The “Silent” Global Burden of Congenital Cytomegalovirus

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SUMMARY

Human cytomegalovirus (CMV) is a leading cause of congenital infections worldwide. In the developed world, following the virtual elimination of circulating rubella, it is the commonest nongenetic cause of childhood hearing loss and an important cause of neurodevelopmental delay. The seroprevalence of CMV in adults and the incidence of congenital CMV infection are highest in developing countries (1 to 5% of births) and are most likely driven by nonprimary maternal infections. However, reliable estimates of prevalence and outcome from developing countries are not available. This is largely due to the dogma that maternal preexisting seroimmunity virtually eliminates the risk for sequelae. However, recent data demonstrating similar rates of sequelae, especially hearing loss, following primary and nonprimary maternal infection have underscored the importance of congenital CMV infection in resource-poor settings. Although a significant proportion of congenital CMV infections are attributable to maternal primary infection in well-resourced settings, the absence of specific interventions for seronegative mothers and uncertainty about fetal prognosis have discouraged routine maternal antibody screening. Despite these challenges, encouraging results from prototype vaccines have been reported, and the first randomized phase III trials of prenatal interventions and prolonged postnatal antiviral therapy are under way. Successful implementation of strategies to prevent or reduce the burden of congenital CMV infection will require heightened global awareness among clinicians and the general population. In this review, we highlight the global epidemiology of congenital CMV and the implications of growing knowledge in areas of prevention, diagnosis, prognosis, and management for both low (50 to 70%)- and high (>70%)-seroprevalence settings.

INTRODUCTION

Cytomegalovirus (CMV) is highly adapted to its human host. A full appreciation of CMV as a pathogen contributing to morbidity and mortality in a variety of immunocompromised hosts is well established. In contrast, the fact that CMV is also a leading cause of congenital infections worldwide is barely appreciated, as is the socioeconomic impact of CMV as the commonest nongenetic cause of childhood hearing loss in the postrubella era and a significant cause of neurodevelopmental delay (1–4). Indeed, CMV causes more cases of congenital disease than the combination of 29 currently screened conditions in most American states (5) and is more common than several disorders included in newborn screening in European Union countries (6).

The worldwide neglect of this problem is underscored by the continued lack of awareness of congenital CMV among health care workers and the public. The low profile of congenital CMV can be explained by the following factors. First, most maternal and newborn infections are asymptomatic and therefore are not recognized at birth. Second, sequelae from congenital CMV infection are frequently delayed in onset, at which point a retrospective diagnosis is challenging. Third, the dogma that congenitally infected children who are born to women with preexisting antibodies have normal outcomes has led to inattention to congenital CMV in developing countries. Emerging data from highly seropositive populations, which are usually in developing countries,
however, suggest that not only is the rate of congenital CMV infection higher than in developed countries but it is an important cause of hearing loss in resource-limited settings (7, 8). In fact, the higher prevalence of congenital CMV infection in highly seropositive populations coupled with recent hearing outcome data from Brazil suggests that the resource-limited settings may bear the greatest burden of congenital CMV infection (7, 8). However, population-based natural history studies that accurately estimate disease, disability, and mortality burden in resource-limited settings are lacking. Moreover, there are insufficient data about the feasibility of newborn screening and antiviral therapy and the cost of long-term care for affected children in developing countries.

The quest for active and passive immunization strategies that can prevent in utero infection remains an ongoing challenge. High virus diversity and the propensity for infection with multiple different virus strains pose an important biological barrier to the development of effective vaccines (9–13). Moreover, at the population level, the fact that most congenitally infected newborns are born to mothers with preexisting immunity limits the benefit of these approaches (14, 15). Therefore, interventions that can reduce the global burden of disease are presently restricted to behavioral measures (16–18).

In this review, we highlight the global epidemiology of congenital CMV and the implications of growing knowledge in areas of prevention, diagnosis, prognosis, and management for both low (50 to 70%)- and high (>= 70%)-seroprevalence settings.

BIOLGY

CMV is a host-restricted member of the Herpesviridae family of viruses (19). Primary infection is characterized by a period of active virus replication with virus shedding in saliva, urine, milk, and genital secretions, a viremic phase, and, in some, an infectious mononucleosis syndrome (19, 20). This is followed by the development of a broad immune response involving all arms of the adaptive immune system, and after several weeks, viral latency is established (19). Latent infection is characterized by either a low level or absence of detectable virus replication with the maintenance of viral genomes as episomes in CD14+ peripheral blood mononuclear cells and CD34+ and CD33+ cells in the bone marrow, which will allow subsequent production of endogenous virus (reactivation) (21, 22). Sequence variability across the large viral genome generates extensive viral strain diversity (genotypes), the biological and clinical significance of which remains unknown (11, 23). In immunocompetent mothers, reactivation of endogenous virus and/or reinfection with new strains occurs periodically, and DNAemia and viruria may be present in both (24).

