45% to 55% to 95.5%.<sup>52</sup>

AF can be evaluated with CMV viral culture and CMV polymerase chain reaction (PCR). The drawback of viral culture is that it can yield false negative results, and it requires a lengthy period of time to grow the virus in the laboratory (up to 6 weeks). PCR of the AF to detect CMV has a sensitivity and specificity of 90% to 98% and 92% to 98%, respectively.<sup>54</sup> Studies attempting to determine the



**Figure 1** Suggested guidelines for management and counseling of women with primary cytomegalovirus (CMV) infection during pregnancy. While no single maternal test is diagnostic, they can be useful in aiding further management and counseling of pregnant women with suspected primary CMV infection. Fetal ultrasound and definitive diagnostic testing for congenital infection should be offered when primary maternal CMV infection is suspected.

threshold of CMV viral load that is predictive of symptomatic congenital infection and/or sequelae have yielded conflicting results. Lazzarotto et al demonstrated that if the AF viral load is  $\geq 10^3$  copies/mL the risk for congenital infection is 100%, and that the presence of viral load of  $\geq 10^5$  copies/mL is predictive of symptomatic congenital infection.<sup>55</sup> Additionally, Lazzarotto et al found that an AF PCR level of  $< 500$  copies/mL is unlikely to be associated with symptomatic congenital infection.<sup>54</sup> Other studies have not demonstrated clear viral load cut-offs correlating with fetal outcomes.<sup>56,57</sup> While the viral load is generally greater in symptomatic infections when compared to asymptomatic infections, considerable overlap exists.<sup>54,56</sup> The analysis of viral load by PCR is also fraught with uncertainty given that there are a great number of PCR assays, differing in primer

sequence, method of PCR, and technique for quantification. If the AF CMV PCR and viral culture are negative, then congenital CMV infection is unlikely.

Counseling women with primary CMV infection accurately in pregnancy is important. Guerra et al demonstrated that accurate interpretation and counseling of women with positive CMV screening can decrease the rate of termination of pregnancy.<sup>58</sup> Women with no evidence of CMV infection in the AF are likely to have an uninfected neonate. Women with CMV present in the AF should be counseled that there is a 10% risk of symptomatic congenital infection and a 90% chance of an asymptomatic congenital infection at birth. However, if the primary maternal CMV infection occurred in early pregnancy, the risk of an infected fetus demonstrating symptoms at birth is increased to between 20% and 30%.<sup>38,59</sup>



Fetuses with documented congenital CMV infection and ultrasound abnormalities have a poor prognosis, especially if cerebral abnormalities are detected.<sup>60</sup>

## Postnatal diagnosis of congenital infection

In the evaluation of a newborn infant with possible congenital CMV, care must be taken to *not* rely on antibody titers in the infant (so-called “TORCH” titers) because these are seldom of value in establishing the diagnosis of congenital CMV. The finding of CMV antibodies in an infant may simply reflect transplacental transfer of IgG, and IgM assays are fraught with the same issues regarding sensitivity and specificity in the newborn as they are in the mother.<sup>44,45</sup> The most important diagnostic studies in the evaluation of suspected CMV disease are virologic studies, not serologic studies, including viral culture and PCR. CMV may be cultured from virtually any body fluid or organ system. Blood, urine, saliva, cerebrospinal fluid, bronchoalveolar lavage fluid, and tissues from biopsy specimens are all appropriate specimens for culture. The specimen is inoculated on to human cells (usually human foreskin fibroblasts) and the cell culture is monitored for the development of the characteristic CMV-associated cytopathic effect. CMV may grow slowly in culture, requiring up to 6 weeks of incubation. Culture identification is enhanced by centrifugation techniques, followed by monoclonal-antibody detection, referred to as the “shell-vial” assay. PCR amplification of CMV DNA from clinical specimens is a useful adjunct to culture techniques, and in recent years is increasingly being employed in place of viral culture for diagnosis of CMV infection.<sup>54</sup> The information derived from PCR not only helps establish the diagnosis of CMV infection, but quantitative data may be of value in establishing prognosis, since there is evidence that infants with higher viral loads may portend an increased risk of development of SNHL; these infants, accordingly, may be more likely than infants with low viral load to benefit from antiviral therapy with ganciclovir.<sup>61</sup>

