Cytomegalovirus infection during pregnancy: state of the science

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Cytomegalovirus is the most common congenital infection, affecting 0.5—2% of all live births and the main nongenetic cause of congenital sensorineural hearing loss and neurological damage. Congenital cytomegalovirus can follow maternal primary infection or nonprimary infection. Sensorineurological morbidity is confined to the first trimester with up to 40—50% of infected neonates developing sequelae after first-trimester primary infection. Serological testing before 14 weeks is critical to identify primary infection within 3 months around conception but is not informative in women already immune before pregnancy. In Europe and the United States, primary infection in the first trimester are mainly seen in young parous women with a previous child younger than 3 years. Congenital cytomegalovirus should be evoked on prenatal ultrasound when the fetus is small for gestation and shows echogenic bowel, effusions, or any cerebral anomaly. Although the sensitivity of routine ultrasound in predicting neonatal symptoms is around 25%, serial targeted ultrasound and magnetic resonance imaging of known infected fetuses show greater than 95% sensitivity for brain anomalies. Fetal diagnosis is done by amniocentesis from 17 weeks. Prevention consists of both parents avoiding contact with body fluids from infected individuals, especially toddlers, from before conception until 14 weeks. Candidate vaccines failed to provide more than 75% protection for >2 years in preventing cytomegalovirus infection. Medical therapies such as cytomegalovirus hyperimmune globulins aim to reduce the risk of vertical transmission but 2 randomized controlled trials have not found any benefit. Valaciclovir given from the diagnosis of primary infection up to amniocentesis decreased vertical transmission rates from 29.8% to 11.1% in the treatment group in a randomized controlled trial of 90 pregnant women. In a phase II open-label trial, oral valaciclovir (8 g/d) given to pregnant women with a mildly symptomatic fetus was associated with a higher chance of delivering an asymptomatic neonate (82%), compared with an untreated historical cohort (43%). Valganciclovir given to symptomatic neonates is likely to improve hearing and neurological symptoms, the extent of which and the duration of treatment are still debated. In conclusion, congenital cytomegalovirus infection is a public health challenge. In view of recent knowledge on diagnosis and pre- and postnatal management, health care providers should reevaluate screening programs in early pregnancy and at birth.

Key words: brain imaging, congenital infection, cytomegalovirus, diagnostic, epidemiology, gestational age, handicap, immunoglobulin G avidity, nonprimary infection, prenatal diagnosis, primary infection, sensorineural hearing loss, serology, valaciclovir

Cytomegalovirus (CMV; *Herpesvirus 5)* is a member of the *Herpesviridae* family. Human CMV (HCMV) is highly species specific, and humans are its only host. The virus is acquired at mucosal sites (community exposure) or by blood-borne transmission (blood transfusion or transplantation). Cell-mediated spread of the virus begins after a replication phase.

The main host cells infected by CMV are the monocytes, the macrophages, and the endothelial cells, but CMV can replicates in most cells' type. The dissemination of the virus is hematogenous. The main secondary sites of host replication are the spleen and liver. Dissemination and replication are not completely controlled by host immunity, and after primary infection, HCMV remains latent mainly in monocytes.

Episodes of reactivation with viral replication may happen in seropositive hosts. These episodes are asymptomatic in nonimmunocompromised individuals but may lead to severe disease in immunocompromised hosts. During primary infection and episodes of reactivation, the virus is found in body fluids (urine, saliva, vaginal secretion, semen, breast milk), and the seropositive subject is therefore a reservoir of virus. Moreover, because the CMV genome is highly variable and immunity against infection incomplete, reinfections with different strains is possible.

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TABLE 1
Prevalence at birth in different settings and burden of nonprimary CMV infection

Variables	Leruez-Ville et al, 2017[23]	Puhakka et al, 2018[20]	Mussi Pinhata et al, 2018[25]
Country	France	Finland	Brazil
CMV seroprevalence in pregnant women	60%	72%	98%
Neonates screened, n	11,715	19,868	1721
Prevalence of congenital CMV infection	0.37%	0.2%	0,5%
Proportion of congenital CMV infection following maternal primary infection	52%	47%	10%
Proportion of congenital CMV infection following maternal nonprimary infection	48%	53%	90%

CMV, cytomegalovirus.

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CMV is the most common cause of congenital infection, the leading nongenetic cause of sensorineural hearing loss (SNHL), the major infection-related cause of congenital malformations in high-income countries, and a major cause of neurological disability. It accounts for up to 10% of all cases of cerebral palsy[1] and 8−21% of all congenital SNHL at birth[2−4]; this increases to 25% by the age of 4 yeasrs because of late-onset hearing loss.[5]

Without any recommendations for systematic screening of maternal CMV infections, fetal infections are mainly revealed by ultrasound abnormalities. Nevertheless, maternal seroconversion is sometimes diagnosed when serology is electively performed in pregnancy or following maternal symptoms.

Congenital CMV epidemiology revisited

HCMVs have humans as the only host and remain latent after primary infection. HCMV genome is highly variable and immunity against infection is incomplete; reinfection with different strains is possible as well as reactivation of endogen latent strain. Those 2 types of infections are named nonprimary infections.

Transmission of the virus occurs by direct or indirect person-to-person contact via urine, oropharyngeal, cervical, and vaginal secretions; semen; milk; tears; blood products; or organ transplants. It is endemic worldwide and has no seasonal variation. There is a prolonged shedding of the virus after primary infection.

Seroprevalence in pregnant women

Seroprevalence increases with age and is higher in individuals with lower socioeconomic status both in high- and low-middle−income countries. Seroprevalence among women of childbearing age varies also accordingly with those factors. Seropositivity ranges between 50% and 85% in the United States and in Western Europe.[6]

Epidemiology of maternal infection

The prevalence of CMV primary infection in pregnancy is around 1−2% in Western Europe and in the United States.[7,8] Being young and having at least 1 child are risk factors for primary infection in pregnancy. The annual risk of primary infection during pregnancy in women seronegative in their previous pregnancy was 5.9% in the United States.[9] In a recent French study, women seronegative at their first pregnancy and conceiving within 2 years had a 19-fold and 5-fold higher risk of primary fetal infection in the first trimester and of related sequelae in their infant, respectively, than the general population.[10]

The prevalence of maternal nonprimary infection is ill defined and was 10% per year in young women in a 3 year study in the United States.[11]

Mother to fetus vertical transmission rate

The transplacental transmission rate after maternal primary infection is around 32%.[12] Early reports found an increased transmission with advancing gestation of 26%, 28%, and 65% in the first, second, and third trimesters, respectively.[13−19] However, systematic neonatal screening yields similar proportions of infected neonates following primary infection at all 3 trimesters.[20,21] The risk of transmission is low following maternal infection occurring more than 11 weeks before conception.[15,22] The transplacental transmission rate after nonprimary infection is unknown.

Prevalence of congenital CMV at birth: the burden of nonprimary infection

The prevalence of neonatal infection correlates with maternal seroprevalence, ranging from 0.4% to 1% in countries with low or intermediate and high seroprevalence, respectively.[12,20,23,24] Half to 100% of congenital infections are from nonprimary infection in countries with low or intermediate and high seroprevalence, respectively[20,23,25] (Table 1).

In France, the risk of congenital infection after primary infection was increased in younger parous women born in high-income countries and from higher-income groups,[23] whereas congenital infection following

nonprimary infection increased in young and unemployed women.[23]

Prevalence of symptoms at birth and rate of long-term sequelae

The definition of symptomatic or asymptomatic status at birth changed over time. Older studies considered a combination of clinical symptoms (small for gestational age [SGA], hepatomegaly, splenomegaly, petechia, pneumonia, retinitis, neurological symptoms) and biological abnormalities (elevated liver enzymes, thrombocytopenia) and/or severe abnormalities on cerebral imaging. SNHL was not included in this definition. Therefore, neonates with isolated SNHL were unduly classified as asymptomatic. The recent definition, includes the results of SNHL evaluation.[26]

Approximately 20% of infected neonates suffer neurological and/or audiological sequelae (Figure 1). Of 117,986 infected neonates from 15 studies, 12.7% were symptomatic at birth and 40−50% developed at least 1 long-term sequelae, including 13.5% of asymptomatic neonates.[27] Hearing loss develops in 7.2−15% of infected children, including 30−60% and 5−10% of symptomatic and asymptomatic neonates respectively.[3,28−33] However, SNHL can be equally severe, unilateral or bilateral, and from mild to profound, in symptomatic as well as in asymptomatic children. Based on birth prevalence of 0.7%, 3.5 per 10,000 children born each year in high-income countries develop moderate to profound bilateral SNHL related to congenital CMV.[31]

The disease's spectrum, including hearing loss, is similar after primary and nonprimary infection with a 10% and 11% risk, respectively.[11,23,24] This confirms that preexisting maternal immunity provides only limited protection to the fetus.