EPIDEMIOLOGY AND CLINICAL OUTCOMES

CMV is a global infection, although significant differences in the seroepidemiology exist between and within countries. CMV acquisition in a population is characterized by an age-dependent rise in seroprevalence, and correlates most closely with socioeconomic level and race (25–29). As a result, up to 50% of women of childbearing age are seronegative in industrialized countries (25, 30). In this population, CMV acquisition occurs at a rate of 1 to 7% per year (31) and usually follows frequent and prolonged contact with young children (less than 3 years of age) (31–34). By comparison, in resource-poor communities in industrialized countries and in developing countries, CMV is usually acquired very early in life owing to breast milk transmission and crowded living conditions, and far fewer adult women are seronegative (7, 35–41).

The incidence of in utero CMV infection is highly population dependent (Fig. 1) and parallels maternal seroprevalence (Fig. 2), probably due to the fact that seroprevalence rates serve as a marker for the size of the reservoir of viruses. Thus, higher seroprevalence rates lead to an increased chance of either reactivation within a host, reinfection of seropositive hosts (together these constitute nonprimary infection), or primary infection of seronegative hosts within the population. This in turn probably leads to various degrees of maternal viremia and influences the risk for subsequent placental and/or fetal infection (42). In addition, seroprevalence levels in a population may reflect variation in host and environmental factors that also influence the risk of maternal (14, 43) and vertical (27) infection. Therefore, in industrialized countries, where the maternal seroprevalence is relatively low overall, rates of congenital CMV infection average 0.6 to 0.7% of live births (1 in every 100 to 150 newborns) (27, 44). However, even within a geographic region, variable rates of CMV seropositivity in mothers from different racial, ethnic, and socioeconomic backgrounds may translate to distinct epidemiological patterns of congenital infection (26, 27, 29, 45, 46). Similarly, in developing nations with highly seropositive populations, higher rates (1 to 5%) have often been reported (7, 47–51).

Most recent studies report lower transmission rates in early pregnancy (in comparison to later gestation) (52–58), with maternal primary infection leading to infection in 30 to 35% (Fig. 2) of fetuses and nonprimary infection having a transmission rate of 1.4% in study populations predominantly from industrialized countries (1.1 to 1.7%) (27). Data from screened populations indicate that while only one in 10 newborns infected in utero have obvious clinical signs of congenital infection (27, 44, 59), 10% to 15% of those without clinical findings (here referred to as having symptomatic and asymptomatic congenital CMV infection, respectively) develop long-term neurological sequelae (44). Specifically, sensorineural hearing loss (SNHL) occurs in about 35%, cognitive deficits in up to two-thirds, and death in around 4% of children with symptomatic infection. Visual impairment is thought to occur in 22 to 58% (60, 61) of symptomatic infants; however, there are insufficient data for this outcome from unbiased sampling. Far lower rates of sensory and cognitive sequelae have been reported in asymptomatic children. Hearing impairment has been reported in 7% to 10% (44, 62) of such infants, while the risk for cognitive deficits has not been studied systematically and the risk for visual impairment appears to be negligible (61). Overall (symptomatic and asymptomatic infections), permanent childhood hearing impairment is the commonest complication. In developed countries, congenital CMV accounts for 21% and 24% of cases of hearing loss at birth and 4 years of age, respectively (3, 59). Without early detection and prompt rehabilitation, this leads to speech, language, and social impairment in a significant number of children and deployment of continued medical care resources (63–66). Since newborn hearing screening may miss or underestimate hearing loss (the majority of children with CMV-associated SNHL have normal hearing at birth and develop subsequent late-onset hearing loss) and since the hearing loss is frequently progressive (50%), long-term monitoring is necessary (59, 67, 68). The public health impact of hearing loss may be even greater in high-seroprevalence settings where birth rates are sub-
substantially higher, although this has not been systematically studied to date.

The risk for long-term outcomes appears to be highest in infants born to mothers with primary infection in the first half of pregnancy (54, 55, 69–71). Following first-trimester maternal CMV infections, about a quarter of infants (20 to 25%) who are congenitally infected (Fig. 3 shows the risk of infection) will develop sensorineural hearing loss (SNHL), and 30 to 35% will suffer some form of central nervous system (CNS) sequelae (70). Since it is not possible to time nonprimary maternal infection, it is not known whether the timing of maternal infection is associated with the risk for sequelae in this group.

It is estimated that more than two-thirds of infants with congenital CMV infection are born to mothers who are already CMV seropositive (14, 15, 72). Emerging observations demonstrate that the risk for symptomatic infection at birth and sequelae, especially...
hearing loss, in these children may be similar to that in infants born to mothers experiencing a primary infection (8, 73–77). In addition, in resource-poor settings, specific risk subgroups may exist, such as mothers with concomitant immunosuppressive chronic diseases (see below). Unfortunately, maternal and birth CMV prevalence and long-term follow-up data for congenitally infected children for many parts of the world are lacking, likely underestimating the global impact of congenital CMV infection.

**Impact of the HIV Epidemic on Congenital CMV**

HIV-infected women are often CMV seropositive and experience more frequent CMV recurrences with progressive immune impairment (78–80). Studies in Europe and the Americas support an increased risk for congenital CMV infection in neonates born to HIV-CMV-coinfected mothers (79, 81, 82). A French perinatal cohort that included 4,797 HIV-infected mothers between 1993 and 2004 demonstrated an increased risk for congenital CMV in HIV-infected newborns compared with HIV-negative infants (10.3% versus 2.2%). HIV-infected newborns also had a 3-fold-higher risk for symptomatic congenital CMV infection than uninfected newborns (23% versus 6.7%). Furthermore, CMV may act as a cofactor for HIV disease progression. The risk for infant mortality is increased in HIV-CMV-coinfected infants, and there is accelerated progression of CNS disease in survivors, especially developmental delay and worsening motor deficits (83, 84).