## CMV screening

### Maternal CMV screening

Routine maternal screening for CMV infection is controversial. Currently neither the American College of Obstetricians and Gynecologists (ACOG) nor the Centers for Disease Control and Prevention (CDC) recommend routine serologic screening for CMV infection in pregnancy.<sup>62,63</sup> Several problems exist with routine maternal serologic screening for CMV. First, if maternal immunity to CMV is documented, it does not rule out the possibility of congenital CMV infection through infection with a new CMV strain or reactivation

of maternal CMV. Second, diagnosis of an *in utero* CMV infection does not necessarily predict symptomatic disease or sequelae in the infant upon completion of the pregnancy. Third, there is no established, evidence-based treatment available for fetal CMV infection in the pregnant patient. Finally, maternal screening may produce undue anxiety, particularly if screening is performed without adequate counseling to educate the patient about the implications of a positive or negative screening test.

Another consideration in developing a CMV screening program is the cost-effectiveness of such measures. Cahill et al evaluated the cost-effectiveness of 3 screening strategies and found that universal screening for primary maternal CMV infection would be cost effective if treatment of affected pregnancies with CMV specific hyperimmune globulin (HIG) would result in a minimum of a 47% reduction in symptomatic CMV disease.<sup>64</sup> Further studies with randomized controlled trials are needed to evaluate treatment options, including CMV HIG, to determine a screening program's ability to reduce morbidity of congenital CMV disease.

Until evidence-based treatment is available, efforts should be made on emphasizing preventative measures to all women who are attempting pregnancy, or who are currently pregnant. Screening of pregnant women should be limited to instances where there is a clinical suspicion of an active CMV infection, either due to maternal symptoms, or ultrasonographic abnormalities suggesting fetal anomalies or maldevelopment.

### Neonatal CMV screening

Newborn screening (NBS) for endocrine and metabolic disorders has been successfully performed through collection and analysis of dried blood spot (DBS) specimens. Universal newborn hearing screening (UNHS) is currently employed and has been demonstrated to have a positive effect on language outcomes among children with permanent bilateral hearing loss.<sup>65</sup>

Congenital CMV infection is a known cause of SNHL; however, UNHS will miss up to 75% of cases of CMV-associated SNHL, since, as noted, hearing loss may be late-onset. This observation provides support for the concept of universal screening for congenital CMV.

Utilization of NBS for diagnosis of congenital CMV infection is currently being debated. NBS for CMV can be justified based the much higher incidence of congenital CMV infection than many other disorders included in NBS program, and its association with SNHL. The potential benefit of NBS for congenital CMV derives from the opportunity to

identify children who require close surveillance, including audiological evaluation and neurodevelopmental assessment. Early intervention programs for SNHL and developmental concerns can then be instituted, if required. Since the majority of cases of SNHL associated with congenital CMV occur post-natally, as already noted, a normal NHS does not provide complete reassurance in an infant with congenital infection, and serial audiological evaluation is required. Additionally, screening could identify children who may benefit from antiviral treatment to prevent hearing loss.

Prior to the implementation of universal CMV screening, a sensitive, reliable, and cost effective screening test for CMV needs to be optimized needs to be established. CMV testing can be performed utilizing urine, saliva, or DBS. Viral culture of the urine has long been the gold standard laboratory test for the diagnosis of CMV infection in newborns. Given the time-consuming and costliness of viral culture, PCR has been increasingly utilized to detect CMV virus. PCR of DBS has recently been reported by a number of investigators as a useful technique for detecting congenital CMV infection. One objection to the use of DBS as the sole test for detecting congenital CMV is that the viral load in blood may often be lower than in saliva or urine, or that DNAemia may be absent altogether in a congenitally infected baby. This may make detection of DNA in the DBS relatively insensitive compared to urine or saliva in making the diagnosis of congenital CMV.<sup>66</sup> There have been many studies evaluating varying methods for CMV DNA detection by PCR with a wide range of sensitivities reported.<sup>67-69</sup> Continued work toward identification of the optimal screening test is necessary.

Another hurdle in the development of a NBS program for CMV is the creation and oversight of programs to carry out screening. Two of the options that have been posed for congenital CMV NBS are 1) hospital-based program utilizing urine, saliva, or DBS specimens and 2) state NBS program using DBS.<sup>70</sup>

Before universal NBS for congenital CMV can become standard practice, a reliable and sensitive screening test needs to be identified, mechanisms for implementation of this screening test must be validated, and a plan for how to longitudinally monitor children indentified with congenital CMV through screening needs to be identified.

