Review

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Review

CMV in Pregnancy: Effects on the Developing Embryo and Fetus, Diagnosis and Treatment: Where to Go Now?

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Abstract

Cytomegalovirus (CMV) is the most teratogenic human virus often manifested with atypical clinical findings. Fetal damage is highest following primary maternal infection during the first trimester of pregnancy with transfer increasing with pregnancy advancement. CMV damage may continue to intensify during the early postnatal years. Viral load enables prediction of fetal infection; its reduction by maternal treatment with valacyclovir may reduce the rate and severity of fetal infection. Definite fetal infection may be diagnosed by amniocentesis and viral DNA detection and brain involvement by ultrasound or MRI. The most common fetal damage is hearing loss alongside a variety of brain lesions resulting in significant neurological deficits, including intellectual impairment. The clinical manifestations are either evident at birth (cCMV) or gradually appear postnatally. Pharmacological treatment with ganciclovir or valganciclovir, if initiated early after birth, reduces hearing loss progress and possibly ameliorates other neurological deficits. As of today, there is no approved CMV vaccine for prevention. The mRNA-1647's vaccine, that is in phase 3 clinical trial, seems to be promising. The IgG avidity testing improved the ability to define recent from old infection and, indirectly, primary from non-primary infection. These advances should make us raise the need of screening pregnant women in the first trimester and newborn infants of mothers suspected of having primary CMV infection. Alternatively, at least infants who fail the screening hearing test. Such recommendations were made recently by a group of experts in Europe. Infants with clinical manifestations should be offered treatment as early as possible following diagnosis.

Keywords: Cytomegalovirus (CMV); Congenital CMV; Valacyclovir; immunoglobulin; CMV vaccine; Neonatal screening

1. Introduction

Cytomegalovirus (CMV) is found universally throughout all geographic locations and may infect all ethnic and socioeconomic groups. The rate of infection among adults aged 40-50 years is 50–85%. In certain populations in Asia and Africa, especially in densely populated areas and areas of low socio-economic status, it can be as high as 100% [1–3]. It is the most common intrauterine infection, and a common cause of sensorineural hearing loss, intellectual disability and neurological dysfunction [4,5]. In healthy people who acquire CMV, there are only a few or no symptoms and no long-term sequelae. Some experience mononucleosis-like syndrome with prolonged fever, and mild hepatitis. Following primary infection, the virus often remains dormant within the body for life. CMV infection in pregnant women may be dangerous for the developing fetus. Similarly, CMV infection in immunocompromised persons may have severe sequelae [6–8].

CMV is the largest of the herpetoviridae virus family with a diameter of about 200nm. It has a double -stranded DNA core of 200 kilobases enclosed by an icosahedral capsid. The DNA genome

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encodes for over 35 different structural proteins and glycoproteins. Human and higher primates seem to be the only reservoir for the human CMV [9]. There are several strains of CMV that infect humans and therefore recurrent infections by different strains are common even in immuno-competent individuals. Individuals can therefore be infected by more than one strain (reinfection). CMV, like other herpes viruses, can remain latent after primary infection residing mainly in the salivary glands [8,10]. Reactivation of a latent infection may therefore occur alongside reinfection by a different strain [8,10,11].

Transmission of CMV occurs from person to person. CMV has been isolated in all body fluids including saliva, urine, feces, semen, vaginal secretions, breast milk, blood and tears. Infection requires close contact with people excreting the virus in their body fluids. Indirect transmission from toys and items that are in close contact with an infected individual is also possible, especially endangering pregnant women who are in close contact with infected children. CMV can be transmitted sexually or from transplanted organs, and rarely, from blood transfusions [1,8,12].

Seroconversion (primary or non-primary CMV) occurs in 1–4% of all pregnancies [13]. The virus can also be transmitted to the infant at delivery from contact with genital secretions or through breast milk [14]. Primary infection is defined as CMV infection in a seronegative person who was never infected before; non-primary (or secondary) infection is defined as a rise in IgG or IgM antibody titers in a person who was previously infected. Following CMV infection IgM antibodies can persist for a long time (6-18 months) in both primary and non-primary infections, which makes the diagnosis of a recent infection difficult [8]. Following primary infection in the first trimester of pregnancy, lack of IgM antibodies in the second trimester or high IgG avidity does not exclude congenital CMV (cCMV) [15]. Both primary or non-primary infections can lead to symptomatic cCMV [16,17]. There is always a certain percentage of women with IgG and IgM antibodies in pregnancy in whom it is impossible to define whether they have primary or non - primary infection [10,11,18].

2. Congenital CMV (cCMV)

The range of cCMV varies between 0.2–2.5% of live births [8] with an average prevalence of 0.67% [19]. Young maternal age, single marital status, and nonwhite race are associated with higher rates of cCMV infection [20,21].

Transmission to the embryo and fetus is more common in primary infection than in non- primary infection. However, the number of pregnant women with non-primary infection is higher than those with primary infection [22]. Therefore, the number of infants with cCMV due to non-primary infection is equal to or higher than those due to primary infection [23]. Hematogenous spread appears to be the most likely pathway of vertical transmission to the fetus. Placental infection occurs first, followed by replication of the virus. The virus is then transferred to the fetus, where it replicates in the renal tubular epithelium and enters the amniotic fluid [18]. CMV spread via ascending infection from maternal genital organs is rare, but possible. The virus, after entering the amniotic fluid, replicates in the fetal oropharynx [24]. If the pregnant woman is seronegative at the time of infection, the probability of transmission to the fetus is 30–50% [10]. Infection in the first trimester of pregnancy bears a significantly greater risk of symptomatic fetal involvement and severe fetal damage, although fetal transfer increases with advancement of pregnancy [9,25–28]. CMV can be transmitted from mother to fetus even if the mother had primary infection several months before pregnancy [26–29].

3. Neonatal Presentation of cCMV: Hearing Loss

The clinical findings in CMV affected newborn infants may be manifested at birth or gradually emerge during the first years of life. The embryo and fetus are most susceptible to teratogens during active organogenesis, which in humans is the first trimester of pregnancy. CMV is an example of a highly teratogenic virus that can affect fetal organs after major organogenesis as the infection and inflammation may continue for years after birth, increasing the damage [8,26–28,30,31]. Only 10–15% of newborns of mothers with primary CMV infection present the typical clinical findings of cCMV at

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birth [9,10,32]. They may include intrauterine growth restriction (IUGR), microcephaly, hepatosplenomegaly, petechiae, jaundice, chorioretinitis, thrombocytopenia, anemia and/or other atypical findings [8,33–40]. Maternal Viral load is an important predictor of neonatal cCMV, as increased viral load increases the chances for neonatal symptoms and for higher severity [31]. Salome et al. [31] found in a most recent meta-analysis that the maternal blood and urine viral load are good predictors of neonatal cCMV and severity. However, blood is a better predictor in comparison to urine.

The most common handicap of cCMV is sensorineural hearing loss that may be progressive due to continuous damage to the cochlea [41]. Progressive hearing loss is manifested by extensive infection of the inner ear, especially affecting the epithelial cells in the organ of Corti [42–45]. It is often accompanied by various degrees of brain infection [43,44]. CMV infection of the vestibular labyrinth was also described with damage to sensory cells in the utricle and in the crista ampullaris. As the virus resides in the inner ear for relatively long periods of time, hearing loss often tends to worsen with time.

Various degrees of brain infection were also observed in 14/20 fetuses, mostly those with high viral loads, demonstrating a strong correlation between brain infection and cochlear damage. Craegs et al. [46], while evaluating ultrasound and/or MRI brain imaging studies from 411 patients with cCMV in association with audiometric studies, found a positive correlation between brain abnormalities and early onset hearing loss, but not with late onset hearing loss. Hence, brain abnormalities predict early hearing loss with 84% specificity and 43% sensitivity. Similar findings were described by Hranilovich et al. [47]. It is therefore advised that infants with cCMV and early hearing loss should have brain imaging studies. These are indeed the recent recommendations of the European group of experts [26], as negative findings have almost 100% accurate predictive value. Although urine and saliva viral load in infants with cCMV infection is higher in symptomatic children in comparison to non-symptomatic children, viral load cannot predict hearing loss or vestibular damage [48,49].

A summary of the main studies is given in Table 1.

Table 1. Fetal CMV infection and auditory system damage.