The French perinatal cohort study also showed that in the era of highly active antiretroviral therapy (HAART), the incidence of vertical CMV infection in HIV-positive mothers was falling, which was associated with improvements in CD4 count (81). However, in a more recent study, Frederick et al. have not observed a significant decrease in the prevalence of congenital CMV infection in children of HIV-infected mothers receiving prenatal antiretroviral therapy (85). The overall prevalence of congenital CMV infection in that study was 3.6%.

In resource-limited settings, the high rate of coinfections in pregnant women with HIV-1 and bacterial and parasitic pathogens likely influences the transplacental transmissibility of CMV (51, 79, 86–88). In sub-Saharan Africa, the burden of HIV-1 in women of reproductive age is alarming, reaching 40% in some regions (89). Despite improvements in, and access to, antiretroviral therapy, maternal HIV acquisition and mother-to-child transmission (MTCT) of HIV in developing countries continue, leading to a sizeable proportion of infants born HIV exposed or infected. In studies of HIV-1-infected and HIV-1-exposed Kenyan women and children, a strong correlation between CMV and HIV loads was observed in the mother being associated with an increased risk of maternal mortality and mortality in the HIV-infected infants by 24 months (88).

To our knowledge, there are no published data on the risk of transmission of CMV in HIV-positive mothers in resource-lim-
edited settings. Therefore, in order to illustrate the potential impact of HIV infection on congenital CMV infection, we extrapolate from findings in high-resource settings and use South Africa and Thailand as examples (Table 1). In South Africa, maternal HIV seroprevalence is about 30\%, with the most recently reported overall HIV MTCT at around 3.5\% (90). Assuming a 1\%, 3\%, and 10\% (79, 81, 85) risk of CMV transmission in HIV-unexposed, HIV-exposed uninfected, and HIV-infected newborns, respectively, we estimate that around 18,450 newborns (an excess of 8,450 infected newborns due to maternal HIV) are born congenitally infected with CMV each year. The number of cases in South Africa equates to just over 40\% of the total annual number of congenital CMV cases in the United States. In other words, South Africa is likely to have roughly 2.5 times the number of congenital CMV infections per capita as in the United States. In Thailand, which has an annual birth rate of 830,000 and a maternal HIV seroprevalence of 0.7\% (91), assuming the above congenital CMV transmission risks and an HIV MTCT of 2.8\% (T. Naiwatanakul, N. Punsuwan, N. Kullerk, W. Faikratok, R. Lolekha, and O. Sangwanloy, presented at the 5th International AIDS Society Conference on HIV Pathogenesis and Treatment, Cape Town, South Africa, 19 to 22 July

FIG 3 Graph representing the risk of intrauterine CMV transmission following maternal primary infection from 15 studies. The transmission risk is the proportion of mothers undergoing a primary infection in a given trimester and/or the preconception period who transmitted CMV to the fetus. The risk is therefore uniform (represented by a flat line) for the time period defined as preconception (from 12 or more weeks prior to conception), first trimester (up to the 12th gestational week), second trimester (from 12 to 26 weeks), and third trimester (26 weeks to delivery) in each of the studies. Studies were grouped according to the number of weeks for which data were collected and are represented by lines of different colors: yellow, studies with late-gestation data; green, studies with preconception and/or first-trimester data; red, studies with first-, second-, and third-trimester data; blue, studies with preconception and first-, second-, and third-trimester data. The black dotted line represents pooling of the data (excluding unpublished data) for each gestational week. The denominator is the sum of mothers undergoing a primary infection from studies with data available for a particular gestational week. The numerator is the total number of transmitter mothers across these studies for that gestational week. Risks are shown as percentages. The number of women undergoing a primary infection in each study is shown in parentheses. (See references 43, 54, 55, 56, 71, 137, 143, 184, 209, 210, 211, 212, 213, and 214.)

TABLE 1 Estimates of the prevalence of congenital CMV infection in two resource-poor settings (South Africa and Thailand) according to maternal HIV-CMV coinfection

<table>
<thead>
<tr>
<th>Parameter</th>
<th>South Africa</th>
<th>Thailand</th>
</tr>
</thead>
<tbody>
<tr>
<td>Annual birth rate</td>
<td>1,000,000</td>
<td>830,000</td>
</tr>
<tr>
<td>Antenatal HIV prevalence (%)</td>
<td>30</td>
<td>0.7</td>
</tr>
<tr>
<td>HIV perinatal transmission rate (%)</td>
<td>3.5</td>
<td>2.8</td>
</tr>
<tr>
<td>No. (no. of congenital CMV infections)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIV unexposed (risk, 1%)</td>
<td>700,000 (7,000)</td>
<td>824,190 (8,242)</td>
</tr>
<tr>
<td>HIV exposed (risk, 3%)</td>
<td>265,000 (7,950)</td>
<td>5,647 (169)</td>
</tr>
<tr>
<td>HIV infected (risk, 10%)</td>
<td>35,000 (3,500)</td>
<td>163 (16)</td>
</tr>
<tr>
<td>Total no. of congenital CMV infections</td>
<td>18,450</td>
<td>8,427</td>
</tr>
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</table>
deficits (100). However, these findings were limited by the absence of a control group. It is anticipated that the randomized multi-center placebo-controlled trial (CASG112) (NCT00466817) commenced in 2008 to compare the clinical benefit of 6 weeks versus 6 months of valganciclovir in symptomatic infants will define the role of prolonged antiviral therapy. A role for antiviral therapy in the prevention of SHNL and adverse psychomotor outcomes in asymptomatic infants has also been suggested (101). However, formally evaluating a toxic drug in a large cohort of asymptomatic children, most of whom will not go on to develop sequelae, remains problematic.