Predictors of long-term sequelae

The main prognostic factors in infected neonates are gestational age at maternal infection and the presence of neonatal symptoms. The relationship between gestational age at maternal infection and outcome of congenital CMV was ill defined until recently. In studies between 1980 and the early 2000's,[14,29,34,35] accurate serological timing of primary CMV infection was difficult because immunoglobulin G (IgG) avidity assays were either not available or at an early stage of development. Although those studies underlined the first trimester as a significant risk factor for sequelae,[14,19,29,34,35] they also reported neurological sequelae and SNHL in 6−15% and in 1−8% of children infected following second- or third-trimester maternal infection, respectively.[14,29,34]

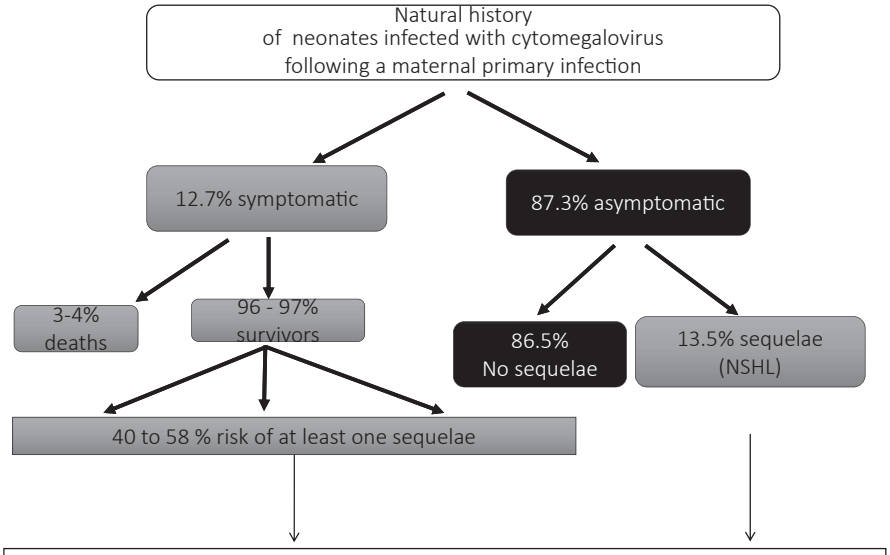
In the largest study of 250 children infected following maternal primary infection, long-term sequelae were solely seen in those infected after primary infection in the first trimester, while no long-term sequelae were reported in more than 100 children infected after primary infection in the second or third trimester.[36] Therefore, if primary infection and transmission are equally frequent in the 3 trimesters, long-term sequelae would develop in 51−57% of cases from first-trimester primary infection (Figure 1).

This is compatible with the only study describing the natural history of children infected following first-trimester primary infection.[34] Those developed neurological sequelae, mental deficiency (intelligence quotient < 70%), and SNHL in 32%, 17%, and 23%, respectively.[34]

This suggests that congenital CMV infection is severe only when the virus hits the fetus in the embryonic or early fetal period. When SNHL or neurologic symptoms are found in children with an infection later in pregnancy, it would be wise to check for middle ear problems and to exclude etiologies other than congenital CMV-related lesions of the forming membranous labyrinth.[30,36]

The risk of long-term sequelae according to the time of maternal

FIGURE 1
Natural history of congenital infection following maternal primary infection with CMV



In this study children with isolated hearing loss are classified in the asymptomatic group. The proportion of long-term sequelae in neonates infected following maternal first-trimester infection are based on the hypothesis that maternal primary infection and transmission are equally frequent in the 3 trimesters of pregnancy. Adapted from Dollard et al (2007).[27]

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nonprimary infection is unknown, but because the spectrum of symptoms is similar, it is likely to be the same than for primary infection.

SNHL develops in 42.9% and in 6.6% of asymptomatic neonates with and without abnormalities on cerebral ultrasound and/or magnetic resonance imaging (MRI).[30] Sensitivity and specificity of abnormal imaging for SNHL are around 52.9% and 90%, respectively.[30] Viral loads are higher in symptomatic neonates,[37,38] and neonatal blood viral load greater than 10,000 copies/mL seems associated with long-term sequelae.[39]

Pathophysiology of fetal infection and related long-term sequelae

Pathophysiology of hearing loss

It is unclear whether late-onset SNHL is caused by viral reactivation or by the immunological host response. Inner ear, particularly cochlear, lesions in fetuses are diffuse, consisting of both cytomegalic cells containing inclusion bodies, and inflammation.[40,41] Vestibular and cochlear infections are frequent, and sensory structures are further altered by dysregulation in the potassium and ion circulation.[40] The importance of the host immune response may be of greater importance than viral destruction in CMV labyrinthitis. This was confirmed in animal studies and suggests that in addition to antiviral treatment, an immunosuppressive agent might be useful as a therapeutic adjuvant.[42]

Pathophysiology of brain lesions

A recent study examined multiple tissues from 45 infected fetuses at midgestation.[43] CMV-positive cells were found in 62% of brains and involved neurons, neuroblasts, glia, endothelium, ependyma, and meninges. In the most severely affected brains, there were cortical necrotic areas. White matter abnormalities included periventricular leukomalacia and deposition of iron and calcium in neurons, axons, and dendrites. Extensive necrosis was associated with both viral inclusions in cells and inflammatory infiltrate with abundant cytotoxic T lymphocytes. Brains from fetuses with moderate cerebral damage had only focal necrosis, much less evidence of inflammation, less notable white matter changes, and abundant microglia and gliosis.

The mechanisms involved have been studied using in vitro infection of primary human cell cultures and in animal models.[44] Neural stem cells are consistently and predominantly affected. Because these cells differentiate into both neurons and glia, the impact of their death or damage will result in both loss of brain mass and abnormal neuronal migration, leading to abnormal organization and communication between brain areas. Recent research has focused on identifying molecular mechanisms through which CMV infection results in impaired differentiation and proliferation of neuronal stem cells.[45−48]

Laboratory diagnosis of infection

Diagnosis of maternal primary infection

Diagnostic tools for maternal primary infection. The diagnosis of primary infection is based on serology. Seroconversion identifies primary infection. When seroconversion cannot be demonstrated, the diagnosis is based on a combination of IgG and immunoglobulin M (IgM) testing pattern. The sensitivity and specificity of the diagnosis of maternal primary infection will therefore highly depend on the performance of IgG and IgM assays.

IgG assays performance. Agreement and respective sensitivity (97−100%) and specificity (96−100%) between CMV IgG commercial assays are high.[49−57] However, interpretation of low IgG levels around the positivity threshold remains difficult because there is no gold-standard technique. No algorithm (retest, test with other assays, etc) is validated to help conclude in these cases. Among 4938 sera tested for IgG with 2 different assays, 1.8% yielded low IgG level and the serology result was discordant with the 2 assays (positive vs negative) in 50% of these sera.[58] It would seem prudent to classify women with low IgG value as seronegative, the higher-risk group.

IgM assay performance. Concordance between CMV IgM assays is 84−95%.[51,52,54,56,57,59,60] Studies from the early 2000s reported relative sensitivity and specificity of those commercial assays, ranging from 54% to 100% and 62% to 100%, respectively.[49,52,53,55,61−64] However, most of those studies used assays that have now undergone upgrades or are no longer available. In a recent study, comparing the 5 most currently used assays in Europe, IgM sensitivity was higher, ranging from 80% for the least sensitive assay to greater than 95% for the other 4 assays.[54] The presence of positive IgM is not specific of a recent primary infection because IgM may persist for months or be related to assays' cross-reactivity. Therefore, it is recommended to request IgG avidity in cases with positive IgM to exclude or confirm a recent primary infection.