Study / Author	Main Findings	Implication for Hearing
Teissier et al. (2011)	CMV infected key inner ear structures (notably the stria vascularis and vestibular epithelia) in all fetuses, with severity correlating with CNS damage.	Disruption of potassium homeostasis in the inner ear may drive sensory cell degeneration and SNHL, with risk linked to the extent and duration of infection.
Gabrielli et al. (2013)	CMV infected the inner ear in 45% of fetuses, especially the stria vascularis and organ of Corti, disrupting cochlear ion balance.	Inner ear infection can lead to SNHL even without brain ultrasound abnormalities, underscoring the need for comprehensive evaluation.
Bartlett et al. (2017)	Asymptomatic infants have 7-11% hearing loss vs. 34-41% in symptomatic infants; early detection and treatment improves outcomes.	There is no reliable viral marker to predict outcome. Consistent follow-up until school age is recommended for both symptomatic and asymptomatic children.
Hranilovich et al. (2020)	MRI abnormalities are associated with failed newborn hearing screening and early hearing loss.	Brain neuroimaging should be considered as part of the evaluation for cCMV infants, even when asymptomatic at birth

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Craeghe et al. (2021)	Brain abnormalities correlate with early hearing loss (84% specificity, 43% sensitivity); do not predict late-onset loss.	Neuroimaging can identify infants at risk for early hearing loss.
Corazzi et al. (2022)	Children with cCMV often show vestibular and postural disorders, the symptoms can vary greatly between individuals.	Vestibular impairment can occur independently of hearing loss, underscoring the importance of assessing both systems.
Kabani et al. (2023)	Viral load in urine and saliva is higher in symptomatic infants, but does not predict hearing loss.	Viral load alone is insufficient to predict hearing loss.
Keymeulen et al. (2023)	Hearing loss was present in 29.2 % of the asymptomatic children and in 70.8 % of the symptomatic children. Only 70.4% of CMV-infected children had normal development; 2.5% had ASD; speech issues found even without hearing loss .	Neurodevelopmental issues including hearing problems, can emerge later. All children with cCMV should receive multidisciplinary neurodevelopmental follow-up
Smyrli et al. (2024)	10-15% of the children asymptomatic at birth had low rates of neurodevelopmental disorders, most commonly sensorineural hearing loss.	Low but present risk of hearing loss in asymptomatic infants.
Gabrielli et al. (2024)	CMV showed tropism for the auditory pathway, infecting the stria vascularis and activating microglia in the auditory cortex, especially in cases with high brain viral load.	Both peripheral (cochlear) and central (cortical) auditory damage may contribute to CMV-related SNHL, highlighting a dual mechanism of injury.

4. Fetal Brain Damage and Neurological Sequelae Caused by CMV

Many brain imaging lesions have been described in children with cCMV. They are best visualized by MRI examinations but may also be observed by ultrasound. The ultrasonographic and MRI brain imaging findings in neonates with cCMV, can be used "to predict the future neurodevelopmental outcome"[50].

Malinger et al. [51] summarized the typical ultrasonographic brain findings. The changes are better visualized in the second half of pregnancy, as early in the second trimester of pregnancy, it may be too early for the possible demonstration of most ultrasonographic findings. Of the more common findings, the authors list ventriculomegaly, microcephaly, and increased periventricular echogenicity. Brain calcifications are a relatively common finding in several intrauterine infections (i.e., Toxoplasmosis, Rubella, Varicella) and, in CMV, they are found in 21%-43% of cases. These are disseminated in different parts of the brain, including the basal ganglia and cerebellum, or in a periventricular location. Periventricular pseudocysts and intraventricular synechia may also be observed. Cerebellar changes are also common, generally with damage to cerebellar structures.

Similar changes were also described by Boppana et al. [52] examining cranial CT scans. Most of the infants with abnormal CT scans at birth developed at least one sequela of cCMV, with 59% suffering from intellectual disability. The authors concluded that a cranial CT scan is a good predictor of adverse neurodevelopmental outcomes because in children with normal CT scans only 29% had neurological sequelae [52]. Other investigators [50,53] came to similar conclusions. It can be

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concluded that brain imaging (CT, MRI) can be a good tool for the prediction of neurological sequelae in children with symptomatic cCMV.

5. Neurodevelopmental Outcome

One of the major problems of cCMV is the neuro-developmental damage, including various degrees of intellectual disability. Learning disabilities may also be found in CMV-infected children even with normal intellectual abilities and no other clinical symptoms of cCMV [24,54,55]. A possible association between cCMV and autism was also suggested [56,57]. It is, however, impossible to accurately predict at birth the extent of the neurodevelopmental impairment since several sequelae, especially hearing impairment and intellectual disabilities, may be progressive due to the continuous inflammatory process.

The rate of neurodevelopmental problems differs among children with cCMV and those without neonatal symptoms. Keymeulen et al. [58] studied the developmental outcome of 753 children with cCMV at different ages. Of them, only 70.4% had normal development. Of their cohort, 16.9% children had mild neurodevelopmental problems, 7.4% had moderate, and 5.2% had severe neurodevelopmental problems. Many had hearing loss. Speech and language impairment were, however, found even in the absence of hearing impairment. 2.5% of the children had autism spectrum disorder (ASD) in comparison to 0.7% in the general population.

Barlett et al. [59] found that In children born with asymptomatic congenital CMV, 7-11% had some degree of hearing impairment as opposed to 34-41% in children with symptomatic CMV at birth. The asymptomatic children had normal development. Normal development at 12 months of age without microcephaly makes subsequent neurodevelopmental or intellectual impairment unlikely [60]. A systematic review assessing the developmental outcome of children with congenital CMV who were asymptomatic at birth concluded that children with congenital CMV only low rates of neurodevelopmental disorders [61].

In contrast, infants with symptomatic CMV infection at birth are likely to have severe CNS sequelae, including intellectual and motor impairment, microcephaly, sensorineural hearing loss, chorioretinitis and sometimes seizures. These sequelae evolve in the early years of life, with 45–90% experiencing these neurologic abnormalities [4,5,28,38,62,63].

A summary of the neurodevelopmental findings is given in Table 2.

Table 2. Fetal CMV infection and neurodevelopmental problems. Studies presented in Table 1 are not discussed here.

Study / Author	Main Findings	Implication for Neurodevelopment
Ivarsson et al. (1990)	Case report of two children with congenital CMV infection who had severe disabilities, including autism	The study suggests that autism may be among the neurodevelopmental sequelae of severe cCMV infection, supporting further investigation into this association.
Boppana et al. (1997)	Among 56 symptomatic cCMV-infected newborns, 70% had abnormal cranial CT scans. 90% of these developed at least one sequela. IQ < 70 occurred in 59% of those with CT abnormalities, versus 1 child with normal CT. CT abnormalities were also linked to hearing loss, but	Newborn cranial CT is a strong predictor of later neurodevelopmental impairment in symptomatic cCMV, whereas clinical signs alone are unreliable for prognosis.

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	clinical findings at birth were not predictive of abnormal imaging.	
Noyola et al. (2001)	In a longitudinal cohort of 41 children with symptomatic cCMV, microcephaly and abnormal head CT at birth were strong predictors of later intellectual and motor disability. 29% had normal IQ/DQ. Sensorineural hearing loss at follow-up was associated with lower IQ, but hearing loss at birth was not.	Microcephaly and abnormal neonatal brain imaging strongly predict poor neurodevelopmental outcome. Early head circumference and CT findings can guide prognosis and intervention.
Lipitz et al. (2002)	Among 18 live-born infants with confirmed cCMV, 4 had neurologic abnormalities; notably, 3 of these had normal prenatal ultrasound. Overall, 19% of infants without prenatal US findings developed postnatal neurologic sequelae.	Normal prenatal ultrasound does not exclude risk of later neurologic impairment. Neurodevelopmental follow-up is warranted even in apparently normal cCMV cases.
Yamashita et al. (2003)	Out of 7 children with symptomatic cCMV, 2 (28.6%) were later diagnosed with autism. Both had subependymal cysts detected neonatally and developed global developmental delays with MRI evidence of disturbed myelination.	There is a potential link between cCMV-related brain injury (possibly in the third trimester) and ASD later in life. Neuroimaging abnormalities may support early identification.
Bartlett et al. (2017)	Children with asymptomatic cCMV performed similarly to healthy controls in neurodevelopmental assessments.	Despite overall good outcomes, long-term neurodevelopmental follow-up is recommended as no reliable marker predicts later sequelae.
Craeghs et al. (2021)	Brain MRI was useful for predicting early neurological risk, though not definitive for long-term outcomes.	Even with normal early imaging, continued neurodevelopmental surveillance is essential.
Keymeulen et al. (2023)	In a cohort of 753 children with cCMV, 29.6% had some level of neurodevelopmental impairment. Adverse outcomes were seen in both symptomatic (53.5%) and asymptomatic (17.8%) children. ASD was diagnosed in 2.5%, and 2% had speech/language delay without hearing loss.	Neurodevelopmental follow-up is essential for all cCMV-infected children, with particular attention to hypotonia, ASD, and speech delays — even in the absence of hearing loss.
Smyrli et al. (2024)	Low rate of children asymptomatic at birth still	All cCMV-exposed children should receive long-term neurodevelopmental follow-up.

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	showed neurodevelopmental impairments later in life.	
Salome et al. (2025)	Higher maternal blood CMV viral load was associated with more severe neonatal disease and adverse neurodevelopmental outcomes.	Maternal viral load could be used as an early indicator for identifying infants at risk of neurodevelopmental sequelae.

6. Diagnosis of Maternal CMV Infection:

Primary infection is defined as CMV infection in a seronegative person who was never infected before; non-primary infection is defined as a rise in IgG or IgM antibody titters in a person who was previously infected [8].