The prognostic value of clinical signs, imaging findings, and laboratory parameters in the newborn with confirmed congenital CMV has been extensively evaluated. Among infants with symptomatic congenital CMV infection, microcephaly (102, 103), choriorretinitis (102), abnormal neurological examination findings (102, 104), abnormal auditory brain stem evoked response (105), and petechiae and thrombocytopenia (104, 105) are each associated with an unfavorable clinical outcome. Furthermore, newborn neuroimaging (ultrasound [US], computed tomography [CT], and magnetic resonance imaging [MRI]) abnormalities (105, 106) carry a high risk for CNS sequelae. In asymptomatic infants, on the other hand, clinical or laboratory predictors of adverse outcomes have not been identified. However, low CMV blood viral loads (<10⁶ copies/10⁵ polymorphonuclear leukocytes) appear to predict normal development with reasonable certainty (>95%) (107–110). In the absence of well-defined predictors of outcome, monitoring of all congenitally infected newborns is advised. This includes regular neurological, developmental, auditory, and visual assessments at least until school age in symptomatic newborns, whereas recommendations for follow-up of asymptomatic newborns are usually restricted to audiology. Regular monitoring for progressive and late-onset deficits permits early rehabilitation (93, 94). At 1 year of follow-up, the absence of neurodevelopmental delay appears to predict a normal intellectual outcome (111).

Owing to the late presentation of most congenital CMV sequelae, diagnosing vertical infection in children beyond the newborn period is a key challenge for both the clinician and the epidemiologist. Dried blood spots (DBS), or Guthrie cards, which are collected routinely at birth in certain countries for newborn genetic and metabolic diseases screening, can be stored for extended periods of time. Since CMV DNA is stable on such DBS cards for up to 18 years, they offer an attractive tool for the retrospective molecular diagnosis of congenital infection in individual children who present with delayed-onset sequelae (112–115). DBS also have appeal for use in newborn congenital CMV screening programs. However, the variable and disappointing rates of detection of CMV DNA (34 to 100%) have made DBS unsuitable for these purposes (112, 114, 116–122), possibly because not all newborns are viremic at birth or due to technical factors (118, 122–124). However, a positive DBS PCR finding is diagnostic of congenital CMV infection and accordingly can be useful to retrospectively diagnose congenital CMV infection beyond the neonatal period.

The recent demonstration that real-time PCR detection of CMV in saliva swabs, either air dried or in viral transport medium, is equally sensitive as virus culture techniques has made wide-scale newborn screening realizable (125). Universal newborn CMV screening would identify infants at risk for hearing loss, who can then be targeted for prompt interventions that prevent significant speech and language deficits (44, 126). However, the cost of testing, the modest efficacy of available antiviral therapy, the high}

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2009), we estimate that 8,427 newborns are born congenitally infected with CMV each year.

**ADVANCES IN DIAGNOSIS AND MANAGEMENT OF THE NEWBORN**

The majority of congenital CMV infections from both resource-poor and upper-income settings are asymptomatic at birth, and the diagnosis of intrauterine infection relies on virus detection by culture-based methods or PCR. Saliva or urine (see below) specimens should be obtained within the first 2 weeks of life (92), as virological testing cannot discriminate intrauterine from postnatal CMV infection beyond 2 weeks. When an early specimen is not available for testing, clinical features highly indicative of congenital CMV infection, such as CNS, retinal, or auditory findings, can suggest the diagnosis in symptomatic infants.

The presence of CNS disease in the symptomatic neonate with laboratory-confirmed congenital infection warrants the consideration of specific antiviral therapy. While there is no evidence for the effectiveness of treatment in children without CNS disease, it is reasonable to consider antiviral treatment in those with disseminated disease which is life threatening (93–95). Ganciclovir (GCV) or its prodrug valganciclovir (VGCV), an acyclic nucleoside analogue, is the preferred antiviral agent for the treatment of CMV disease (96). The efficacy of ganciclovir for the prevention of progressive hearing loss in infants with proven congenital CMV CNS disease as evidenced by microcephaly, other neurological findings, neuroimaging abnormalities, or hearing loss was evaluated in a randomized trial nearly a decade ago. At 12 months of follow-up, a considerably higher rate of preserved normal hearing, as well as improved hearing and prevention of worsening of hearing in those with a baseline hearing deficit, was demonstrated following a 6-week course of intravenous GCV, compared with no therapy (95). However, the frequency of drug toxicity, the absence of a placebo group, and high attrition rates in this study limit the significance of the findings. More recently, a secondary analysis on the same study population showed that infants who received GCV therapy appeared to have fewer developmental delays at 6 and 12 months than untreated infants (97). Based on these findings, a 6-week course of intravenous ganciclovir or oral valganciclovir (VGCV) is considered for children with CNS involvement (93, 98). Pharmaceutical liquid preparations of VGCV provide stable systemic exposure, and plasma levels equivalent to those for intravenous therapy can be achieved; however, a head-to-head comparison of efficacy has not been performed (98, 99). Rates of neutropenia during a 6-week course are, however, substantial (63% for ganciclovir and 38% for valganciclovir (99), and biochemical/hematological parameters should be carefully monitored when either drug formulation is used (93). Such toxicity also precludes the treatment of neonates with asymptomatic infection because their risk of longer-term sequelae is only about 13% (44).