IgG avidity assay performance. Automated assays available on high throughput platforms yield sensitivity, specificity, and concordance of 82−100%, 90−100% and 80−100%, respectively.[56,57,65] However, pitfalls remain: avidity may be falsely low in past infections with very low IgG levels,[66] and in very recent seroconversion, avidity can in turn be falsely high.[65] An intermediate (neither high nor low) IgG avidity result in the first trimester of pregnancy cannot rule out the occurrence of a primary infection in early pregnancy or in the periconceptional period. In those cases a negative CMV polymerase chain reaction (PCR) in maternal whole blood excludes a primary infection in the previous month with greater than 80% sensitivity.[67] Conversely, a positive CMV PCR does not always indicate a recent primary infection because the DNA-emia may also remain positive.[67]

To conclude, most recent automated avidity assays identify women with primary infection at high risk of fetal infection with high sensitivity, although

some lack specificity to exclude a primary infection within 3 months.

What is the performance of serology screening in pregnancy?

Serology screening in pregnancy is based on IgG and IgM testing followed by IgG avidity testing in cases with positive IgM. In avidity tests a mild denaturing agent (usually urea) is added to the antibody-antigen mixture. Antibodies of low avidity that are present in acute infection (less than 3 months) are more likely to dissociate from the antibody-antigen complexes than those with higher avidity present in past infection (more than 3 months).

Two different algorithms have been evaluated: (1) search for IgG and IgM and, if positive, then measure IgG avidity, or (2) measure IgG alone and, if positive, measure IgG avidity systematically.[68] The first strategy proved superior because IgG avidity alone and IgM detection combined with IgG avidity failed to exclude seroconversion in 26% and only 1% of cases, respectively.[68]

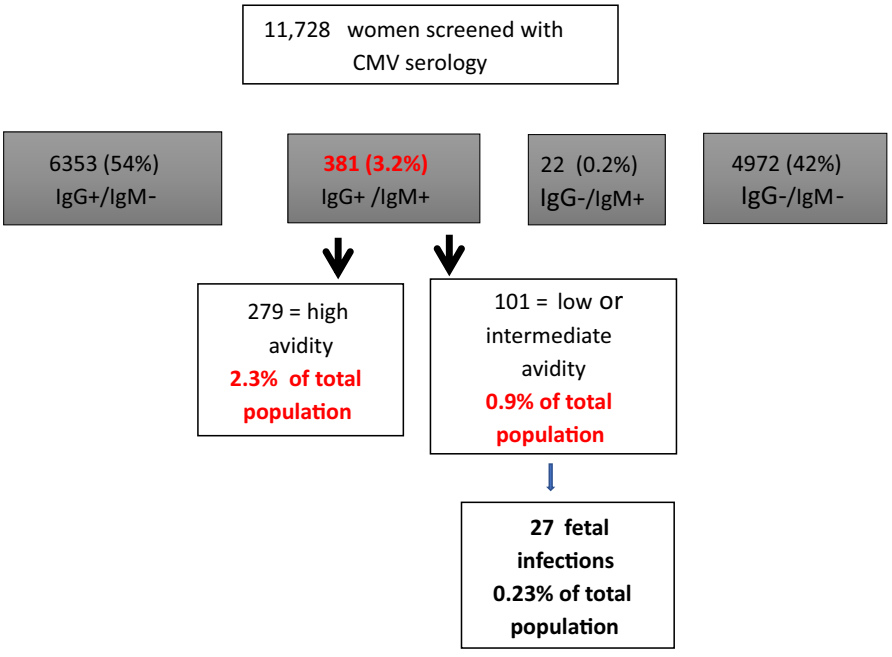
The prevalence of positive CMV IgM in more than 10,000 pregnant women screened was 0.9−5.7%.[69−72] In women with positive IgM, a high avidity index excluded a recent primary infection in 65%. A low avidity index indicated a primary infection in 19%. An intermediate avidity was reported in 15% and was therefore inconclusive in only around 0.5% of all women screened.[69−72]

This strategy has a good specificity to exclude primary infection, but its sensitivity to diagnose primary infection has not been evaluated. Although IgM usually persists for months after primary infection, IgM can become undetectable within 3 months. Therefore, a screening strategy based primarily on IgM detection could miss some primary infections.[60]

When should serology screening be done during pregnancy?

Because second- and third-trimester maternal infections do not lead to long-term sequelae, serology screening beyond 15 weeks is not relevant. If screening should be implemented, it should focus on identifying first-trimester primary infection, adding to the 11−14 week opportunity for risks assessment in early pregnancy (Figure 2), although some primary infection in very early pregnancy may be missed. Earlier serology screening may become justified if the efficacy of valaciclovir to avoid vertical transmission is confirmed.[73]

FIGURE 2
Results of maternal CMV serology screening between 11 and 14 weeks from 2011 to 2016 in Necker center



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Is it possible to diagnose maternal nonprimary infection with serology?

The diagnosis of maternal nonprimary infection is based on a positive CMV PCR in blood/urine or saliva in a woman known seropositive before pregnancy. In 32 neonates infected after nonprimary infection, none of their mothers had detectable IgM and only 6% showed significantly increased IgG levels in the first or second trimester.[23,74,75] Conversely, 7 of 205 known seropositive women with positive IgM and positive CMV PCR in blood or urine (3.4%) delivered an infected neonate.[76] In-house assays detecting strain-specific serological responses have a low sensitivity for screening.[77] Therefore, in known seropositive pregnant women, serology is not useful and is misleading. The value of other diagnostic tests such as CMV PCR in urine, saliva, or blood to identify seropositive women at risk of giving birth to an infected neonate should be assessed.

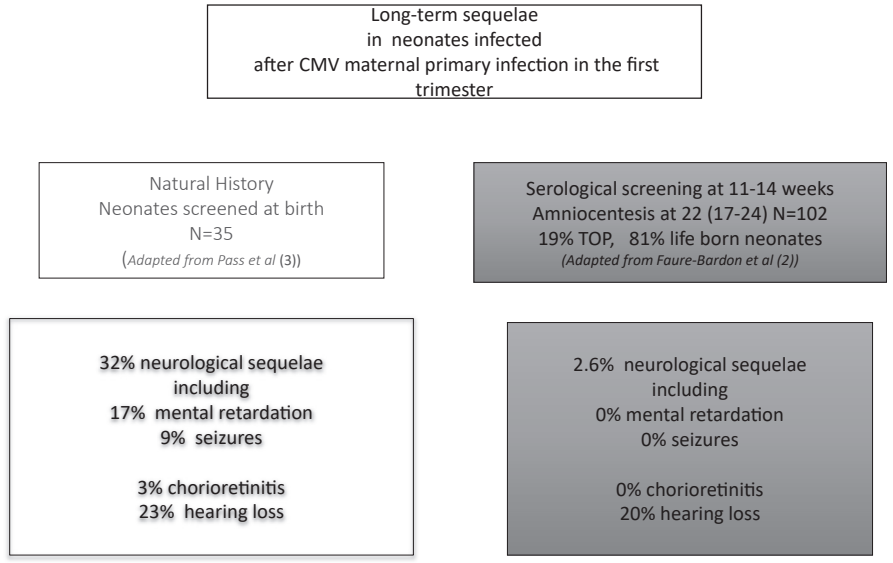
Diagnosis of fetal infection

Detection of CMV DNA in the amniotic fluid is the gold standard for prenatal diagnosis because infected fetuses pass the virus in their urine.

Should PCR be performed in maternal blood before amniocentesis?

The rationale is the potential risk of viral inoculation from maternal blood to a noninfected fetus. The same proportion of neonatal infections followed amniocentesis vs no amniocentesis, irrespective of maternal blood PCR results.[78] In addition, positive PCR in maternal blood in primary infection increases the probability of a positive CMV PCR in amniotic fluid.[79,80] However, maternal CMV blood PCR is also often

FIGURE 3
Comparison of the outcome of children infected following maternal primary CMV infection in the first trimester between a group with no antenatal intervention and a group with antenatal management[34,107]



This figure compares the reported outcomes in children born after first-trimester primary infection following natural history, with those born following CMV serology screening in the first trimester and prenatal diagnosis. The proportions of neurological sequelae in both groups (32% vs 2.6%) clearly suggest that screening is likely to identify the most severe cases and give parents the choice of terminating the pregnancy or anticipating the birth of a potentially severely handicapped child.

CMV, cytomegalovirus; *TOP*, termination of pregnancy.