In contrast to many primary viral or bacterial infections where IgM is elevated only for several weeks, following CMV infection IgM antibodies may remain elevated for a relatively long period of time (6–18 months) in both primary and non-primary infections [13–15,24]. Hence, IgM antibody levels determination is not reliable. Studying viral load in blood or body secretions in every pregnant woman is also impractical. In addition, it is almost impossible to diagnose CMV infection based on clinical findings since these findings are not-specific and often absent [8,64,65]. When there are both IgG and IgM antibodies and the previous antibody status is unknown (which is often the case), it is difficult to judge whether this is a primary or non-primary infection. Avidity testing to assess the production time of the IgG antibodies will, in most cases, define old infection with high avidity from newer infection with low avidity, Avidity lower than 30-35% is considered to be a sign of relatively recent infection. Non-primary CMV infection is usually diagnosed if there is either a significant rise in IgG antibodies in a person with previous positive IgG, or positive IgG and positive IgM that was previously negative [8,64–68]. In spite of this important tool there is a certain percentage of women with IgG and IgM antibodies in pregnancy in whom it is impossible to define whether we are dealing with primary or non-primary infection.

A low avidity index, particularly when assessed early in pregnancy, reliably indicates recent infection and helps stratify fetal risk, especially if there are also fetal ultrasonographic abnormalities [64]. Viral DNA detection in cervical secretions may offer supplementary information when serological results are unclear [65].

Prince and Lapé-Nixon [66] emphasized that avidity testing is superior to IgM alone in determining infection timing, particularly in the first trimester. When avidity results are intermediate or late in pregnancy, monitoring viral load (e.g., via CMV DNA detection) can improve diagnostic accuracy. A review by Lazzarotto et al. . [68] further supports the use of CMV IgG avidity testing in IgM-positive pregnant women to distinguish primary from non-primary infections. A low avidity index within the first 3–4 months of pregnancy indicates recent primary infection. They also recommended CMV DNA testing as a secondary tool only when avidity is inconclusive or unreliable. Lastly, Sapuan et al. [67] highlighted that CMV shedding, most commonly in cervicovaginal secretions, occurs in about 21.5% of seropositive pregnant women. Although shedding alone is not diagnostic of primary infection, it may reflect non-primary viral activity and contribute to vertical transmission, offering a useful complement to serological testing in complex cases. Together, these findings reinforce the use of IgG avidity as a first-line diagnostic tool, with CMV DNA detection serving as a valuable adjunct in the small subset of pregnancies where avidity results are non-informative.

For the screening and treatment of pregnant women with primary or non-primary CMV infection see Tables 3 and 4.

Table 3. Recommendations for screening/treating pregnant women with no prior knowledge of their CMV antibody status or are known to be seronegative.

Recommendation/Step	Details

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Initial screening	Serological screening of all pregnant women for CMV antibodies early in pregnancy
Tests to perform	Screen for IgM, IgG and IgG avidity
If serology is negative	Continue screening each 4-5 weeks up to weeks 16–18 If still negative, no need to continue screening due to low possible fetal damage following late infections
If seroconversion occurs (suggesting primary infection)	Start treatment with valacyclovir, 8 grams/day (2 grams X 4 times daily)
If PCR is negative	Consider carrying out amniotic fluid CMV PCR Consider stopping treatment
If PCR is positive	Continue treatment
After delivery	Perform CMV PCR on urine or blood of the newborn infant
If newborn PCR is positive	Start treatment with valgancyclovir for at least 6 weeks Perform hearing assessment, ophthalmologic examination and brain imaging (preferable MRI) and check for clinical signs of cCMV
If signs are observed	Continue treatment with valgancyclovir for up to 6 months
Follow-up of asymptomatic infants	Should continue for at least two years
Hearing follow-up	Up to the age of 5 years

Table 4. Recommendations for screening/treating pregnant women who were seropositive prior to pregnancy.

Recommendation/Step	Details
General recommendation	Since reactivation or reinfection of CMV is common (non-primary CMV), in spite of the low rate of fetal damage, it is advised to reassess the CMV antibody status early in pregnancy
Tests to perform	Screen for IgM, IgG and IgG avidity
If no serological signs of non-primary infection	No need for additional studies
If study suggests reactivation/reinfection	Start treatment with valacyclovir, 8 grams/day (2 grams X 4 times daily) Consider carrying out amniotic fluid CMV PCR for possible fetal infection
If PCR is negative	Stop treatment
If PCR is positive	Consider continued treatment
After delivery	Perform CMV PCR on urine or blood of the newborn infant
If newborn PCR is positive	Start treatment with valgancyclovir for 6 weeks
	Perform hearing assessment, ophthalmologic examination and brain imaging (preferable MRI) and check for clinical signs of cCMV
If signs are observed	Continue treatment with valgancyclovir for up to 6 months
Follow-up of asymptomatic infants	Should continue for at least two years

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7. Diagnosis of Intrauterine Fetal CMV Infection:

The most effective way for diagnosing fetal infection is by molecular diagnosis of viral DNA [8,32,37,69]. Studies using Rt PCR correlate between the "viral load" in the amniotic fluid and the degree of fetal damage. As it takes 5–7 weeks since fetal infection and replication of the virus in the kidney until enough of the virus is secreted to the amniotic fluid, and the testing is not sufficiently reliable before the 21st week of pregnancy, PCR should be performed not before the 21st week. However, in most cases of primary CMV infection, detection of the virus from week 17 of pregnancy is also reliable if 6 weeks have passed from the time of infection [26,27]. If infection occurs later in pregnancy, detection of the virus from amniotic fluid should be attempted at least 6 weeks after maternal infection [37,70] While it is agreed to perform PCR and virus isolation in primary maternal infection, [8,26–28,32,37,69], there is no agreement in cases of non-primary maternal infection, when the risk of fetal damage is relatively low although fetal damage may be severe. Moreover, treating the pregnant mother with valaciclovir may reduce fetal infection by 60%-70% [26,27]. It is therefore suggested to carry out antenatal diagnosis even in cases of non-primary infections, if the infection occurred in the first trimester of pregnancy and/or there are signs of fetal damage [26,71] .

8. Predicting Signs of Fetal Infection and Damage:

As stated earlier, the simplest and most accurate way to assess fetal infection is by studying viral DNA in the amniotic fluid [26,27,69]. However, this is an invasive procedure. Tanimura and Yamada found [72] that CMV DNA can also be detected in uterine cervical secretions which is a non - invasive procedure. However, its accuracy is yet not established.

Ultrasonographic findings in the second or third trimesters of pregnancy are helpful to detect possible fetal damage and its extent, but are not diagnostic, as they are observed in less than half of the infected fetuses [52,53,63,73,74]. The viral load in the amniotic fluid often reflects the severity of fetal infection and may be used for predicting the possible degree of fetal damage. Quantitative determination of CMV DNA in the amniotic fluid of at least 1000 genome equivalents gave a 100% certainty of detecting an infected fetus. Higher viral loads with the presence of 100,000 genome equivalents or more, predicted the development of a symptomatic infection [75]. Viral load in maternal blood is also a relatively good predictor. A similar relation with viral load was also described in studies on the quantity of CMV particles in the urine of infants infected in utero with CMV, where infants with high CMV levels were symptomatic, while those with low counts were not [76]. It is, however, unknown why some women transmit the virus to the fetus, while others do not. Mortality is relatively high among neonates with cCMV following primary maternal infection, but rare among infants with cCMV born after maternal non-primary infection, even with a similar degree of neurological damage, as well as in asymptomatic children with primary infection [34]. There seems to be no data on mortality of children with cCMV following treatment or of children born to valacyclovir treated mothers.

9. Reduction of CMV Transfer in Pregnancy

9. a. Prevention of Maternal Infection in Pregnancy

General recommendations for pregnant women regarding CMV infection include practicing good personal hygiene, especially handwashing with soap and water after contact with diapers or oral secretions, particularly with a child who is in day care [26,63]. Women who develop a mononucleosis-like illness during pregnancy should be evaluated for CMV infection and, if positive, counseled about the possible risks in pregnancy. If a woman has primary CMV infection with persistent IgM antibodies and/or virus shedding in the urine, it is advisable to wait with future pregnancies for 3-6 months [26,28]. The time to wait for future pregnancies is in some debate since Fowler et al. [77] have shown that fetuses may be (rarely) infected by CMV even years after maternal primary infection.

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9. b. Prevention by Cytomegalovirus Hyperimmune Globulin (CMV HIG)

CMV HIG is a specialized immunoglobulin preparation enriched with high titers of antibodies against CMV. It is administered to individuals at high risk of CMV infection, such as organ transplant recipients and immunocompromised patients [78]. CMV HIG is generally administered once a month during pregnancy at a dose of 100 IU/kg.

CMV HIG acts primarily by providing passive immunity through the transfer of high-titer CMV-specific antibodies that neutralize circulating CMV virions, inhibiting viral replication and dissemination. Additionally, they may enhance immune-mediated clearance of infected cells through mechanisms such as opsonization and antibody-dependent cellular cytotoxicity [79]. CMV HIG is thought to reduce vertical transmission by limiting viral load in maternal circulation and potentially decreasing viral spread across the placenta. However, clinical studies have shown mixed results regarding its effectiveness [80].