It has been suggested that ongoing viral replication in end organs may contribute to adverse long-term outcomes (progressive hearing impairment was reported for 21% of treated patients) (95), and prolonged antiviral therapy has been considered. A recent retrospective study of 6 weeks of intravenous GCV followed by VGCV up to a year showed that prolonged antiviral therapy may prevent hearing loss in children with normal baseline hearing and result in lower rates of deterioration in those with baseline deficits (100). However, these findings were limited by the absence of a control group. It is anticipated that the randomized multi-
proportion of asymptomatic infections, and potentially adverse psychosocial effects are considered barriers to implementation, even in countries with newborn screening programs for the detection of genetic and metabolic disorders and hearing loss (126, 127). In spite of these issues, newborn virological screening can be justified on the grounds that congenital CMV infection is likely the most common nongenetic cause of sensorineural hearing loss and screening can now be undertaken noninvasively (125). In addition, the delayed onset of most cases of CMV-associated hearing loss makes newborn hearing screening an inadequate tool for the detection of CMV-associated hearing loss (67, 68). In resource-limited settings, reliable estimates of prevalence and disease burden from congenital CMV infection are needed before the cost-effectiveness and utility of newborn CMV screening can be determined.

ADVANCES IN PREVENTION OF ADVERSE OUTCOMES

Prenatal Screening and Diagnosis of Infection in the Mother and Fetus

Maternal (prenatal) screening may permit early identification of at-risk pregnancies or infected infants and thus the use of interventions to reduce morbidity has attracted increasing interest in recent years (128). The feasibility of prenatal screening has been argued on the basis that eight European countries have overcome commonly cited obstacles to this strategy (129, 130). However, universal antibody screening of pregnant women in most resource-rich countries has not been recommended because of the absence of proven specific interventions for maternal primary infection, and challenges in deciphering the prognosis of an individual mother and fetus have been discouraging. It is also becoming increasingly apparent that at a population level, the effectiveness of such prenatal screening programs will be limited, as around two-thirds of infants with congenital CMV infection in the United States and the vast majority in resource-limited settings are born to women who are seropositive preconceptionally (14, 15). In these settings, it has been assumed that reactivation of endogenous virus or reinfection with a different strain leads to intrauterine transmission (9, 13). However, since such events are clinically silent and simple virological or immunological markers for nonprimary infection do not exist, identifying women at risk of transmission is presently not possible.

Clinical suspicion of maternal primary infection, i.e., glandular fever or flu-like illness, and the detection during routine ultrasound screening of abnormalities suggestive of intrauterine CMV that lack an apparent cause are the common indications for specific diagnostic testing (131). Maternal primary infection can be confirmed reliably by the demonstration of seroconversion (CMV IgG negative to CMV IgG positive) when a baseline serum sample from either the earliest antenatal visit or prior to conception is available (Fig. 4). When such a comparison serum is not available, the detection of both CMV IgG and IgM antibodies may indicate a recent primary infection (132). However, as a reactive CMV IgM may be found in both primary and nonprimary infections and may persist for many months following primary infection, it does not reliably predict the risk for congenital infection (133). Therefore, a reactive CMV IgM should be further evaluated by determining the maturity of the CMV IgG antibodies using the avidity assay. Low-affinity CMV IgG antibodies (those that bind less tightly with their target protein) are produced in the first 18 to 20 weeks after infection (134). A subsequent maturation process generates IgG antibodies with higher avidities (affinity maturation). A high CMV IgG avidity index therefore excludes a recent primary infection and when detected before 12 to 16 weeks of gestation indicates a significantly lower risk of congenital infection (134, 135). Conversely, low-avidity IgG antibodies together with a reactive CMV IgM strongly supports the diagnosis of maternal primary infection in the preceding 3 or 4 months (136).

The substantial risk of vertical transmission following primary maternal infection justifies invasive prenatal testing. Amniotic fluid (AF) CMV PCR is the test of choice for confirming fetal infection. As the interval between maternal and detectable fetal infection is at least 6 to 8 weeks, amniocentesis should be performed at 20 to 21 weeks of gestation and at least 7 weeks following maternal infection (69, 137–143). It is well established that the sensitivity of PCR (70 to 90%) for prenatal diagnosis is superior to that of virus culture techniques; when correctly timed, it approaches 100% (137, 143). However, as PCR may occasionally give false-positive results, it is generally recommended that screening be performed using a combination of PCR and virus culture or, where culture-based testing is not available, a second (confirmatory) molecular test (139, 144, 145) (Fig. 4). When both PCR and virus isolation tests are positive, congenital infection can be diagnosed with 100% certainty. On the other hand, when both tests are negative, fetal infection can be ruled out with a high degree of certainty (negative predictive value, >94%) (42). False-negative culture and PCR results have occasionally been reported and may be a result of delayed transmission of CMV to the fetus (69, 146, 147). Invasive prenatal testing may also be justified in nonprimary infections when sonographic findings suggest in utero CMV abnormalities (77). However, at present, firm guidelines in this area are lacking.