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negative when the fetus is infected.[80] Therefore, testing CMV PCR in maternal blood before amniocentesis is not relevant and should not be performed.

When should amniocentesis be scheduled?

Amniocentesis should be performed at least 6 weeks after the presumed date of primary infectionand after 20−21 weeks, with asensitivity of 85−95%.[17,81,82] An infected neonate is born after a negative result in 5−15% of cases, resulting from late transplancental passage of the virus, therefore later than the first trimester and up to 19 weeks after primary infection.[78] None of these neonates develop any long-term sequelae.[83] In a recent monocentric study, the risk was similar between 17 and longer than 20 weeks, and only an interval shorter than 8 weeks from primary infection yielded more false-negative results.[84] Therefore, amniocentesis can be scheduled 8 weeks following primary infection and from 17 weeks onward.

Neonatal diagnosis and neonatal screening

Who?

Universal neonatal screening is not recommended in any country. Neonatal diagnosis is recommended when maternal primary infection has been documented, in the presence of compatible neonatal symptoms, and in neonates failing universal hearing screening. However, neonatal hearing screening will detect only 20−60% of congenital CMV-related SNHL, missing late-onset cases.[85−87]

How and when?

CMV PCR in saliva collected within 3 weeks of life, has the same sensitivity than that in urine, and is the recommended tool for neonatal diagnosis.[26,88−91] The presence of a low amount of CMV DNA from breast milk or genital secretion may contaminate saliva samples, and a positive result should be controlled in a second sample of saliva or urine.[21,23,91]

Although symptomatic neonates have high blood viral loads, levels in children with late-onset SNHL is not known. The sensitivity of CMV PCR in dried blood spot is of 30−100% for a retrospective diagnosis of congenital CMV infection,[92−96] and its use as a screening tool is still debated.

Fetal CMV infection

There are 2 main circumstances leading to the prenatal diagnosis of fetal CMV infection: (1) maternal primary infection is revealed by maternal symptoms or following prenatal serology screening and (2) prenatal ultrasound is suggestive of fetal infection.

Maternal symptoms are noticed in only one third of the cases and they are nonspecific, encompassing mild fever, asthenia, myalgia, and flu-like syndrome. Biological disturbances are present in 50% of cases, mainly lymphocytosis greater than 40%, and elevated liver enzymes.[97]

Prenatal ultrasound findings can be gross or subtle. Although the full-blown picture of a severely affected fetus with marked growth restriction, microcephaly and hydrocephalus is unlikely to go unnoticed, prenatal ultrasound of neonates with symptomatic congenital CMV infection usually fails to identify any abnormality. Moreover, when features compatible with congenital CMV are mentioned in the report, they are recognized as such in less than half of the cases. This is in contrast with the prognostic value of targeted ultrasound examination when the fetus is known to be infected with greater than 90% negative predictive value[98−102] (Figure 3).

Ultrasound findings

Ultrasound features can be labeled as extracerebral and cerebral findings, respectively (Table 2).

TABLE 2
Ultrasound findings in fetal infection with CMV in the literature

Variables	Liesnard et al, 2000[17]	Enders et al, 2001[193]	Lipitz et al, 2002[35]	Azam et al, 2001[194]	Gouarin et al, 2002[195]	Benoist et al, 2008[98]	Guerra et al, 2008[196]	Lipitz et al, 2010[100]	Picone et al, 2013[19]	Leyder et al, 2016[197]	Enders et al, 2017[84]	Total
Number of cases of congenital CMV infection	55	57	51	20	30	73	154	38	60	61	38	637
Overall ultrasound findings	14	39	11	5	15	37	23	9	23	30	16	222 (35%)
SGA	5	12	6	0	10	7	4	2	8	1	1	**56 (9%)**
Hydrops	0	4	2	0	0	1	1	0	0	0	0	8 (1.2%)
Ascitis	0	15	0	2	1	3	0	0	5	0	1	27 (4.2%)
Pericardial effusion	0	3	0	0	0	1	0	0	3	1	0	8 (1.2%)
Pleural effusion	0	0	1	0	0	0	0	0	0	0	0	1 (<1%)
Skin edema	0	2	0	0	0	0	0	0	0	0	0	2 (<1%)
Hyperechogenic bowel	8	2	3	1	6	19	10	5	8	11	9	**82 (13%)**
Hepatomegaly splenomegaly	1	3	0	1	0	10	1	1	3	1	3	24 (3.8%)
Liver calcifications	**0**	**0**	**1**	**0**	**0**	2	0	0	1	2	2	8 (1.2%)
Placentomegaly	0	2	0	1	0	2	0	0	3	2	3	13 (2.0%)
Oligohydramnios /hydramnios	1	5	4	0	0	5	0	0	0	4	3	22 (3.4%)
Polyhydramnios	1	1	1	0	0	1	0	0	1	0	0	5 (<1%)
Others extracerebral findings[a]	0	2	0	0	0	2	1	0	3	0	2	11 (1.7%)
Microcephaly	2	11	0	1	5	7	0	0	9	2	0	**37 (6%)**
Hydrocephaly	2	9	0	0	2	13	0	0	0	0	0	26 (3.6%)
Ventriculomegaly	1	7	4	1	3	2	10	0	6	2	3	**39 (6.1%)**

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(continued)

TABLE 2
Ultrasound findings in fetal infection with CMV in the literature *(continued)*

Variables	Liesnard et al, 2000[17]	Enders et al, 2001[193]	Lipitz et al, 2002[35]	Azam et al, 2001[194]	Gouarin et al, 2002[195]	Benoist et al, 2008[98]	Guerra et al, 2008[196]	Lipitz et al, 2010[100]	Picone et al, 2013[19]	Leyder et al, 2016[197]	Enders et al, 2017[84]	Total
Cerebral calcification(s)	0	NA	2	0	9	13	0	0	9	4	0	**37 (6.3%)** (37/580)
Hyperechogenic periventricular halo	0	0	0	0	0	0	1	0	4	4	10	19 (3%)
Subependymal cysts	0	0	0	0	0	5	0	1	5	0	0	11 (1.7%)
Abnormal gyration	0	0	0	0	0	1	0	0	4	0	0	5 (<1%)
LSV	0	0	0	0	0	0	0	1	2	0	0	3 (<1%)
Other brain structural abnormalities[b]	1	10	1	0	0	3	0	3	1	6	0	36 (5.6%)

The most frequent symptoms are indicated in bold.

CMV, cytomegalovirus; *LSV*, lenticulostriate vessel; *SGA*, small for gestional age.

[a] Others extracerebral findings are hyperechogenic kidneys, hydronephrosis, cardiomegaly, and club foot; [b] Other brain structures abnormalities are temporal cyst; irregular ventricular wall, holoprosencephaly, cystic occipital lesion; small cyst in parietal lobe periventricular cysts, cerebral; and cerebellar hypoplasia, choroid plexus cyst,

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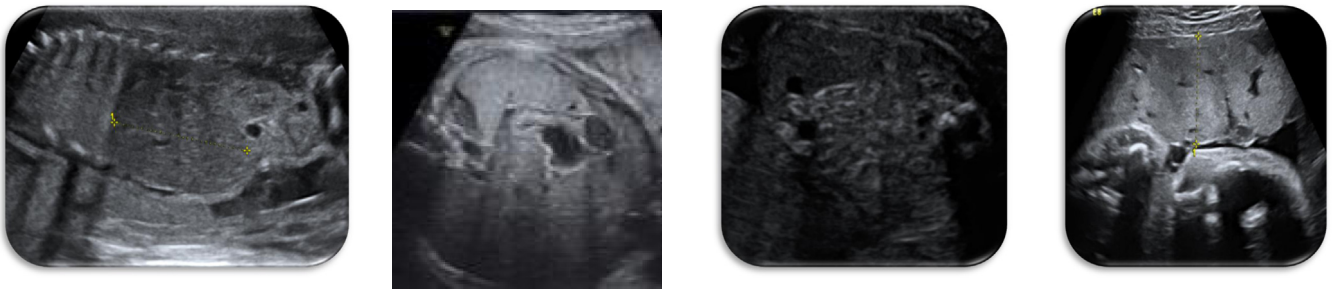
Extracerebral findings (Figure 4 and video) can be described while following the natural history and progression of the disease, although ultrasound is unlikely to show all of the following steps. The fetus becomes infected when the placental barrier fails to contain the viral replication within 2−3 months of the maternal infection. Placentitis shows a thick (>40 mm) heterogeneous placenta.[103] Fetal viremia leads to preferential invasion of the kidneys with nephritis that can cause transient oligohydramnios. Hepatosplenomegaly either compresses the stomach bubble or displaces it toward the middle of the abdomen. The spleen can be measured in the left quadrant of an axial plane of the abdomen and is contained between the spine, the lowest rib, and the stomach bubble. Color Doppler can show the splenic vascular pedicle.