Nigro et al. [79] were the first to suggest that HIG is a potential prenatal therapy for cCMV infection. In their non-randomized study in 2005 they demonstrated that treatment with CMV HIG in pregnant women experiencing CMV seroconversion reduced the vertical transmission rate from 40% (19/47) in untreated women to 16% (6/37) in those receiving CMV HIG. Several later studies indeed supported the potential efficacy of CMV HIG in preventing mother-to-fetus transmission of the virus [81–83].

In subsequent studies [84–86], no significant benefit was observed following the administration of CMV HIG regarding the rate of transplacental infection following primary CMV infection. However, there were some indications of a reduction in the severity of cCMV symptoms. In addition, some complications related to the CMV HIG were noted in a few pregnant women.

Due to its uncertain efficacy and potential risks, HIG is currently not recommended as standard treatment during pregnancy. The recent European consensus recommendations [26] advise against routine monthly administration of HIG at a dose of 100 IU/kg. However, they suggest that biweekly administration at a higher dose (200 IU/kg) may be considered in select cases involving very recent primary infections. Further research is needed to determine if some patient subgroups—such as those with high maternal viral loads or particular immune profiles—might benefit from targeted HIG therapy.

9. c. Prevention by Maternal Antiviral Treatment (Valacyclovir)

Acyclovir and valacyclovir are antiviral medications widely used to treat herpes simplex and varicella-zoster infections. Valacyclovir, a prodrug of acyclovir, offers improved bioavailability and more convenient dosing, making it effective in treating herpes zoster and genital herpes [87,88].

Valacyclovir is rapidly converted into its active form-acyclovir after oral absorption in the digestive tract. Once inside a virally infected cell, acyclovir is phosphorylated by viral thymidine kinase into acyclovir triphosphate, its active metabolite. Having minimal effects on human DNA polymerase, it is an effective and well-tolerated antiviral agent. Additionally, over 80% of acyclovir is excreted unchanged in the urine, reducing the risk of cellular toxicity [89–91]. In pregnant women, valacyclovir administration results in significantly increased plasma acyclovir levels compared to direct acyclovir administration, with higher peak concentrations and greater overall daily exposure, enhancing its antiviral efficacy [92]. Clinical data over several decades on valacyclovir in pregnant women have not shown any drug-related increase in the risk of major birth defects.

In recent years, an increasing number of studies have demonstrated the effectiveness of valacyclovir in lowering the rate of fetal cytomegalovirus infection during pregnancy [88,93]. In a randomized, double-blind, placebo-controlled trial, Shahar-Nissan et al. [93] evaluated the effectiveness of valacyclovir (8 g/day) in preventing vertical CMV transmission in pregnant women with primary CMV infection during early pregnancy. Only 11% of amniocenteses in the valacyclovir group were CMV-positive compared to 30% in the placebo group, demonstrating a 71% relative reduction in transmission risk. The effect was more pronounced in first-trimester infections, where

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valacyclovir reduced transmission from 48% in the placebo group to 11%. Treatment was well tolerated, with no significant adverse events reported.

Zammarchi at al [88] similarly found that in 205 pregnant women treated with valacyclovir there was a relative reduction of transplacental transmission by 61%, in comparison to 242 untreated women. Furthermore, symptomatic cCMV infection at birth was decreased by 83%. Similar results were reported by Egloff et al. [94]. Maternal side effects were generally mild, with one case of reversible renal toxicity.

Two recent meta-analyses published in 2023 [94,95], provide additional evidence supporting the effectiveness of valacyclovir in reducing the risk of cCMV infection when administered during pregnancy. Notably, these meta-analyses included overlapping datasets, analyzing similar patient populations based in the same key studies.

Overall, these findings suggest that valacyclovir at high doses of 8 g/day, serves as a promising preventive strategy for cCMV, with the strongest benefit observed when initiated early in pregnancy. Valaciclovir administered to the CMV positive mother was found not only to reduce transplacental passage of the virus, but it also decreased viral load in the infected fetal blood [96,97]. These recent clinical trials demonstrating valacyclovir's effectiveness in reducing CMV transmission should possibly prompt a re-evaluation of global screening policies [98], as indeed suggested by the group of European experts [26]. As ongoing studies continue to explore potential antiviral options to reduce the risk of vertical transmission [95], we have probably reached the time to expand the European recommendations globally.

10. Treatment of Infants with cCMV

Currently, there are two agents which are effective in the treatment of infants with cCMV, Ganciclovir and valganciclovir. Both can also be given orally [32]. Neonatal treatment must be initiated as early as possible [31]. If possible, no later than the first month; initiation later, up to 14-16 weeks, is of partial beneficial effect [31,32]. In one of the first trials, ganciclovir treatment of neonates with symptomatic congenital CMV infection had beneficial effects on hearing, preventing hearing deterioration, which was observed more frequently in nontreated patients [99]. A similar encouraging result, preventing the progression of hearing loss was described by Michaels et al. [100] in nine treated children with congenital CMV. However, no hearing improvement was noticed. Intravenous ganciclovir treatment also had some benefits in neonatal CMV retinitis [101]. As of today, there seems to be no solid evidence of improvement in the neurodevelopmental sequelae. Similarly, there are apparently no proven preventive effects of treatment on children that are not symptomatic at birth [8,23]. The duration of treatment should be no less than 6 weeks. The European expert's consensus recommendations are from 6 weeks up to 6 months [26].

11. Postnatal Cytomegalovirus Transmission via Breast Milk

Unlike cCMV, postnatal CMV (pCMV) infection is generally considered to have minimal clinical significance. Most cases are acquired through breastfeeding—a common and typically benign route of transmission. In full-term infants pCMV infections are usually asymptomatic and are not associated with long-term sequelae such as hearing loss or neurodevelopmental impairment, largely due to sufficient transplacental transfer of maternal antibodies and more robust immune responses. Therefore, routine intervention is generally not recommended for full-term newborns. In contrast, pCMV infection in preterm and low birth weight infants can result in substantial clinical complications, and potential long-term neurodevelopmental impairments [102]. Thus, pCMV transmission via breast milk represents a meaningful clinical concern, particularly in very low birth weight and preterm populations. Multiple recent studies have highlighted both the epidemiological relevance and clinical implications of this transmission pathway.

A comprehensive review by Osterholm and Schleiss [102] explored the pathogenesis, prevention, and clinical consequences of breast milk-acquired CMV infection in premature infants.

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In preterm neonates pCMV can lead to sepsis-like illness, hematologic abnormalities, hepatitis, pneumonitis, and bronchopulmonary dysplasia, particularly those born before 32 weeks' gestation or weighing less than 1500 grams [120]. CMV shedding occurs in up to 90% of breast milk samples from infected mothers, although only around 20% of exposed infants become infected.

Several factors modulate transmission risk, including viral load, mucosal integrity, and maternal antibody levels. While short-term morbidity is well documented, long-term neurodevelopmental outcomes remain uncertain, with studies showing mixed results. Preventive strategies such as short-term milk pasteurization have demonstrated efficacy in reducing viral infectivity while preserving nutritional benefits. However, freezing breast milk appears less effective, and there is no consensus regarding routine antiviral therapy in pCMV.

A systematic review and meta-analysis by Hu et al. [103] assessed the transmission of CMV via breast milk and the effectiveness of various feeding practices in reducing infection among LBW and preterm infants. Analyzing data from 21 studies involving 1,920 infants, the authors reported a CMV infection rate of 19.3% in infants fed untreated breast milk, compared to 13.5% and 9.1% in those receiving frozen or mixed feeding, respectively. CMV-related symptoms and CMV sepsis-like syndrome, were also less frequent in the frozen and mixed feeding groups. Although severe CMV disease was relatively uncommon, the findings underscore the vulnerability of preterm infants and support the adoption of modified milk-handling practices to minimize transmission risks. The study supports the use of frozen breast milk or mixed feeding as a practical approach to reduce CMV transmission in high-risk infants. Several investigators studied various aspects of the effects of CMV positive breast milk on premature infants [104–106]. The main conclusions were that the milk viral load correlates with the rate of pCMV infection, Affected infants had lower birth weight and a higher incidence of maternal chorioamnionitis [104]. Song et al. [117] recommend for routine screening of CMV in the breast milk of mothers who gave birth to a premature infant. Since the rate of shedding into breast milk in seropositive women is about 80%, it is advised not to feed infants with fresh milk if it is infected by CMV but with frozen milk or combined diet as they decrease the rate of CMV transmission to the infant [118].

The described studies highlight the importance of carefully balancing the well-documented benefits of breastfeeding with the potential risks of CMV transmission in high-risk neonatal populations, as preterm and very low birth weight (VLBW) infants remain particularly susceptible to CMV-related complications. Risk-reduction strategies such as breast milk screening, pasteurization, or modified feeding practices may be appropriate in these cases.

12. Vaccines for Prevention of CMV Infection:

To date, no effective vaccine for the prevention of CMV disease is approved for use. However, there are several promising vaccines that are in clinical trials.