In the case of confirmed fetal infection, since a significant proportion of infected infants have a normal outcome, parents should be counseled on the established risks of symptomatic infection and long-term morbidity following intrauterine CMV infection, in order to guide decision-making regarding the options of termination of pregnancy (TOP) or expectant management (131), while intrauterine therapies (discussed below) remain experimental. In the absence of virological correlates or biomarkers that can definitively distinguish a symptomatic from an asymptomatic course of infection, defining the prognosis for an infected fetus may be aided by 2 to 4 weekly fetal ultrasound (US) examinations and appropriately timed (see above) amniotic fluid viral load testing (131). It has been shown that cerebral ultrasound abnormalities are strongly associated with a poor prognosis (148), and recent findings also show that combining ultrasound with magnetic resonance imaging improves the sensitivity of prenatal screening for cerebral lesions, in particular, after 30 to 34 weeks of gestation (149, 150). On the other hand, the predictive value of nonspecific findings, such as intrauterine growth restriction (IUGR), bowel hyperechogeneity, or isolated other noncerebral abnormalities, for symptomatic infection or adverse outcomes is relatively low (69, 148, 151). Amir et al. suggested that lenticulostriate vasculopathy (LSV) is a possible marker of hearing loss in congenital CMV infection (152). However, LSV is nonspecific, and other studies have not confirmed the prognostic value of this finding (148, 153). When no ultrasound findings are detected, the risk for symptomatic congenital infection and sequelae is significantly reduced, but these cannot be excluded (69, 139, 149, 151, 154).
FIG 4 Proposed diagnostic and management algorithm for maternal and congenital CMV infection. The presence of high-avidity CMV IgG antibodies before 16 weeks of gestation excludes primary infection; however, nonprimary infection is still a possibility. Indications for prenatal testing in nonprimary infections are less clear, and decisions should be made on a case-by-case basis when sonographic findings are suggestive of congenital infection. Baseline investigations for newborns with symptomatic congenital CMV infection should include complete blood count, liver function tests, CMV real-time PCR (blood and urine), audiometry, ophthalmology screen, and cranial US/CT/MRI. A low CMV DNA blood viral load in the first month of life can predict a normal development in asymptomatic newborns. Since the cutoff values for amniotic fluid viral load measurements were derived from a few studies and have not been validated with international standards, they may not be generalizable.
Several studies suggest that low AF virus loads can provide reassurance for lower risks of both symptomatic infection and long-term sequelae (42, 140, 143, 155, 156). Although an association between high AF virus loads and symptomatic infection at birth has been documented in some studies (42, 140, 155), other studies have failed to show such an association (146, 157, 158). Virus load was also found to correlate with gestational age (146, 157). It is important to bear in mind that in the absence of international PCR quantification standard, the different assays deployed in these studies would have suffered from substantial inter- and intralaboratory variations, making the use of predictive cutoff values less generalizable. In addition, differing study designs make it difficult to compare the data among the various studies. The recently approved first WHO international standards for CMV PCR will reduce this variability and should be used to reevaluate the prognostic role of AF virus levels in future multicenter studies (159). Even if the predictive role for low AF virus load in symptomatic disease and sequelae is confirmed, these invasive diagnostics are beyond the reach of most public health systems in low-income countries, and therefore it is unlikely that the diagnosis and treatment of in utero CMV infection will become part of routine obstetric practice in these settings.

**Antiviral Therapy and Passive Immunization**

The results of ongoing controlled trials involving oral valaciclovir (NCT01037712), and CMV hyperimmune globulin (HIG) (NCT00881517) for prenatal intervention are awaited (160). Ganciclovir cannot be used for prenatal therapy due to its mutagenic potential in animals, but oral valaciclovir administered to mothers with evidence of fetal infection appears to be safe and decreases the circulating fetal viral load (161). However, evidence for improved outcomes with treatment has yet to be demonstrated.

The rationale for passive immunization of seronegative mothers comes from the observed lower risk of fetal infection in mothers with preexisting antibodies (162). This is further supported by evidence that CMV HIG can inhibit viral spread in vitro (163, 164), restore placental health in mothers with primary infection (165), and lead to regression of cerebral ultrasound abnormalities (166). A prospective study has demonstrated that monthly intravenous infusions of CMV HIG to mothers with confirmed primary infection (including those with virological evidence of fetal infection) are safe and can both prevent (adjusted odds ratio [OR], 0.32) and treat (adjusted OR, 0.02) fetal infection (167). Furthermore, recent retrospective studies have suggested that CMV HIG can protect against poor outcomes in infants (168, 169). In spite of these promising findings, though, a recent Cochrane Library Review underscored the lack of data from randomized controlled studies and accordingly the need for further research to assess the efficacy of antenatal interventions for the prevention of intratubarine transmission and adverse outcomes (170). Therefore, the results from two randomized controlled trials of CMV HIG that are under way (NCT00881517 and NCT01326778) should be awaited to confirm the effect on transmission or prevention of disease. Regardless of the outcome of these studies, since most seropositive individuals appear to have high levels of antiviral antibodies (as a result of boosting following frequent reactivation and/or reinfection), it can be inferred that CMV HIG will have little to no role in high-seroprevalence populations.