Measurements of the maximal transverse diameter increases with gestation.[104] The right lobe of the liver can be measured in a parasagittal plane between the gallbladder and the diaphragm.[105] Stomach compression can lead to mild polyhydramnios. Direct viral perforation of the small bowel can cause hyperechogenic bowel[106] in relation with transient meconial ileus or meconial peritonitis with mild ascites.

Impaired growth can develop at any trimester, usually showing moderate SGA <10th centile with normal Doppler. Milliary thin calcifications can be seen in any fetal tissue including the myocardium. A dilated heart could reflect mild anemia, fetal cardiomyopathy, or both, often with pericardial and pleural effusion, more rarely part of full-blown hydrops fetalis. Those extracerebral findings can be seen following maternal primary or nonprimary infection at any trimester of the pregnancy. However, when they follow on maternal infection before 14 weeks, they are associated with a high risk of developing SNHL and brain lesions leading to neurodevelopmental abnormalities.

Brain lesions (Figure 5) develop only following maternal infection in the first trimester of pregnancy.[102,107] Isolated

FIGURE 4
Extracerebral findings in a 22 week infected fetus with CMV



A Hepatomegaly, right fepatic lobe measured in a right parasagittal plane

B Splenomegaly, long axis of the spleen shoxn in an axial plane. The stomac bubble is small and displaced to the center

C Hyperechogenic bowel, persistance of the echogenicity of the small bowel similar to that of the spine while turning down the gain

D Placentomegaly

CMV, cytomegalovirus.

Leruez-Ville. Cytomegalovirus infection during pregnancy. Am J Obstet Gynecol 2020.

features can include either one of the following: mild to moderate ventriculomegaly <15 mm, subependymal cysts, calcification of the lenticulostriate vessels, parenchymal calcifications, and septation of the posterior horn of a lateral ventricle. When any one of the above remains strictly isolated, the prognosis remains that of an asymptomatic neonate who may present or develop partial SNHL.

The severity of brain lesions can be anticipated when second-trimester ultrasound examination shows progressive marked ventriculomegaly (>20 mm), periventricular hyperechogenicities, and thickened, irregular ventricular rims, defining ventriculitis. Cerebellar hyperechogenicity could result from inflammation or hemorrhage. In the third trimester, increased pericerebral spaces, especially in an SGA fetus, is suggestive of microencephaly.[102]

Abnormal neuronal migration is challenging for prenatal ultrasound, although transvaginal examination through sagittal and coronal planes can show poor and asymmetrical gyration, particularly at the level of the sylvian fissure.

Heterotopias and polymicrogyria, however, are mainly amenable to MRI, especially because those can be limited to the temporal lobes. In addition to those typical findings, severe ventriculomegaly, periventricular leukomalacia, porencephaly, schizencephaly, and microcephaly below 5 SD can be seen, usually in the third trimester.

The contribution of fetal MRI

MRI features of CMV-related brain lesions have been classified in stages of increasing severity.[108] Functional MRI has been expected to show an early ominous sign of encephalitis through hypersignal of the white matter in T2 sequences (HSWM), especially if this involves the temporal lobes. However, clinical studies suggest that HSWMs are both subjectively and inconsistently reported by radiologists with a 40−60% discordance and mainly when undisputable structural abnormalities are present.[102]

Attempts have been made at reaching a more objective assessment of HSWM by using gray-scale ratios of regions of interest in the thalami (gray matter) over that in the temporal lobes (white matter), borrowing this approach from computed tomography (CT) scan imaging. However, this is an inappropriate use of gray-scale regions of interest ratios because MRI T1 and T2 signal intensity is dependent on multiple tissue properties, including proton densities and magnetic susceptibility among others, and is lacking a calibrated reference tissue, while gray scale in CT imaging is given by the Hounsfield unit relative to water in a well-defined equation.[108]

Apparent coefficient diffusion MRI sequences have been used to overcome this obstacle by quantifying the movements of the water molecules that is impaired in lesional brain edema. However, to date this has not proven more useful clinically.[109] It is therefore advisable not to grade fetal brain MRI accordingly to HSWM but only to anatomical findings for prenatal counselling.

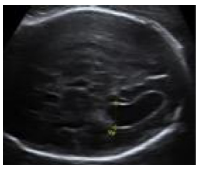
Figure 6 summarizes the management of fetal infection following maternal primary infection in the first trimester of pregnancy.

Congenital CMV infection-related hearing loss

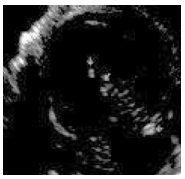
Characteristics of hearing loss in children with congenital CMV infection

An equal degree of hearing loss at all frequencies (flat) is the most common finding in children with congenital CMV-related SNHL,[33,110] although

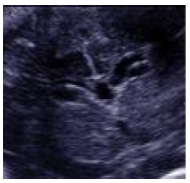
FIGURE 5
Cerebral features of fetal CMV infection



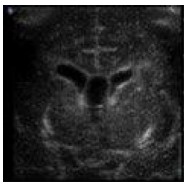
A Isolated mild ventriculomegaly < 15 mm



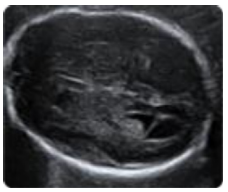
B Parenchymal calcifications in a parasagittal plane



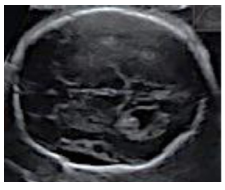
C Bilateral subependymal cysts in a mid-coronal view of the lateral ventricles



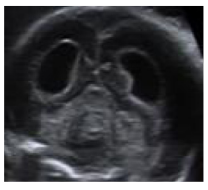
D Calcifications of the lenticulostriate vessels in a mid-coronal view of the thalami



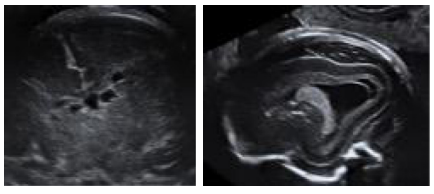
E Isolated intraventricular septation of the posterior horn



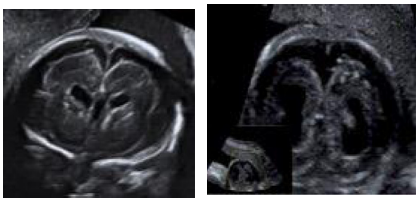
F Hyperechogenic thickened rims of the lateral ventricles and of the sylvian fissure in an axial plane



G And in a posterior cornal plane



H Periventricular cysts in a mid-coronal plane and in I. in a parasagittal plane



K& L Enlarged pericerebral spaces and cortical mantle showing lisencephaly and polymicrogyria in addition to all the above mentioned

CMV, cytomegalovirus.