12. a: Unsuccessful Vaccines:

A live attenuated vaccine using the Towne 125 strain has been developed about 50 years ago [107]. This vaccine was unable to prevent infection in women of child bearing age exposed to young children shedding CMV [108]. There were many concerns regarding reactivation of residing CMV and fear the vaccine strain may be shed from the cervix and in breast milk or of its carcinogenic potential. A recombinant CMV vaccine based on the envelope glycoprotein gBr has been developed and appeared to be safe and immunogenic in early trials [38]. Vaccines based only on small parts of the virus eliminate the concerns of viral reactivation and oncogenicity but were ineffective. Other non -successful vaccines were Plasmid based DNA vaccines- ASP0113 vaccine [109] and other trials. Following these disappointing results, their development was discontinued. However, the data from these trials underscore the challenges of eliciting protective immunity in immunocompromised populations, especially without strong neutralizing antibody responses or inclusion of the pentameric complex [110]. For a review on the vaccines with unsatisfactory effects, see the systematic recent review by Chiavarini et al. [111].

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12. b. Very Recent Promising Vaccines, Under Clinical Trials:

12. b1. Virus -Like Particle Platform:

A recent Phase 1, first-in-human study by Langley et al. [112] evaluated the safety and immunogenicity of a novel enveloped virus-like particle (eVLP) cytomegalovirus (CMV) vaccine in healthy, CMV-seronegative adults. The vaccine was well tolerated, and most adverse events were mild and transient. Immunogenicity results showed robust, dose- and adjuvant-dependent antibody responses, with the alum-adjuvanted 2.0 μg formulation producing the strongest and most durable immune response. Participants developed neutralizing antibodies targeting fibroblast and epithelial cell entry, key mechanisms in CMV infection. Additionally, a subset of participants developed antibodies against the AD-2 epitope of gB, which has been associated with protective immunity. Antibody levels remained elevated six months after the final dose, highlighting the vaccine's potential to induce durable immunity.

Additional evidence supporting VLP platforms includes ongoing clinical evaluation of a pentamer-displaying VLP vaccine (SPYVLP01) using SpyTag/SpyCatcher technology. Preliminary reports indicate promising immunogenicity and good tolerability in early-phase trials. These newer VLP constructs aim to address the limitations of gB-only formulations by incorporating the pentameric complex, which is essential for epithelial cell neutralization and preventing congenital transmission [113].

Animal model studies have further demonstrated that effective protection against congenital CMV requires antibodies against both gB and the pentamer, with gB-only vaccines providing incomplete protection. These findings emphasize the importance of multivalent VLP designs moving forward [114,115].

12. b2. mRNA Vaccine Platform:

Among the most promising candidates in CMV vaccine development is mRNA-1647, a multivalent mRNA-based vaccine developed by Moderna [116–118]. This vaccine encodes six CMV antigens—glycoprotein B (gB) and the pentameric complex (gH/gL/UL128/UL130/UL131A)—which are essential for viral entry into fibroblasts and epithelial cells, the primary routes of CMV infection and dissemination. In a first-in-human Phase 1 randomized trial, Fierro et al. [117] demonstrated that mRNA-1647 was safe and well tolerated, with no vaccine-related serious adverse events. The vaccine elicited robust dose-dependent immune responses, including high levels of neutralizing antibodies, binding antibodies to both gB and pentamer, and polyfunctional CD4+ and CD8+ T-cell responses. These immune responses were sustained for over a year in both CMV-seronegative and seropositive adults. Hu et al. compared mRNA-1647 to the earlier gB/MF59 subunit vaccine, using systems serology to evaluate functional qualities such as antibody-dependent cellular cytotoxicity (ADCC) and neutralization breadth. mRNA-1647 induced more durable and functionally superior immune responses, including broader neutralization against multiple cell types and stronger ADCC activity—key mechanisms potentially linked to protection against CMV transmission. These trials represent the most advanced stage of CMV vaccine evaluation to date and are expected to provide definitive data on mRNA-1647's potential to prevent maternal primary infection and congenital transmission.

Recent advances in CMV vaccine development—particularly through mRNA and virus-like particle platforms—have shown encouraging safety and immunogenicity profiles, reigniting optimism in the field. While no candidate has yet received regulatory approval, the progress of mRNA-1647 into Phase 3 trials and the promising results from VLP-based formulations represent major milestones. However, as of February 2025, mRNA-1647 has not received regulatory approval, and its administration in pregnancy has not been studied.

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13. Routine CMV Screening During Pregnancy and in Neonates: Current Evidence and Recommendations

13. a. Screening in Pregnancy:

Since primary and non-primary CMV infections in the mother often lack clinical symptoms, there is a possibility that infection during pregnancy will ensue unnoticed. However, until recently it was not accepted to carry out routine serological studies for CMV antibodies in pregnancy, not only because economic reasons but also because it was sometimes difficult to define clearly between primary and non-primary infection and there was nothing to offer mothers with positive tests except the possibility to diagnose fetal infection using invasive diagnostic means [8]. The Panel of European experts on CMV [68] supported, even as late as 2020, against routine CMV screening in pregnancy because they are not cost-effective and "there are no vaccines to prevent infection, no efficacious and safe therapies available for the treatment of maternal or fetal infection" However, as in the last few years there seem to be effective pharmacological means to reduce viral transmission to the fetus, brought the panel of European experts in 2024 to advocate for CMV screening in the first trimester of pregnancy and treating pregnant women with primary CMV infection with valaciclovir to reduce CMV transmission to the fetus by 70% [26].

Collinet et al. [119] categorized CMV screening strategies into primary, secondary, and tertiary prevention frameworks. While they recognized the potential of routine prenatal serological screening in reducing severe sequelae, the authors cautioned against associated risks, including increased anxiety, overuse of invasive diagnostics, and potential for unnecessary pregnancy terminations. They suggested that universal neonatal screening and integration with hearing screening programs might serve as more practical tertiary prevention strategies, emphasizing the need for accurate epidemiological data and well-defined clinical pathways.

Several recent review articles and meta-analyses have examined and debated the question of whether routine screening for CMV should be implemented during pregnancy and in the neonatal period, addressing the rationale, benefits, and potential limitations of such approaches for both, pregnant women and newborns.

A systematic review by Xie et al. [120] examined 11 international clinical guidelines and two consensus statements, revealing that universal maternal CMV screening is still not routinely practiced due to insufficient data and ethical concerns. However, some guidelines support targeted screening for high-risk women, particularly those exposed to young children. The authors call for urgent updates of clinical guidelines, in light of recent therapeutic advances and the growing evidence base.

The consensus recommendations of the European experts [26] address almost all facets of CMV infection in pregnancy and emphasize two important recent findings: 1. The highest damage of primary CMV infection is in the first trimester of pregnancy and 2. Antiviral treatment with valacyclovir reduces vertical CMV transmission by about 70%. Based on these findings, the authors recommend that pregnant women should be screened for primary CMV infection in the first trimester of pregnancy starting as early in pregnancy, and, if negative, should continue at monthly intervals until weeks 14-16 of gestation. They also recommend carrying out IgG avidity testing to be able to differentiate between recent and less recent primary CMV infection. They additionally recommend that all pregnant women with proven primary CMV infection be treated daily with 8 grams/day of valacyclovir.

See Tables 3 and 4 for recommendations of screening.

13. b. Screening of Newborn Infants:

A comprehensive review by Chiopris et al.[23] highlights significant diagnostic challenges, including the absence of standardized prenatal screening protocols, the limited sensitivity of existing diagnostic tools, and the high prevalence of asymptomatic infections at birth. While the authors do not support universal screening of pregnant women, they recommend neonatal screening using PCR

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testing of blood, urine, or saliva in infants with suspected cCMV infection, given that routine clinical examinations and newborn hearing screening often fail to identify infected cases. They support their view by the long-term benefits for hearing and neurodevelopmental outcomes by early postnatal treatment with oral valganciclovir. However, effectiveness of treatment for asymptomatic cases remains unproven and carries risks of hematologic and potential long-term toxicities.

Lantos et al. [121] present a novel geographically weighted cost-effectiveness analysis evaluating universal newborn screening for cCMV. Using data from over 96,000 infants across seven U.S. metropolitan areas. Universal screening reduced severe-to-profound hearing loss and resulted in higher overall cost savings per infant screened. They claim that relying solely on hearing-targeted neonatal screening leads to underdiagnosis, particularly among asymptomatic infants who are still at risk for late-onset sequelae. Cost-effectiveness arguments in favor of universal screening hold true even after accounting for geographic variation in prevalence and healthcare access, positioning it as the optimal strategy to reduce CMV-related morbidity and improve long-term child health outcomes

Most recently, Schleiss and Blázquez-Gamero [122] provided a comprehensive, policy-oriented review of universal newborn CMV screening proposing it as the emerging standard of care as they seem to be more effective and cost-efficient than hearing-evaluation targeted approaches. Moreover, they emphasize the importance of early identification and treatment to support longitudinal audiological and developmental care. However, they also caution against overmedicalization, overtreatment, and psychosocial stress on families, particularly in cases of clinically inapparent infection. They also call for dual maternal-newborn screening models.