## Prevention

Prevention of CMV transmission in women of childbearing age is of utmost importance in order to reduce the rate of congenital infection. Both the CDC workgroup and

ACOG recommend education on hygienic practices to prevent CMV viral transmission.<sup>62,71</sup> Hygienic strategies are important in preventing CMV transmission given that the saliva and urine of infected children are significant sources of CMV infection among women who are pregnant.<sup>25</sup> Preventative strategies include washing hands whenever there is contact with a child's saliva or urine, not sharing food, utensils, or cups, and not kissing a child on the mouth or cheek.<sup>4,72</sup>

Education of women about the implications of acquiring CMV infection, particularly during pregnancy, is vital. A survey of women in 2005 showed that only 14% of women knew what CMV was, but reported that following preventative measures for preventing an infection that could harm an unborn baby would generally be acceptable.<sup>73</sup> The effectiveness of educating pregnant women on methods to prevent CMV transmission has been demonstrated.<sup>74-76</sup> In a study where seronegative mothers with a child in group day care were instructed on measures to prevent CMV transmission, pregnant mothers had a significantly lower rate of CMV infection when compared to non-pregnant mothers attempting conception.<sup>75</sup> Additionally, Vauloup-Fellous et al recently demonstrated a lower CMV seroconversion rate after counseling pregnant women on hygienic measures.<sup>76,77</sup>

Despite the demonstrated success of education of pregnant women on hygienic measures to prevent CMV transmission, obstetricians are not providing uniform, appropriate counseling. A recent survey of the ACOG Collaborative Ambulatory Research Network (CARN) members revealed that less than half counseled their patients on methods to prevent CMV transmission and the importance of prevention of transmission.<sup>63</sup> Additionally, results of the survey of the ACOG CARN members and a survey of physicians in the Netherlands demonstrated gaps in their knowledge of CMV transmission.<sup>63,78</sup> Continued education of providers on CMV infection and prevention is important in order for accurate counseling of pregnant women.

Effective strategies to increase awareness of CMV and the methods to prevent its transmission are needed. Additionally, a systematic means to measure the success of these programs is required. Recently, Bate and Cannon have proposed a plan to identify effective behavioral interventions for prevention of congenital CMV based on a social marketing model.<sup>79</sup> This paper provides a framework to identify and evaluate behavioral interventions aimed at encouraging pregnant women to follow preventative measures.<sup>79</sup> Identification of effective means to educate pregnant women on CMV would

allow for more focused use of resources, and could result in a reduction in CMV transmission.

## Therapy

### Antiviral

Antiviral agents currently licensed for the treatment of CMV infections include ganciclovir and its prodrugs valganciclovir, foscarnet, and cidofovir. Although there has been significant experience with the use of ganciclovir in the infected newborn (reviewed below), none of these agents have been formally approved by the US Food and Drug Administration (FDA) specifically for the treatment or prevention of CMV infections during pregnancy or for congenital CMV.<sup>80</sup> There has been concern reported regarding the safety of treatment with antiviral medications in pregnancy. A case report of use of oral ganciclovir in a liver transplant patient in pregnancy did not show any evidence of teratogenicity.<sup>81</sup> Ganciclovir has been demonstrated to cross the placenta, and therefore could in theory, be utilized to treat *in utero* congenital CMV infection.<sup>82</sup> An observational study of 20 women with 21 fetuses with confirmed *in utero* CMV infection treated with oral valganciclovir demonstrated placental transfer of the drug, with therapeutic concentrations in the AF and a reduction of viral load in the fetal blood.<sup>83</sup> There have been several case reports of treatment of congenital CMV infection *in utero* with oral, parenteral, or intra-amniotic ganciclovir with varying degrees of success.<sup>84–88</sup> Although it is probably safe, prenatal treatment of fetal CMV infection with ganciclovir is currently not supported by the available data; further study with a randomized controlled trial is needed.