**Maternal Antiviral Immune Responses and Intrauterine Transmission**

CMV infection and risk of transmission to the fetus are intimately linked to immunity, although the temporal appearance and quality of the humoral and T-cell-mediated responses against CMV during primary and nonprimary maternal infection remain incompletely understood.

The importance of immune responses in protecting against intrauterine transmission of CMV is borne out by the significantly decreased risk of congenital infection in infants born to women who were seropositive prior to pregnancy (~1%) in contrast to those with primary infection during pregnancy (~30%) (14, 27) and the beneficial effects of administering hyperimmune globulin (HIG) in women with primary infection during pregnancy (167). The observation that differential levels of neutralizing anti-glycoprotein B titers exist at the time of delivery in transmitter and nontransmitter mothers undergoing a primary infection also supports the role of the humoral arm of the immune system in modulating intrauterine transmission of CMV (171). The gB protein is relatively well conserved among different virus strains and is considered the major target of the neutralizing antibody response to CMV (172). Recent data, however, suggest that the pentameric complex comprising gH, gL, UL128, UL130, and UL131 is the most important antigenic complex for neutralizing antibody responses (173). High titers of neutralizing antibody are thought to protect against transmission by blocking receptor-mediated transcytosis of CMV in the placenta (174) and by reducing viral replication (87). It will be interesting to investigate the temporal appearance of humoral responses against the pentameric glycoprotein complex in both primary and recurrent infections in pregnant women and to determine whether they are a potential marker for risk of transmission and/or congenital CMV disease.

There is increasing evidence in transplant recipients that high levels of virus replication and disease are associated with the suboptimal quality of the T-cell response against CMV (175, 176). In addition, in healthy adolescents, it has been shown that the plasma CMV DNAemia was still evident despite the detection of a strong neutralizing antibody response within 6 to 8 weeks following primary infection, although lymphoproliferative responses were weak (177). Therefore, cellular immunity is indispensable during the acute phase of infection, as well as for the control of chronic infection and the prevention of reinfection (10, 178–180). Although a range of CMV proteins are targeted by the CD4 and CD8 immune system (181), major targets include the pp65 tegument protein and the IE1 antigen (and to a lesser extent gB) (182). In pregnant women experiencing primary infection, the evolution of the lymphoproliferative response has been shown to be relatively slow until a memory T-cell response develops (183). The cytokine profile of these T cells is dominated by gamma interferon producers, with relatively little interleukin-2 (IL-2) production. In mothers who experienced primary infection and who transmitted the virus to their fetuses, CMV CD4+ T-cell responses appear to be delayed and of lower frequency, and there were lower levels of CMV CD45RA− cells in mothers who transmitted CMV to their fetuses (183, 184). In seropositive pregnant women, it has been shown that naive CD8 T cells were reduced by 50%, with the CD45RA effector population showing a more highly differentiated state (CD27 and CD28 low) while the CD45RA revertant
Vaccines
The economic impact of congenital CMV was assessed by the Institute of Medicine nearly a decade ago. They estimated that the costs of medical and educational care for the thousands of children with asymptomatic and symptomatic congenital infection in the United States amounted to $1.9 billion per year, whereas the investment needed to develop a CMV vaccine would be approximately $360 million. The Institute of Medicine accordingly ranked the development of a CMV vaccine as the highest priority (4). Knowledge that CMV exhibits a high level of molecular diversity and carries an extensive array of virus immune evasion genes is increasing (10, 23, 186, 187). Consistent with this, it has been demonstrated that infection within a host can occur with multiple virus strains concomitantly, including at the time of initial infection, or sequentially (10, 23, 186, 187). Broad and cross-neutralizing cellular and humoral responses have therefore become a major goal of vaccine design (188). Whereas the traditional focus of CMV vaccines has been the prevention of primary maternal infection, this view has been challenged by recent data demonstrating that nonprimary infection drives most congenital infections, and that the rates of symptomatic infection at birth and hearing loss are similar in infants infected following primary and nonprimary maternal infections (7, 8, 74).

A recent phase II trial evaluated the efficacy of a recombinant genetically modified gB protein in a novel adjuvant, MF59 (189), in seronegative women and found a modest (~50%) reduction in the rate of primary maternal infection in the vaccinated group compared to the placebo group (190). However, this protection was observed predominantly within the first year after immunization. Although boosting of both antibody and CD4 T-cell responses by the gB vaccine was also demonstrated in CMV-seropositive women, whether such boosting will provide protection against nonprimary infection in mothers with preexisting immunity is not known (191). The same vaccine deployed in patients awaiting solid organ transplantation (the cohort consisted of seropositive and seronegative patients) was immunogenic and reduced the duration of viremia in patients with CMV infection posttransplantation (192).