In summary, recent evidence highlights the growing importance of early detection strategies for congenital CMV, with increasing support for universal newborn screening due to its clinical benefits, cost-effectiveness, and potential to reduce health disparities. In addition, routine maternal screening in the first trimester of pregnancy is advocated as well. These recommendations are valid as long as an efficient and safe immunization is yet unavailable.

14. Conclusions

Cytomegalovirus (CMV) is the most teratogenic human virus known today, as it may affect the developing embryo and fetus following primary and non-primary maternal infection. Often the damage continues to increase in the early postnatal years affecting different neurological functions such as hearing, vision and intellectual ability. Due to lack of typical clinical findings, both maternal infection and cCMV of the newborn infant often pass unnoticed, making prevention or reduction of damage very difficult. Since both Valaciclovir and valganciclovir are effective in reducing the viral load, thus reducing transplacental passage of the virus or the emerging neurological sequelae to the newborn infant, we probably reached the time when attempts should be made to routinely screen in the first trimester of pregnancy for maternal primary CMV infection and carry out newborn screening to detect all CMV positive infants, including those that manifest only few or no clinical symptoms at birth. This seems to be preferable to screening only those that fail the neonatal hearing screening. This should probably be the policy as long as there is no effective immunization.

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Abbreviations

The following abbreviations are used in this manuscript:

CMV	Cytomegalovirus
cCMV	Congenital cytomegalic virus
pCMV	Postnatal Cytomegalovirus
IUGR	Intrauterine Growth Restriction
HIG	Hyperimmune Globulin
ASD	Autism Spectrum Disorder
VLBW	Very Low Birth Weight
eVLP	Enveloped Virus-Like Particle
ADCC	Antibody-Dependent Cellular Cytotoxicity

References

1. Taechowisan, T., et al., *Immune status in congenital infections by TORCH agents in pregnant Thais.* Asian Pac J Allergy Immunol, 1997. **15**(2): p. 93-7.
2. Gargouri, J., et al., *Prevalence of anti-CMV antibodies in blood donors in the Sfax region (value in blood transfusion.* French]. Tunis Med, 2000. **78**: p. 512-7.
3. Pultoo, A., et al., *Seroprevalence of cytomegalovirus infection in Mauritian volunteer blood donors.* In dian J Med Sci, 2001. **55**: p. 73-8.
4. Barbi, M., et al., *A wider role of cytomegalovirus infection in sensorineural hearing loss.* Pediatr Infect Dis J, 2003. **22**: p. 39-42.
5. Pultoo, A., et al., *Detec tion of cytomegalovirus in urine of hearing impaired and men tally retarded children by PCR and cell culture.* J Commun Dis, 2000. **32**: p. 101-8.
6. de Jong, M.D., et al., *Summary of the international consensus symposium on management of HIV, CMV and hepatitis virus infections.* Antiviral Res, 1998. **37**(1): p. 1-16.
7. Raxonable, R.R. and C.V. Paya, *Herpesvirus infections in trans plant recipients: current challenges in the clinical management of cytomegalovirus and Epstein–Barr virus infections.* Herpes, 2003. **10**: p. 60-5.
8. Ornoy, A. and O. Diav-Citrin, *Fetal effects of primary and secondary cytomegalovirus infection in pregnancy.* Reprod Toxicol, 2006. **21**(4): p. 399-409.
9. Gaytant, M.A., et al., *Congenital cytomegalovirus infections: review of the epidemiology and outcome.* Obstet Gynecol Surv, 2002. **57**: p. 245-56.
10. Alford, C.A., et al., *Congenital and peri natal cytomegalovirus infections.* Rev Infect Dis, 1990. **12**(Suppl. 7): p. 745-51.
11. Daniel, Y., et al., *Congenital cytomegalovirus infection.* Eur J Obstet Gynecol Reprod Biol, 1995. **63**: p. 7-16.
12. Kotton, C.N. and N. Kamar, *New Insights on CMV Management in Solid Organ Transplant Patients: Prevention, Treatment, and Management of Resistant/Refractory Disease.* Infect Dis Ther, 2023. **12**(2): p. 333-342.
13. Hagay, Z.J., et al., *Congenital cytomegalovirus infection: a long-standing problem still seeking a solution.* Am J Obstet Gynecol, 1996. **174**: p. 241-5.
14. Vollmer, B., et al., *Postnatally acquired cytomegalovirus infection* via *breast mill: effects on hearing and development in preterm infants.* Pediatr Infect Dis J, 2004. **23**: p. 322-7.
15. Chan, E.S., et al., *Maternal Cytomegalovirus (CMV) Serology: The Diagnostic Limitations of CMV IgM and IgG Avidity in Detecting Congenital CMV Infection.* Pediatr Dev Pathol, 2024. **27**(6): p. 530-544.
16. Boppana, S.B., et al., *Symp tomatic congenital cytomegalovirus infection in infants born to mothers with preexisting immunity to cytomegalovirus.* Pediatrics, 1999. **104**: p. 55-60.
17. Boppana, S.B., et al., *Intraute ine transmission of cytomegalovirus to infants of women with pre conceptional immunity.* N Engl J Med, 2001. **344**: p. 1366-71.
18. Lazzarotto, T., et al., *Prenatal indicators of congenital cytomegalovirus infection.* J Pediatr, 2000. **137**: p. 90-5.
19. Ssentongo, P., et al., *Congenital Cytomegalovirus Infection Burden and Epidemiologic Risk Factors in Countries With Universal Screening: A Systematic Review and Meta-analysis.* JAMA Netw Open, 2021. **4**(8): p. e2120736.
20. Preece, P.M., et al., *Congenital cytomegalovirus infection: predisposing maternal factors.* J Epidemiol Community Health, 1986. **40**: p. 205-9.

 doi:10.20944/preprints202507.1510.v1

21. Fowler, K.B., S. Stagno, and R.F. Pass, *Maternal age and congenital cytomegalovirus infection: screening of two diverse newborn popu lations.* J Infect Dis, 1993. **168**: p. 552-6.
22. Rahav, G., et al., *Primary versus nonprimary cytomegalovirus infection during pregnancy, Israel.* Emerg Infect Dis, 2007. **13**(11): p. 1791-3.
23. Chiopris, G., et al., *Congenital Cytomegalovirus Infection: Update on Diagnosis and Treatment.* Microorganisms, 2020. **8**(10): p. 1516.
24. Raynor, B.D., *Cytomegalovirus infection in pregnancy*, in *Semin Peri natol ;17:394–402*. 1993.
25. Revello, M.G. and G. Gerna, *Diagnosis and management of human cytomegalovirus infection in the mother, fetus, and newborn infant.* Clin Microbiol Rev, 2002. **15**: p. 680-715.
26. Leruez-Ville, M., et al., *Consensus recommendation for prenatal, neonatal and postnatal management of congenital cytomegalovirus infection from the European congenital infection initiative (ECCI).* Lancet Reg Health Eur, 2024. **40**: p. 100892.
27. Chandrasekaran, P., et al., *Prenatal and postnatal antiviral therapies for the prevention and treatment of congenital cytomegalovirus infections.* Curr Opin Infect Dis, 2024. **37**(6): p. 494-505.
28. Chatzakis, C., et al., *Timing of primary maternal cytomegalovirus infection and rates of vertical transmission and fetal consequences.* Am J Obstet Gynecol, 2020. **223**(6): p. 870-883 e11.
29. Scaramuzzino, F., et al., *Secondary cytomegalovirus infections: How much do we still not know? Comparison of children with symptomatic congenital cytomegalovirus born to mothers with primary and secondary infection.* Front Pediatr, 2022. **10**: p. 885926.
30. Benshushan, A., et al., *Early re current CMV infection with severe outcome to the fetus.* Acta Obstet Gynecol Scand, 1998. **77**: p. 694-5.
31. Salome, S., et al., *Can viral load predict a symptomatic congenital CMV infection? A systematic review and meta-analysis.* Eur J Pediatr, 2025. **184**(2): p. 188.
32. Fowler, K.B., S. Stagno, and R.F. Pass, *Maternal immunity and prevention of congenital cytomegalovirus infection.* JAMA, 2003. **289**(1008): p. 11.
33. Rousseau, T., et al., *Severe fatal cytomegalic inclusion disease after docu mented maternal reactivation of cytomegalovirus infection during pregnancy.* Prenat Diagn, 2000. **20**: p. 333-6.
34. Gaytant, M.A., et al., *Congenital cytomegalovirus infection after recurrent infection case reports and review of the literature.* Eur J Pediatr, 2003. **162**: p. 248-53.
35. Fowler, K.B., S. Stagno, and R.F. Pass, *The outcome of congenital cytomegalovirus infection in relation to maternal antibody status.* N Engl J Med, 1992. **326**: p. 663-7.
36. Enders, G., et al., *Prenatal diagnosis of congenital cytomegalovirus infection in 189 pregnancies with known outcome.* Prenat Diagn, 2001. **21**: p. 362-77.
37. Nigro, G., et al., *Prenatal diagnosis of fetal cytomegalovirus infec tion after primary or recurrent maternal infection.* Obstet Gynecol, 1999. **94**: p. 909-14.
38. Pass, R.F., *Cytomegalovirus infection.* Pediatr Rev, 2002. **23**: p. 163-70.
39. Yow, M.D., et al., *Epidemiologic characteristics of cytomegalovirus infection in mothers and their infants.* Am J Obstet Gynecol, 1988. **158**: p. 1189-95.
40. Ivarsson, S.A., K. Jonsson, and B. Jonsson, *Birth characteristics and growth pattern in children with congenital cytomegalovirus infec tion.* J Pediatr Endocrinol Metab, 2003. **16**: p. 1233-8.
41. Witters, I., M. Ranst, and J.P. Fryns, *Cytomegalovirus reactivation in pregnancy and subsequent isolated bilateral hearing loss in the infant.* Genet Couns, 2000. **11**: p. 375-8.
42. Teissier, N., et al., *Inner ear lesions in congenital cytomegalovirus infection of human fetuses.* Acta Neuropathol, 2011. **122**(6): p. 763-74.
43. Gabrielli, L., et al., *Congenital cytomegalovirus infection: patterns of fetal brain damage.* Clin Microbiol Infect, 2012. **18**(10): p. E419-27.
44. Gabrielli, L., et al., *Human fetal inner ear involvement in congenital cytomegalovirus infection.* Acta Neuropathol Commun, 2013. **1**: p. 63.
45. Gabrielli, L., et al., *The Auditory Pathway in Congenitally Cytomegalovirus-Infected Human Fetuses.* Int J Mol Sci, 2024. **25**(5).