Despite a lack of formal FDA approval, there is good evidence supporting the treatment of congenital CMV infection with ganciclovir, based upon its demonstrated impact on SNHL outcomes. A randomized, controlled trial of 100 neonates with symptomatic congenital CMV infection treated with intravenous ganciclovir at a dose of 6 mg/kg every 12 hours for 6 weeks showed prevention of deterioration of hearing at 6 months.<sup>89</sup> In a retrospective review of 9 children with symptomatic congenital CMV, none had progression of hearing loss following prolonged ganciclovir therapy.<sup>90</sup> In another study, children with asymptomatic congenital CMV infection were treated with ganciclovir versus observation and followed over time.<sup>91</sup> No children in the ganciclovir group had SNHL, while 11.1% in the observation group demonstrated evidence of SNHL over time. Valganciclovir has been more recently studied for the postnatal treatment of symptomatic congenital CMV, and is an attractive alternative to ganciclovir, because it can be administered orally.<sup>92</sup>

A trial is currently in progress comparing treatment with valganciclovir for 6 weeks versus 6 months, with the goal of examining whether prolonged therapy further improves neurodevelopmental outcomes, including SNHL.<sup>92</sup>

### Passive immunization

Immunoglobulin therapy has been used to treat several conditions in pregnancy and is generally well tolerated. The mechanisms by which immunoglobulins act to treat many pregnancy-related conditions remain largely unknown. Treatment of a variety of viral infections with immunoglobulin has been valuable in disease control. Theoretical mechanisms include direct neutralization of virus particles; facilitation of antibody-directed natural killer cell activity; antibody-dependent cellular cytotoxicity; and blocking of viral entry at the cell surface.<sup>93</sup>

Passive immunization with hyper-immune globulin (CMV HIG) has been studied for the *in utero* treatment and prevention of congenital CMV infection. CMV HIG is a pooled, high-titer immunoglobulin preparation derived from donors with high levels of CMV antibody. Improved perinatal outcomes after treatment of congenital CMV infection was first demonstrated in animal models.<sup>94,95</sup> Nigro and colleagues completed a prospective study of CMV HIG for the treatment of pregnant women with primary CMV infection, including some women with confirmed fetal CMV infection.<sup>96</sup> The women were enrolled in the “therapy” group if they had an amniocentesis and confirmed congenital CMV infection, as evidenced by a positive PCR in the AF, or the “prevention” group if they did not have an amniocentesis. In the therapy group only 1/31 of the treated mothers delivered an infant with congenital CMV disease, compared to 7/14 mothers who were not treated with HIG. In the prevention group 6/37 mothers receiving HIG delivered infants with congenital CMV, compared to 19/47 mothers who did not receive treatment. Overall, there was a statistically significant reduction of risk for congenital CMV infection in the HIG therapy group, and a reduction in CMV infection in the HIG prevention group. In a subsequent study by Nigro and colleagues, 3 fetuses treated with HIG had resolution of their ultrasonographically detected cerebral abnormalities; in contrast 2 untreated fetuses had persistence of their cerebral abnormalities.<sup>97</sup>

In addition to fetal effects, CMV HIG has been demonstrated to affect the placenta. In pregnancies treated with HIG there has been demonstration of significant reductions in placental thickness.<sup>97,98</sup> The reduction in placental thickness observed following HIG treatment suggests that improved fetal outcomes are mediated, at least in part,



through improved placental health and function, and not (only) through the salutary effect of immune globulin present in the fetal circulation.<sup>99</sup>

The mechanisms by which HIG works for the treatment and prevention of congenital CMV may be due to the virus neutralizing effect. Nigro et al demonstrated that women in the treatment group had significantly higher levels of CMV specific IgG concentration and IgG avidity after HIG treatment when compared to the untreated group.<sup>96</sup> Symptomatic CMV disease may be secondary to inflammation in response to CMV infection.<sup>100,101</sup> The immunomodulatory effects of HIG may decrease the inflammation, and subsequent tissue damage from CMV. Randomized controlled trials of HIG for treatment and prevention of congenital CMV infection are needed. Until such data are available, clinicians could give consideration to treatment with CMV HIG in pregnant patients with confirmed fetal CMV infection.

## Vaccines

### Live attenuated virus vaccines

It has been over three decades since the first live CMV vaccine candidate was tested in humans.<sup>102</sup> A subsequent live attenuated CMV vaccine candidate, the Towne strain, was found to have a good safety profile and was immunogenic.<sup>103</sup> A clinical trial of the Towne strain CMV vaccine in kidney transplant patients showed that the vaccine did not prevent CMV infection, but it did reduce the severity of CMV disease when compared to placebo controls.<sup>104</sup> However, the Towne vaccine failed to prevent CMV infection in seronegative women who had children attending group day care when it was tested in a placebo-controlled trial.<sup>105</sup> The lack of efficacy in this study was attributed to the decreased neutralizing titers produced after Towne vaccination when compared to infection by wild type virus. In addition, the laboratory-adapted Towne strain of CMV has genomic deletions which may affect the immunogenicity of the vaccine. With the goal of improving the immunogenicity of live, attenuated CMV vaccines, 4 “chimeric” vaccines were created which represented hybrids of the Towne strain and the less attenuated Toledo strain of CMV, and tested in a phase one study; these vaccines were all well tolerated, although immunogenicity was difficult to assess.<sup>106</sup> Additional study of the chimeric vaccines need to be performed in seronegative controls.