Several proof-of-concept studies of various candidate vaccines have also been conducted in recent years. A two-component alphavirus replicon vaccine containing gB and a pp65/IE1 fusion protein has shown to be immunogenic in phase I clinical trials. In seronegative subjects, the vaccine elicited neutralizing antibodies and multifunctional T-cell responses (193), and it also boosted T-cell responses in CMV-seropositive renal transplant patients (194). A DNA vaccine comprising both gB and pp65 has also undergone phase I studies and a placebo-controlled phase II trial in stem cell transplant recipients. Although there was no difference in the number of vaccine and placebo recipients who received CMV-specific antiviral therapy, a significant reduction in the incidence and recurrence of DNAemia was seen (195). More recently, combining gB with a Toll-like receptor 9 (TLR9) agonist has produced durable polyfunctional cellular and cross-neutralizing humoral responses in transgenic mice (196). While there is some evidence for protection against nonprimary infection in these studies, evaluation of these candidate vaccines for the prevention of maternal and congenital infection seems to be far in the future. Even in low-seroprevalence settings, where vaccination of seronegative mothers could be cost-effective, it is unclear, in light of emerging findings on the epidemiology of congenital CMV, whether a CMV vaccine would provide substantial reductions in morbidity.

Behavioral Measures
In the absence of effective immunization strategies, the restriction of maternal infection relies predominantly on behavioral measures such as frequent hand washing after exposure to young children’s body fluids and avoiding intimate contact with young children (197). Children, when infected vertically or in the first few years of life, can shed virus in urine and saliva for many years either continuously or intermittently (108, 198–200). CMV therefore spreads readily in settings where preschool children are concentrated, with fomites on wet absorbent surfaces most able to harbor viable viruses (33, 201). This places seronegative pregnant women who work in child care centers or who have a young child in the home or in day care at increased risk of seroconversion (31, 32, 34, 202). Accordingly, specific advice to seronegative women on measures that interrupt child-to-mother transmission has been shown to be effective (16, 18, 203). Besides contact with young children, sexual transmission from a seropositive male partner is an additional established route by which women may be infected with CMV (25, 160, 197, 204–207). It is quite likely that these modes of transmission are also responsible for reinfection of seropositive mothers with new or different virus strains. Indeed, sexual transmission probably frequently results in maternal reinfection in high-seroprevalence populations, where young women often report multiple sex partners and unsafe sex practices. However, the contribution of sexual and child-to-mother transmission to maternal reinfection in these settings remains to be virologically documented. Moreover, the relative role of reinfection compared with reactivation in delivering a child with CMV is also unknown. It is therefore difficult to speculate on the impact of behavioral changes in resource-limited settings. On the whole, promoting education and awareness of congenital CMV infection and ways to avoid exposure for all prospective mothers remains a key health educational objective (160, 208).

CONCLUSIONS
Congenital CMV is a major cause of disability in children, with little evidence for change in disease burden over time in high- and middle-income countries despite large scientific and clinical advances in the CMV field. This results from a general neglect of the problem, contributed to by the absence of clinical disease at birth in the majority of babies who develop complications and the lack of safe and effective antiviral therapy to prevent or reduce sequelae in most children with congenital CMV infection. Therefore, prevention of maternal infection and transmission is the main priority. Vaccines may offer protection against primary infection, and the efficacy of vaccines in mothers following nonprimary infection should be assessed.

The neglect of congenital CMV infection in the developing world reflects not only delayed onset of sequelae but also compet-
ing health priorities in such populations. Given that early detection of hearing loss can limit long-term disabilities, PCR-based newborn screening to identify those at risk of sequelae deserves consideration. However, it would be premature to consider newborn CMV screening in resource-poor settings because the disease burden from congenital CMV and the cost/benefit ratio of long-term follow-up have not been defined. In addition, the cost and the competing health priorities for these settings make it difficult to envision such a screening program. While studies to define the disease burden should be undertaken as a matter of urgency, for the present, raising awareness of congenital CMV should be prioritized.

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Suresh Boppana is a Professor of Pediatrics and Microbiology at the University of Alabama at Birmingham and has been studying the natural history and pathogenesis of maternal and congenital cytomegalovirus (CMV) infection for the past 20 years. Dr. Boppana’s work challenged the dogma that children with congenital CMV infection born to women with primary CMV infection during pregnancy experience most of the disease burden from this intrauterine infection. He has shown that CMV reinfections occur frequently in healthy seropositive women and that such reinfections could lead to intrauterine infection, symptomatic disease, and sequelae. His work with collaborators in Brazil and India is beginning to document the impact of congenital CMV infection in highly seropositive settings, including developing countries. He is currently the principal investigator of large multicenter study to define the contribution of congenital CMV infection to overall hearing loss and to develop diagnostic methodologies that can be used to screen large number of newborns. The findings from this study, demonstrating low sensitivity of the dried blood spot PCR assay and the development of a highly sensitive and specific saliva real-time PCR assay for congenital CMV infection, have been published in the Journal of the American Medical Association and the New England Journal of Medicine, respectively. His current research is focused on understanding the pathogenesis of CMV-associated hearing loss. He mentored several undergraduate, graduate, and postdoctoral trainees. He served as a consultant member of several NIH study sections.

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