 doi:10.20944/preprints202507.1510.v1

46. Craeghs, L., et al., *Congenital CMV-Associated Hearing Loss: Can Brain Imaging Predict Hearing Outcome?* Ear Hear, 2021. **42**(2): p. 373-380.
47. Hranilovich, J.A., et al., *Brain Magnetic Resonance Imaging in Congenital Cytomegalovirus With Failed Newborn Hearing Screen.* Pediatr Neurol, 2020. **110**: p. 55-58.
48. Kabani, N., et al., *Urine and Saliva Viral Load in Children with Congenital Cytomegalovirus Infection.* J Pediatric Infect Dis Soc, 2023. **12**(4): p. 230-233.
49. Corazzi, V., et al., *Vestibular and postural impairment in congenital Cytomegalovirus infection.* Int J Pediatr Otorhinolaryngol, 2022. **152**: p. 111005.
50. de Vries, L.S., et al., *The spectrum of cranial ultrasound and magnetic resonance imaging abnormalities in congenital cytomegalovirus infection.* Neuropediatrics, 2004. **35**(2): p. 113-9.
51. Malinger, G., D. Lev, and T. Lerman-Sagie, *Imaging of fetal cytomegalovirus infection.* Fetal Diagn Ther, 2011. **29**(2): p. 117-26.
52. Boppana, S.B., et al., *Neuroradiographic findings in the newborn period and long-term outcome in children with symptomatic cytomegalovirus infection.* Pediatrics, 1997. **99**: p. 409-14.
53. Noyola, D.E., et al., *Houston Congenital CMV Longitudinal Study Group. Early predictors of neurodevelopmental outcome in symptomatic congenital cytomegalovirus infection.* J Pediatr, 2001. **138**: p. 325-31.
54. Damato, E.G. and C.W. Winnen, *Cytomegalovirus infection: perinatal implications.* J Obstet Gynecol Neonatal Nurs, 2002. **31**: p. 86-92.
55. Marsico, C. and D.W. Kimberlin, *Congenital Cytomegalovirus infection: advances and challenges in diagnosis, prevention and treatment.* Ital J Pediatr, 2017. **43**(1): p. 38.
56. Ivarsson, S.A., et al., *Autism as one of several disabilities in two children with congenital cytomegalovirus infection.* Neuropediatrics, 1990. **21**: p. 102-3.
57. Yamashita, Y., et al., *Pos sible association between congenital cytomegalovirus and autistic disorder.* J Autism Dev Disord, 2003. **33**: p. 455-9.
58. Keymeulen, A., et al., *Neurodevelopmental outcome in children with congenital cytomegalovirus infection: A prospective multicenter cohort study.* Early Hum Dev, 2023. **182**: p. 105777.
59. Bartlett, A.W., et al., *Hearing and neurodevelopmental outcomes for children with asymptomatic congenital cytomegalovirus infection: A systematic review.* Rev Med Virol, 2017.
60. Dahle, A.J., et al., *Longitudinal investigation of hearing disorders in children with congenital cytomegalovirus.* J Am Acad Audiol, 2000. **11**: p. 283-90.
61. Smyrli, A., et al., *What are the neurodevelopmental outcomes of children with asymptomatic congenital cytomegalovirus infection at birth? A systematic literature review.* Rev Med Virol, 2024. **34**(4): p. e2555.
62. Nigro, G., et al., *Multi-system cytomegalovirus fetopathy by recurrent infection in pregnant women with hepatitis B.* Prenat Diagn, 1999. **19**: p. 1070-2.
63. Lipitz, S., et al., *Outcome of pregnancies with vertical transmission of primary cytomegalovirus infection.* Obstet Gynecol, 2002. **100**: p. 428-33.
64. Sonoyama, A., et al., *Low IgG avidity and ultrasound fetal abnormality predict congenital cytomegalovirus infection.* J Med Virol, 2012. **84**(12): p. 1928-33.
65. Ebina, Y., et al., *The IgG avidity value for the prediction of congenital cytomegalovirus infection in a prospective cohort study.* J Perinat Med, 2014. **42**(6): p. 755-9.
66. Prince, H.E. and M. Lape-Nixon, *Role of cytomegalovirus (CMV) IgG avidity testing in diagnosing primary CMV infection during pregnancy.* Clin Vaccine Immunol, 2014. **21**(10): p. 1377-84.
67. Sapuan, S., et al., *A systematic review and meta-analysis of the prevalence of human cytomegalovirus shedding in seropositive pregnant women.* Rev Med Virol, 2022. **32**(6): p. e2399.
68. Lazzarotto, T., et al., *Congenital Cytomegalovirus Infection: A Narrative Review of the Issues in Screening and Management From a Panel of European Experts.* Front Pediatr, 2020. **8**: p. 13.
69. Khalil, A., et al., *Congenital Cytomegalovirus Infection: Update on Screening, Diagnosis and Treatment: Scientific Impact Paper No. 56.* BJOG, 2025. **132**(2): p. e42-e52.
70. Arav-Boger, R. and R.F. Pass, *Diagnosis and management of cytomegalovirus infection in the newborn.* Pediatr Ann, 2002. **31**(11): p. 719-25.