### Subunit vaccines

#### Purified recombinant glycoprotein B

Additional vaccine approaches are currently being developed with the goal of inducing a potent virus-neutralizing antibody

response.<sup>107</sup> The CMV envelope glycoprotein B (gB) has been the most studied subunit vaccine candidate for this purpose, since it is a target of neutralizing antibody in all CMV-seropositive individuals. Animal models of gB vaccine have shown its ability to prevent congenital CMV infection and disease.<sup>108,109</sup> Results of a phase II, placebo-controlled, randomized, double-blind trial of the recombinant CMV envelope gB with MF59 adjuvant were recently published.<sup>110</sup> In this study, three doses of the CMV vaccine or placebo were administered at 0, 1, and 6 months to healthy women within 12 months postpartum. Women in the vaccine group were less likely to become infected with CMV than the placebo group ( $P = 0.02$ ) with 18/225 women becoming infected with CMV in the vaccine group, compared to 31/216 in the placebo group.

### DNA vaccines

DNA vaccines have been studied in both animal models and humans. In the guinea pig model gB DNA vaccination administered prior to conception offered some protection against congenital CMV transmission in the liveborn pups.<sup>111</sup> A phase I study of a bivalent CMV DNA vaccine demonstrated safety and immunogenicity of the DNA vaccine.<sup>112</sup> The effect of immune priming with a CMV DNA vaccine encoding pp65, IE1, and gB was tested by administering the Towne strain after vaccination and evaluating the immune response.<sup>113</sup> There was a significant decrease in time to pp65 T-cell and gB antibody response in DNA primed subjects when compared to control subjects who were administered Towne strain only. DNA vaccines are currently being evaluated in the hematopoietic stem cell transplantation population toward the goal of reducing CMV disease in these individuals.<sup>114</sup>

### Vector systems

Vector systems utilize non-replicating vectors to express gene products of interest. Two examples of vector systems utilized in CMV vaccine development are the modified vaccinia virus Ankara (MVA) and an alphavirus vector based on an attenuated variant of Venezuelan equine encephalitis virus. MVA utilizes a highly attenuated poxvirus vector. The MVA vector system can express gB, as well as the cell-mediated immunity targets pp65, and IE1.<sup>115</sup> The alphavirus vector system produces virus-like replicon particles (VRP) that can express gB, pp65 and IE1.<sup>116</sup> The alphavirus vector system has been studied in the in the guinea pig model and was found to induce both humoral and cellular immunity and improve pregnancy outcomes.<sup>117</sup>

## Future prospects

Recent advances in the understanding of CMV-cell interactions have led to new insights into potential glycoprotein targets for vaccination. Recently, a new glycoprotein complex, including the gene products of the UL128-131 region of the viral genome, has been characterized. These proteins are necessary for CMV entry into epithelial cells.<sup>118,119</sup> The live, attenuated Towne vaccine and the gB-based protein subunit vaccine are less effective than natural infection in inducing antibodies capable of neutralizing CMV infection in epithelial cells. Future optimization of CMV vaccines may require improvement in the humoral response to virally encoded proteins important in epithelial cell entry, particularly in view of the fact that most CMV infections are acquired at mucosal surfaces.<sup>120</sup>

## Conclusion

CMV remains a significant public health concern. The neurologic disability from congenital CMV infection can be devastating. Antiviral drugs administered postnatally appear to decrease the severity of SNHL. CMV HIG has shown promising results for *in utero* treatment of fetal CMV infection, and possible prevention of congenital transmission, although randomized, blinded, controlled trials are lacking. CMV vaccine development continues to be a major public health priority. Until an effective CMV vaccine is licensed, education of young women regarding hygienic and behavioral approaches that can help prevent CMV transmission is essential. Obstetricians can lead the way in ensuring that appropriate counseling about the risks of CMV becomes a mainstay of prenatal care.

## Disclosures

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