Leruez-Ville. Cytomegalovirus infection during pregnancy. Am J Obstet Gynecol 2020.

high-frequency SNHL was also reported.[11,33] Flat hearing configuration demonstrates damage to the whole cochlea affecting equally high and low frequencies (base and apex of the cochlea, respectively). This is also seen in congenital Rubella syndrome.[111]

The possibility of late-onset hearing loss, progression, improvement, and fluctuation of hearing threshold make it very difficult to predict outcome and standardize follow-up of infected children. Follow-up is recommended up to the age of 4−6 years,[30,32,33,110,112,113] although late-onset SNHL most often develops within the first 2 years of life.[30,113]

Progressive hearing loss is defined as a sensorineural decrease in hearing of 10 dB or more at any one frequency or auditory brainstem response threshold, documented on 2 separate evaluations. Fluctuating hearing loss, seen in 20−30% of SNHL, is a decrease in hearing of greater than 10 dB at 1 or more frequencies, followed by improvement greater than 10 dB measured at 1 or more times.[32,114] Improvement of hearing is seen more often in children with otherwise asymptomatic infections.[33,114] Middle-ear problems are frequently observed in young children and can influence in a significant way the evaluation of hearing thresholds. A thorough ear, nose, and throat examination is therefore warranted, including (high-frequency) tympanometry.[115,116]

Vestibular problems

Congenital CMV-related vestibular dysfunction, including gross motor delay, are more frequent in association with SNHL but can occur independently.[107,112,117−121] All cases followed primary infection in the first trimester.[107,120]

Rehabilitation

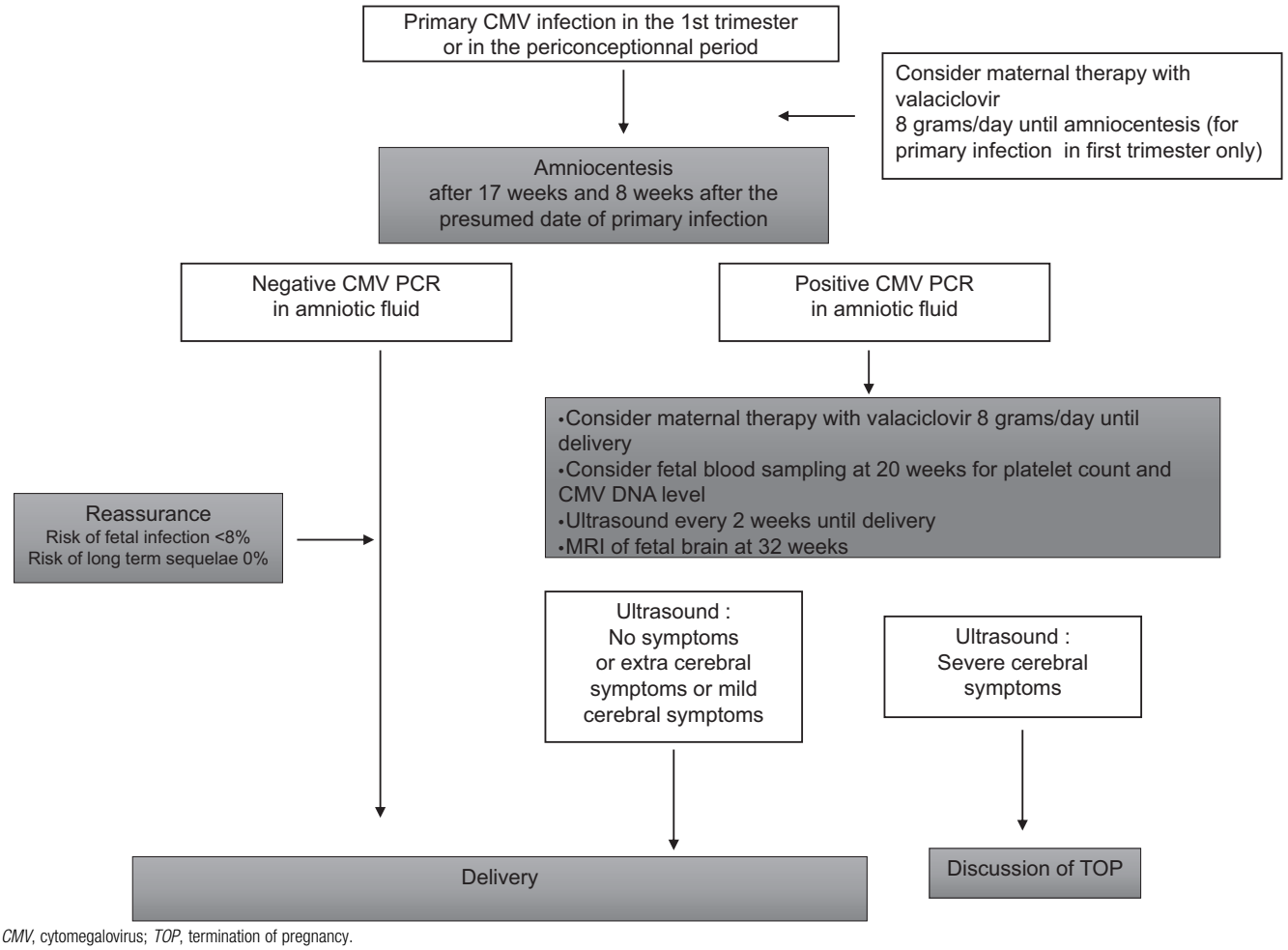
Only 5.7% of congenital CMV-infected children need a hearing aid or cochlear implantation, ranging from 29.3−44.4% to 1.6−3.4% in symptomatic and asymptomatic neonates, respectively.[3,28,30]

Cochlear implantation improves audition and language in cases with severe to profound hearing loss,[122] although those often suffer coexistent cognitive disabilities.[123−125] Asymptomatic infected neonates with late-onset SNHL had nonverbal intelligence and academic achievement scores in math and reading similar to controls.[126]

Neurological sequelae of congenital CMV infection

In addition to SNHL, congenital CMV infection can cause developmental delay, cognitive impairment, neuromuscular dysfunction (cerebral palsy), epilepsy, and impaired vision function, and it has been associated with autism spectrum disorder. In this section of this review, the term neurological sequelae will refer to impairments exclusive of hearing loss

FIGURE 6
Proposed algorithm for the management of congenital CMV infection



CMV, cytomegalovirus; *TOP*, termination of pregnancy.

Leruez-Ville. Cytomegalovirus infection during pregnancy. Am J Obstet Gynecol 2020.

and vestibular dysfunction, which are covered elsewhere.

Public health significance of neurological sequelae of congenital CMV infection

The majority of children with congenital CMV will not have any medical or neurological consequences of infection. However, around 30,000 cases with related disabilities are born every year in the United States and a similar number in the European Union, with an overall mortality and sequelae rates of 0.5% and 17−20%, respectively.[27]

The annual health care cost attributed to congenital CMV infection in the United States and in the United Kingdom was estimated to be $1.86 billion and £495−942 million.[127,128] Congenital CMV infection causes far more long-term sequelae than either *Hemophilus influenza b* or congenital rubella did prior to vaccine control of these infections. Congenital CMV infection continues to lead to more children with disabilities annually in the United States than trisomy 21, fetal alcohol syndrome, or spina bifida.[129]

Neurological sequelae occur primarily in patients with symptomatic congenital CMV

Screening of 117,986 newborns showed that sequelae occurred in 34 of 252 cases that were asymptomatic at birth (13.5%).[27] Only 10 of 252 (4.0%) had cognitive impairment and only 2 (0.8%) had motor deficits, rates that are similar to what one might expect in a control population.

Developmental and cognitive measures between 204 children with asymptomatic congenital CMV infection and 177 uninfected siblings found no difference in developmental or intelligence quotients between cases and controls.[130] A systematic review of outcome of asymptomatic congenital CMV infection included 37 reports that provided data on hearing, neurodevelopmental outcome, or both and reported that there was no difference between cases and a control group in neurodevelopmental outcome (excluding hearing loss).[131] These and other long-term follow-up studies of

children that are asymptomatic at birth (identified by screening newborns for CMV infection) show that these children are at increased risk for hearing loss, but it is not clear that they are at increased risk of mental retardation or motor deficits.[132,133]

Children with symptomatic congenital CMV infection, on the other hand, have a 40−70% risk of having neurological sequelae.[27,134]

Neonatal cranial imaging predicts neurological sequelae

Abnormal postnatal imaging, cranial CT, or MRI, is found in 70% of symptomatic infants,[135] and abnormal cranial CT bears odds ratios of 5.6−24 for severe sequelae and shows also good negative predictive value.[136] Postnatal imaging in symptomatic neonates increasingly refers to transcranial ultrasound showing cystic lesions, calcifications, ventriculomegaly, lenticulostriate vasculopathy, and cerebellar hypoplasia, but MRI is better for detecting white matter abnormalities, polymicrogyria, lissencephaly, hippocampal dysplasia, and cerebellar hypoplasia.[137,138] These and other studies consistently show a strong association between type and number of cranial imaging abnormalities in newborns and permanent neurological sequelae because of cognitive, motor, and sensory impairments.[139,140]

Ocular and visual abnormalities

Ocular abnormalities because of congenital CMV infection, mainly chorioretinitis, often manifest as retinal scarring,[141] have been recognized almost exclusively in patients who were symptomatic at birth, although the frequency of ocular abnormalities has varied widely from around 10% to 40%.[135,142−144] Chorioretinitis was found in 19% of the symptomatic group and in 3.9% of the asymptomatic group. Severe abnormalities (optic atrophy/nystagmus) occurred in 13% of patients who were symptomatic at birth and in 1.2% of asymptomatic patients.