 doi:10.20944/preprints202507.1510.v1

71. Diav-Citrin, O., et al., *Cytomegalovirus in pregnancy: primary versus secondary infection: a prospective Cohort study on 208 pregnancies.* Birth Defects Res Clin Mol Teratol. **67**(317).
72. Tanimura, K. and H. Yamada, *Potential Biomarkers for Predicting Congenital Cytomegalovirus Infection.* Int J Mol Sci, 2018. **19**(12).
73. Malinger, G., et al., *Fetal cytomegalovirus infection of the brain: the spectrum of sonographic findings.* Am J Neuroradiol, 2003. **24**: p. 28-32.
74. Sung, C.Y.W., et al., *Cytomegalovirus infection lengthens the cell cycle of granule cell precursors during postnatal cerebellar development.* JCI Insight, 2024. **9**(11).
75. Lazzarotto, T., et al., *Congenital cytomegalovirus infection in twin pregnancies: viral load in the amniotic fluid and pregnancy outcome.* Pediatrics, 2003. **112**: p. 153-7.
76. Stagno, S., et al., *Comparative serial virologic and serologic studies of symptomatic and subclinical congenitally and natally acquired cy tomegalovirus infections.* J Infect Dis, 1975. **132**: p. 568-77.
77. Fowler, K.B., S. Stagno, and R.F. Pass, *Interval between births and risk of congenital cytomegalovirus infection.* Clin Infect Dis, 2004. **38**: p. 1035-7.
78. Planitzer, C.B., et al., *Cytomegalovirus neutralization by hyperimmune and standard intravenous immunoglobulin preparations.* Transplantation, 2011. **92**(3): p. 267-70.
79. Nigro, G., et al., *Passive immunization during pregnancy for congenital cytomegalovirus infection.* N Engl J Med, 2005. **353**(13): p. 1350-62.
80. Nozawa, N., et al., *Cytomegalovirus-specific, high-avidity IgG with neutralizing activity in maternal circulation enriched in the fetal bloodstream.* J Clin Virol, 2009. **46 Suppl 4**(Suppl 4): p. S58-63.
81. Seidel, V., et al., *Fetal and maternal outcome after hyperimmunoglobulin administration for prevention of maternal-fetal transmission of cytomegalovirus during pregnancy: retrospective cohort analysis.* Arch Gynecol Obstet, 2020. **302**(6): p. 1353-1359.
82. Kagan, K.O., et al., *Outcome of pregnancies with recent primary cytomegalovirus infection in first trimester treated with hyperimmunoglobulin: observational study.* Ultrasound Obstet Gynecol, 2021. **57**(4): p. 560-567.
83. Kagan, K.O., et al., *Prevention of maternal-fetal transmission of cytomegalovirus after primary maternal infection in the first trimester by biweekly hyperimmunoglobulin administration.* Ultrasound Obstet Gynecol, 2019. **53**(3): p. 383-389.
84. Blazquez-Gamero, D., et al., *Prevention and treatment of fetal cytomegalovirus infection with cytomegalovirus hyperimmune globulin: a multicenter study in Madrid.* J Matern Fetal Neonatal Med, 2019. **32**(4): p. 617-625.
85. Revello, M.G., et al., *A randomized trial of hyperimmune globulin to prevent congenital cytomegalovirus.* N Engl J Med, 2014. **370**(14): p. 1316-26.
86. Hughes, B.L., et al., *A Trial of Hyperimmune Globulin to Prevent Congenital Cytomegalovirus Infection.* N Engl J Med, 2021. **385**(5): p. 436-444.
87. Wu, J.J., et al., *Valacyclovir in the treatment of herpes simplex, herpes zoster, and other viral infections.* J Cutan Med Surg, 2003. **7**(5): p. 372-81.
88. Zammarchi, L., et al., *Treatment with valacyclovir during pregnancy for prevention of congenital cytomegalovirus infection: a real-life multicenter Italian observational study.* Am J Obstet Gynecol MFM, 2023. **5**(10): p. 101101.
89. Kausar, S., et al., *A review: Mechanism of action of antiviral drugs.* Int J Immunopathol Pharmacol, 2021. **35**: p. 20587384211002621.
90. Bras, A.P., D.S. Sitar, and F.Y. Aoki, *Comparative bioavailability of acyclovir from oral valacyclovir and acyclovir in patients treated for recurrent genital herpes simplex virus infection.* Can J Clin Pharmacol, 2001. **8**(4): p. 207-11.
91. Perry, C.M. and D. Faulds, *Valaciclovir. A review of its antiviral activity, pharmacokinetic properties and therapeutic efficacy in herpesvirus infections.* Drugs, 1996. **52**(5): p. 754-72.
92. Kimberlin, D.F., et al., *Pharmacokinetics of oral valacyclovir and acyclovir in late pregnancy.* Am J Obstet Gynecol, 1998. **179**(4): p. 846-51.
93. Shahar-Nissan, K., et al., *Valaciclovir to prevent vertical transmission of cytomegalovirus after maternal primary infection during pregnancy: a randomised, double-blind, placebo-controlled trial.* Lancet, 2020. **396**(10253): p. 779-785.

 doi:10.20944/preprints202507.1510.v1

94. Egloff, C., et al., *New data on efficacy of valacyclovir in secondary prevention of maternal-fetal transmission of cytomegalovirus.* Ultrasound Obstet Gynecol, 2023. **61**(1): p. 59-66.
95. Rybak-Krzyszkowska, M., et al., *Cytomegalovirus Infection in Pregnancy Prevention and Treatment Options: A Systematic Review and Meta-Analysis.* Viruses, 2023. **15**(11).
96. Jacquemard, F., et al., *Maternal administration of valaciclovir in symptomatic intrauterine cytomegalovirus infection.* BJOG, 2007. **114**(9): p. 1113-21.
97. Leruez-Ville, M. and Y. Ville, *Fetal cytomegalovirus infection.* Best Pract Res Clin Obstet Gynaecol, 2017. **38**: p. 97-107.
98. Roberts, S.L., et al., *Screening policies for cytomegalovirus in pregnancy in the era of antivirals.* Lancet, 2022. **400**(10351): p. 489-490.
99. Kimberlin, D.W., et al., *National Institute of Allergy and Infectious Dis eases Collaborative Antiviral Study Group. Effect of ganciclovir therapy on hearing in symptomatic congenital cytomegalovirus dis ease involving the central nervous system: a randomized, controlled trial.* J Pediatr, 2003. **143**: p. 16-25.
100. Michaels, M.G., et al., *Treatment of children with congenital cytomegalovirus infection with gan ciclovir.* Pediatr Infect Dis J, 2003. **22**: p. 504-9.
101. Barampouti, F., M. Rajan, and W. Aclimandos, *Should active CMV re tinitis in non-immunocompromised newborn babies be treated?* Br J Ophthalmol, 2002. **86**: p. 248-9.
102. Osterholm, E.A. and M.R. Schleiss, *Impact of breast milk-acquired cytomegalovirus infection in premature infants: Pathogenesis, prevention, and clinical consequences?* Rev Med Virol, 2020. **30**(6): p. 1-11.
103. Hu, X., et al., *Transmission of cytomegalovirus* via *breast milk in low birth weight and premature infants: a systematic review and meta-analysis.* BMC Pediatr, 2021. **21**(1): p. 520.
104. Lee, J.E., et al., *Clinical presentation and transmission of postnatal cytomegalovirus infection in preterm infants.* Front Pediatr, 2022. **10**: p. 1022869.
105. Song, J., et al., *Routine breast milk monitoring using automated molecular assay system reduced postnatal CMV infection in preterm infants.* Front Microbiol, 2023. **14**: p. 1257124.
106. Park, H.W., et al., *Incidence of Postnatal CMV Infection among Breastfed Preterm Infants: a Systematic Review and Meta-analysis.* J Korean Med Sci, 2021. **36**(12): p. e84.
107. Plotkin, S.A., et al., *Candi date cytomegalovirus strain for human vaccination.* Infect Immun, 1975. **12**: p. 521-7.
108. Adler, S.P., et al., *Immunity induced by primary human cytomegalovirus infection protects against secondary infection among women of childbearing age.* J Infect Dis, 1995. **171**: p. 26-32.
109. Ljungman, P., et al., *A randomised, placebo-controlled phase 3 study to evaluate the efficacy and safety of ASP0113, a DNA-based CMV vaccine, in seropositive allogeneic haematopoietic cell transplant recipients.* EClinicalMedicine, 2021. **33**: p. 100787.
110. Emery, V.C., *Vaccination against cytomegalovirus: Still at base camp?* Am J Transplant, 2018. **18**(12): p. 2847-2848.
111. Chiavarini, M., et al., *Safety, Immunogenicity, and Efficacy of Cytomegalovirus Vaccines: A Systematic Review of Randomized Controlled Trials.* Vaccines (Basel), 2025. **13**(1).
112. Langley, J.M., et al., *An enveloped virus-like particle alum-adjuvanted cytomegalovirus vaccine is safe and immunogenic: A first-in-humans Canadian Immunization Research Network (CIRN) study.* Vaccine, 2024. **42**(3): p. 713-722.
113. Permar, S.R., M.R. Schleiss, and S.A. Plotkin, *A vaccine against cytomegalovirus: how close are we?* J Clin Invest, 2025. **135**(1).
114. Choi, K.Y., N.S. El-Hamdi, and A. McGregor, *Inclusion of the Viral Pentamer Complex in a Vaccine Design Greatly Improves Protection against Congenital Cytomegalovirus in the Guinea Pig Model.* J Virol, 2019. **93**(22).
115. Valencia, S., et al., *Comparison of vaccination with rhesus CMV (RhCMV) soluble gB with a RhCMV replication-defective virus deleted for MHC class I immune evasion genes in a RhCMV challenge model.* Vaccine, 2019. **37**(2): p. 333-342.
116. Akingbola, A., et al., *The mRNA-1647 vaccine: A promising step toward the prevention of cytomegalovirus infection (CMV).* Hum Vaccin Immunother, 2025. **21**(1): p. 2450045.
117. Fierro, C., et al., *Safety and Immunogenicity of a Messenger RNA-Based Cytomegalovirus Vaccine in Healthy Adults: Results From a Phase 1 Randomized Clinical Trial.* J Infect Dis, 2024. **230**(3): p. e668-e678.

 doi:10.20944/preprints202507.1510.v1

118. Hu, X., et al., *Human Cytomegalovirus mRNA-1647 Vaccine Candidate Elicits Potent and Broad Neutralization and Higher Antibody-Dependent Cellular Cytotoxicity Responses Than the gB/MF59 Vaccine.* J Infect Dis, 2024. **230**(2): p. 455-466.
119. Collinet, P., et al., *Routine CMV screening during pregnancy.* Eur J Obstet Gynecol Reprod Biol, 2004. **114**: p. 3-11.
120. Xie, M., et al., *Serological screening for cytomegalovirus during pregnancy: A systematic review of clinical practice guidelines and consensus statements.* Prenatal Diagnosis, 2023. **43**(7): p. 959-967.
121. Lantos, P.M., et al., *A Geographically Weighted Cost-effectiveness Analysis of Newborn Cytomegalovirus Screening.* Open Forum Infectious Diseases, 2024. **11**(6): p. ofae311.
122. Schleiss, M.R. and D. Blázquez-Gamero, *Universal newborn screening for congenital cytomegalovirus infection.* The Lancet. Child & Adolescent Health, 2025. **9**(1): p. 57-70.