Epilepsy in symptomatic congenital CMV infection

Epilepsy occurs in around 10% of patients with symptomatic congenital CMV infection; these patients often have other evidence of CNS damage. The only imaging findings that were more frequent in the epilepsy group were migration disorders and ventricular dilatation.[144] Cessation of seizures and withdrawal of antiepileptic medications was reported in 59% of patients after treatment with ganciclovir.[145]

Autism and congenital CMV infection

Although congenital CMV infection is not generally considered to be an important cause of autism, there is evidence linking the two. This is not surprising because some patients with congenital CMV infection will have significant cognitive, communication, and sensory abnormalities and could be diagnosed clinically with autism. Studies have recovered dried blood spots, remnants from newborn screening of patients with autism and controls for retrospective diagnosis of congenital CMV, by testing for CMV DNA using PCR. This association has been found in several populations from Italy, Japan, and Sweden with 5.3%, 7.4%, and 3% of autistic patients with congenital CMV.[146−148]

Taking a different approach, an Italian study evaluated patients with proven congenital CMV infection for autism and found a 2- to 3-fold increased prevalence compared with the general population.[149] A systematic review of reports linking congenital CMV infection to autism spectrum disorder provides more information and concludes that further study is needed.[150]

Neurological sequelae of congenital CMV infection: is there more?

A recent study from The Netherlands found more frequent problems in language development, concentration, and quality of life compared with a control group at school age.[151] Given the fact that damage to the CNS because of congenital CMV can be focal and isolated, it is possible that some children with congenital CMV have subtler problems related to processing of visual, auditory, or even sensory information. Normal visual acuity and the ability to detect sound can be present without the ability to decode the visual or auditory input. More studies that extend follow-up into adulthood and use methods capable of detecting problems with processing of input to the CNS are needed.

Treatment of congenital CMV infection

Options for antenatal treatment

Prevention of maternal infection. Caring for preschool children in the year before delivery is a risk factor for delivering a congenitally infected baby after maternal primary infection, however not after nonprimary infection.[23,152] Toddlers show prolonged viral shedding for weeks or even months and are a significant source of infection in seronegative women and therefore a risk for congenital CMV infection in their offspring.[152,153]

Hygienic measures to avoid exchange of body fluids (saliva, tears, urine, etc) are recommended to avoid maternal and paternal contamination. One controlled study demonstrated the benefit of these measures to decrease primary infection.[154] In the intervention arm, CMV-seronegative women were given hygiene information at 11−12 weeks of gestation and prospectively tested for CMV until delivery. The comparison arm consisted of women enrolled at delivery not informed about CMV during pregnancy and tested retrospectively. The 1.2%. rate of seroconversion in the intervention group was significantly lower than 7.6% in the comparison group.

These results are very encouraging; however, to give hygienic counselling in women at 11−12 weeks of gestation is already too late because all potential severe infections have already happened at the end of the first trimester. Indeed, in this study, maternal serology at the time of inclusion showed that in both groups, around 1% of these women already showed positive IgM and IgG at 12 weeks.[154] Increasing awareness in women before pregnancy should be the ultimate aim of information campaigns.

Prevention of fetal transmission by passive immunization with hyperimmune globulin (HIG)

The rationale for HIG efficacy to prevent and/or treat CMV congenital infection is

supported both by in vitro experiments in decidua organ culture and in vivo in the guinea pig and rhesus monkey models.[155–157]

Efficacy of HIG in clinical trials

A nonrandomized trial using HIG at a dose of 100 U/kg intravenously monthly reported a significant decrease of fetal transmission from 40% in untreated women to 16% in treated ones.[158] These results were not confirmed by a double-blind randomized, placebo-controlled trial in 124 pregnancies with primary CMV infection[159]: HIG given monthly at the same dosage than previously was followed by a 30% transmission rate that was not significantly lower than 44% observed with placebo (NaCl). In addition, there was an increase, although not significant, of premature babies in the HIG group.

Another placebo-controlled randomized controlled trial (RCT) trial was conducted in the United States using the same criteria but was designed to include 800 women. The trial was stopped for futility at interim analysis of the first 399 cases showing transmission rates of 22.7% and 19.4% in the HIG and placebo groups, respectively. The rates of preterm birth were similar in both groups (12.2% vs .8.3%).[160]

The debate on the use of HIG was, however, revived on the ground of the pharmacokinetic of CMV HIG showing that CMV IgGs have a shorter half-life of about 11 days in maternal blood compared with 22 days as previously reported.[161,162] Biweekly administration of HIG might therefore be more efficient than monthly administration as used in previous trials.[158–160]

A nonrandomized phase I study reported that biweekly administration of a 200 U HIG decreased maternal-fetal transmission compared with a historical cohort (7.5% vs 35%) in the context of systematic serology screening in pregnancy.[163] In addition to the higher dosage of HIG, the authors of the latter study brought up the issue of the importance of including only cases with a very recent primary infection. This could prove important because maternal and therefore placental viremia precede the appearance of IgM and IgG by 1 week. Those parameters were indeed controlled in the animal studies but not in the 2 RCTs conducted to date.

Therefore, although HIGs seem safe in pregnant women, their efficacy has not been demonstrated in clinical practice and the potential impact of the dosage and timing of administration remains to be established.

Antiviral drugs to prevent or to treat fetal infection

Which antiviral drug?

Treating infected fetuses with antiviral therapy early enough to avoid the development of irreversible CNS injury is an appealing option. However, the development of this strategy has been hampered by the lack of anti-CMV drugs that are both potent and safe to be used during pregnancy.

Ganciclovir *(GCV)*. GCV and its oral prodrug valganciclovir are inhibitors of the DNA-polymerase enzyme that initiates viral replication. It is the most efficient drug currently available to cure CMV disease, but because of its extreme genotoxicity in vitro, it has been classified as a potentially teratogenic drug. In the rat model, GCV induces germ cell deficiency in the exposed embryos with irreversible testicular abnormalities.[164] Ganciclovir adequately crosses the placenta in the perfused cotyledon model but also in vivo.[165,166]

There are 4 reports on its use in transplant or AIDS pregnant patients in first,[167,168] second, and third trimesters.[166,169] None of the 4 live-born babies exposed in utero showed any birth defects. Three pregnant women carrying an infected fetus were also purposely treated with valganciclovir at the dose of 900 mg/d in the third trimester with valganciclovir. Interestingly, viremia and viruria were negative in all 3 neonates.[170]

Acyclovir and valaciclovir. Acyclovir and its prodrug valaciclovir are also inhibitors of the DNA-polymerase. They are less effective to inhibit viral replication than ganciclovir but provide effective prophylaxis against CMV infection in renal transplantation.[171] Acyclovir also has the best safety profile among anti-CMV drugs: it is neither genotoxic nor carcinogenic in vitro or in animals.[172] However, in the rat model, exposure to the highest dose of acyclovir (300 mg/kg) induced incomplete eye opening and tail abnormalities.[164,173] In 2 registries of 596 and 1561 pregnant women exposed in the first trimester and 2379 in the second and third trimesters, the rate of birth defects was 2%, 3.2%, 2%, and 2.4% with no specific pattern and in the same range than in more than 800,000 untreated pregnancies.[174,175] Dose regimens and duration of exposure were not detailed but were likely to be those recommended for herpes simplex virus or varicella infection and therefore around 2 g/d.

New anti-CMV drug. Letermovir is an inhibitor of the terminase viral enzyme, which closes up the viral replication process. It was recently licensed for CMV disease prophylaxis in bone marrow transplant recipients.[176] The target of letermovir is unique to cytomegalovirus, and the drug direct toxicity is very low.[176] The drug showed no teratogenicity in animal studies, but no data on its use in the context of congenital HCMV infection are available to date.

Valaciclovir to prevent maternofetal transmission: clinical experience

The results of a double-blind, placebo-controlled RCT using valacclovir 8 g/d in 100 pregnant women with documented seroconversion during the periconceptional period and in the first trimester have just been released (NCT02351102).[73] Treatment was initiated at the time of serological detection and continued until diagnostic amniocentesis. Five amniocenteses (11.1%) were positive for CMV in the valacclovir group, compared with 14 (29.8%) in the placebo group ($P = .03$), giving an odds ratio of 0.29 (95% confidence interval, 0.09–0.90) for vertical CMV transmission.[73]

Valaciclovir treatment in pregnant women carrying an infected fetus: clinical experience

A pilot study confirmed that valaciclovir crosses the placenta and that a dose

TABLE 3
Three new CMV vaccine candidates in development for prevention of maternal and congenital CMV infection

Antigen	Format	Company	Clinicaltrials.gov number	Publications
gB/eVLP	Virus-like particle	Variation Biotechnology	NCT02826798	Kirchmeier et al, 2014[189]
Virion	Live virus, replication defective	Merck	NCT01986010 NCT03486834 NCT03840174	Adler et al, 2019[191] Wang et al, 2016[190]
gB/pentameric complex	mRNA/lipid nanoparticle	ModernaTX	NCT03382405	John et al, 2018[192]

gB, glycoprotein B; *eVLP*, enveloped virus-like particle vaccine; *mRNA*, messenger RNA.

Leruez-Ville. Cytomegalovirus infection during pregnancy. Am J Obstet Gynecol 2020.

regimen of 8 g/d per os achieved expected concentrations in maternal and fetal blood in the second trimester.[177] Valaciclovir reduced fetal blood viremia by 1 log.[177,178] The results of a phase II multicenter open label study showed that high-dose valaciclovir (8 g/d) administered to women carrying an infected fetus, from the diagnosis of fetal infection in the second trimester to delivery, was associated with a higher proportion of asymptomatic neonates (82%) than that in an untreated historical cohort (43%).[178] No maternal, fetal, or neonatal adverse effects were reported in more than 60 treated women for a median of 90 days from 25 weeks to delivery.[177,178] This trial demonstrated the plausibility of valaciclovir efficacy, but a higher level of evidence is required.

Neonatal treatment

GCV or its prodrug valganciclovir (VGCV) is the preferred antiviral agent for the treatment of congenital CMV disease. European and US guidelines recommend VGCV to be given at a dose of 16 mg/kg twice daily for 6 weeks to 6 months to symptomatic neonates bearing SNHL and/or neurological symptoms. They also recommend against antiviral therapy in asymptomatic neonates without SNHL. The expected effect on hearing is to prevent hearing deterioration and late-onset hearing loss but also improvement of hearing thresholds in affected children. The effect of antiviral therapy on hearing is difficult to determine because unrelated middle ear problems are frequent in young children and also because hearing thresholds in infected children fluctuate (deterioration and improvement) over time, even without treatment.[110]

In 1 RCT in 42 neonates with SNHL, 1 6 week course of ganciclovir prevented hearing deterioration at 6 months compared with a control group; however, hearing thresholds decreased again at 12 months.[179] Oral valganciclovir also improved hearing function in symptomatic children. A 6 month course was not superior to a 6 week course at 6 month follow-up but showed a benefit, although modest, in the longer term.[180]

Other studies did not have untreated controls and report variable efficacy of prolonged treatment with VGCV on long-term assessment of SNHL.[181−183]

To conclude, the effect of antiviral therapy on hearing thresholds is far from clear, and even with treatment, we will still witness children with SNHL who are going to need hearing rehabilitation.

Update on vaccine

Preventing congenital CMV infections is recognized as an important public health goal in the United States and Europe. Using an analysis based on quality-adjusted life years and economic considerations, the Institute of Medicine of the US National Academy of Sciences listed a vaccine given to 12 year old girls with the goal of preventing maternal CMV infections during pregnancy as a top national priority for vaccine development.[184]

The first clinical trial with a CMV vaccine was conducted in the early 1970s, and multiple clinical trials with a variety of candidate CMV vaccines were conducted during the approximately 30 years preceding the Institute of Medicine report.[185,186] However, there is currently no licensed vaccine for the prevention of CMV infection, and a clinical trial that could lead to licensure is yet to be performed. Prevention of CMV infection is an enormous challenge because of the complexity of the virus and the biology of human CMV infection. Cytomegalovirus is a large, complex virus with about 20 times the genetic material of HIV, and a substantial portion of its genome is devoted to interacting with and escaping the host immune system.

Two randomized, double-blind, placebo-controlled clinical trials in young women showed modest efficacy of around 40−50% for prevention of CMV infection, the first evidence that vaccine prevention of CMV infection in humans could be achieved.[187,188] The study vaccine for both of these clinical trials was composed of recombinant subunit envelope glycoprotein B (gB) and a relatively new adjuvant, MF59 (now used in some licensed flu vaccines). Although these results were encouraging, further development has not been pursued, probably because of concern over the modest level of efficacy and declining levels of antibody with passage of time.

New CMV vaccine candidates emerged as a result of efforts to enhance the immunogenicity of CMV gB and to include a wider range of CMV antigens. For the interested reader, a review of CMV vaccines provides lists of multiple candidate vaccines currently being studied as well as those that are not being developed further as of 2017.[186] A

number of CMV vaccines appear to be focused on prevention or treatment of CMV infection in patients in the setting of hematopoietic stem cell transplantation, solid organ transplantation, or cancer, and those vaccines will not be discussed here.

Three novel vaccine candidates aimed at prevention of maternal and congenital CMV infection are listed in the Table 3. Each of these has entered early-phase clinical trials in humans and has advantages over the recombinant subunit gB/MF59 vaccine. The enveloped virus-like particle vaccine (eVLP) expresses the full-length extracellular domain of gB much as occurs with viral infection and stimulates a broader range of neutralizing antibodies than the subunit gB vaccine. A preclinical study with the gB/eVLP vaccine reported potent induction of neutralizing antibodies.[189] A phase I clinical trial with gB/eVLP has been completed; results are not yet published.

The virion vaccine is derived from a live virus strain (AD169) that was attenuated by serial propagation in fibroblast cells. The virus was modified to improve immunogenicity and to limit replication.[190] Improved immunogenicity is based on restoring genes for the CMV proteins that form a pentameric complex in the viral envelope (gH/gL/pUL128/pUL130/pUL131).

This pentameric complex plays a key role in the induction of antibodies that can neutralize CMV preventing infection of epithelial cells. A phase I clinical trial with this vaccine in seronegative, healthy adults demonstrated neutralizing antibody titers 12 months after the third dose of vaccine to be similar to those of unvaccinated subjects with past CMV infection. No viral shedding was detected, evidence that the vaccine virus did not replicate in humans.[191]

The third candidate listed in the table is a messenger RNA vaccine encapsulated in a lipid nanoparticle. The messenger RNAs carry instructions for synthesis of CMV gB and the pentameric complex. Excellent neutralizing antibody responses to in preclinical studies using CMV infected fibroblast and epithelial cells were reported.[192] A vaccine that is safe, well tolerated, and provides a significant improvement in efficacy compared with CMV gB/MF59 is the hoped-for result within the next few years.

If a successful phase 3 clinical trial is based on prevention of CMV in seronegative women of childbearing age, questions will remain: will government agencies approve the vaccine for prevention of congenital CMV infection and will there be data to support use of the vaccine in all women of childbearing age, CMV seropositives as well as seronegatives? Vaccination of women of childbearing age is not a part of their routine health care except for influenza and Tdap immunizations often given during pregnancy.

Achieving widespread use of CMV vaccine for the prevention of maternal infection will require a major public health campaign aimed at women of childbearing age as well as primary care physicians, providers of prenatal care, and obstetricians. Children 12 years of age are routinely immunized now against human papilloma virus, and it would be reasonable to consider simultaneous immunization against CMV. However, if we are to achieve prevention of congenital CMV infection based on immunization of 12 year old girls, a vaccine and immunization schedule that provide protection over a 10- to 20-year interval will be needed.

Children of preschool age are a major source of maternal CMV infections. Immunizations are a regular part of their health maintenance, and immunizing them with the goal of preventing horizontal transmission of CMV could be considered. Because they would not directly benefit from a CMV vaccine, excellent vaccine safety and tolerability would be a necessity. Because there would be an interval of at least 10 years from immunization to onset of sexual activity, waning immunity would be a concern.

There will be problems to solve to determine how to optimize use of a vaccine for prevention of congenital CMV infection. However, after almost 50 years of discussion of that goal, facing those problems with the availability of a safe and efficacious vaccine will be a welcome challenge. ■

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