Cytomegalovirus infection in pregnancy: review of the literature

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Summary

The aim of this review is to summarize the principles of cytomegalovirus (CMV) infection in pregnancy. In particular, the aim of this review is to evaluate:
1) Incidence and mother-to-child transmission
2) The value of screening of pregnant women
3) Diagnosis of CMV maternal infection
4) Diagnosis of fetal infection (evaluate the value of ultrasound examination and amniocentesis and evaluate whether the amniotic viral load of mothers with primary cytomegalovirus infection correlate with fetal or neonatal outcomes)
5) Diagnosis of infection in newborns
6) Therapy in pregnancy, postnatal therapy and prevention

Key Words: prenatal diagnosis, congenital infection, cytomegalovirus, viral load, amniotic fluid.

Recommendations

The quality of evidence reported in this document has been assessed using the evaluation of evidence criteria in the Report of the Canadian Task Force on Preventive Health Care (SOGC CLINICAL PRACTICE GUIDELINE; No 240, April 2010).

I: Evidence obtained from at least one properly randomized controlled trial
II-1: Evidence from well-designed controlled trials without randomization
II-2: Evidence from well-designed cohort (prospective or retrospective) or case–control studies, preferably from more than one centre or research group
II-3: Evidence obtained from comparisons between times or places with or without the intervention. Dramatic results in uncontrolled experiments (such as the results of treatment with penicillin in the 1940s) could also be included in this category
III: Opinions of respected authorities, based on clinical experience, descriptive studies, or reports of expert committees
A. There is good evidence to recommend the clinical preventive action
B. There is fair evidence to recommend the clinical preventive action
C. The existing evidence is conflicting and does not allow to make a recommendation for or against use of the clinical preventive action; however, other factors may influence decision-making
D. There is fair evidence to recommend against the clinical preventive action
E. There is good evidence to recommend against the clinical preventive action
L. There is insufficient evidence (in quantity or quality) to make a recommendation; however, other factors may influence decision-making.

Introduction

Cytomegalovirus is the most common cause of intrauterine infection, and is a common cause of sensorineural hearing loss and mental retardation.
Primary infection is defined as CMV infection in a previously seronegative person.
Secondary infection is defined as intermittent excretion of the virus in the presence of host immunity and may be due to either reactivation of an endogenous virus (1) or exposure to a new virus strain from an exogenous source.
Recently two CMV vaccines are being evaluated, screening programs and interventions have been studied, and more is known about the mechanism of transplacental virus spread and the natural history of congenital infection.
Although the diagnosis of congenital CMV infection is still complex, important goals have been achieved in recent years, among which are: tests to determine the avidity index of anti-CMV IgG, allowing the diagnosis of a primary CMV infection and innovative and traditional virological tests to detect the virus in amniotic fluid.
Here, we review recent developments in our understanding

of the diagnosis, treatment, and prevention of congenital CMV infection.

Incidence, mother-to-child transmission

The congenital cytomegalovirus (CMV) infection in the developed countries occurs with an incidence between 0.3% and 2.4% of all live births (1,2).
Mother-to-child transmission is mainly the result of primary maternal CMV infection which carries a risk of transmission varying from 24% to 75% (mean value 40%) (1-4).
Cases of CMV transmission due to non- primary infection have been reported in 1-2.2% of cases (3,4). Nevertheless, increasing evidence shows that the outcome of non-primary maternal infection may be symptomatic and severe (5,6). Recently, the possibility that recurrences and unfavourable outcome might be related to reinfection by a new viral strain has been suggested (7).
Ten to fifteen percent of congenitally infected infants will have symptoms at birth including intrauterine growth restriction, microcephaly, hepatosplenomegaly, petechiae, jaundice, chorioretinitis, thrombocytopenia, and anemia, and 20% to 30% of them will die, mostly of disseminated intravascular coagulation, hepatic dysfunction, or bacterial superinfection (8,9).
Most of the congenitally infected infants (85-90%) have no signs or symptoms at birth, but 5% to 15% of them will develop sequelae such as sensorineural hearing loss, delay of psychomotor development, and visual impairment (7,10).

The value of screening

The value of screening for fetal CMV infection is still controversial (11-13).
The screening may help in the prevention of congenital infections and seronegative pregnant women could be given basic information on how to avoid sources of infection and the possibility of a prenatal diagnosis could be offered to those who acquire infection (12,13).
Naessens et al. evaluated a screening program for CMV in which serological testing was performed at the first prenatal visit; they showed that such screening allows the detection of 82% of all congenital CMV infections (14).
Nevertheless, routine screening of pregnant women for CMV by serology testing is currently not recommended (III-B).
Serologic testing for CMV may be considered for women who develop influenza-like illness during pregnancy or following detection of sonographic findings suggestive of CMV infection (III-B).
Seronegative health care and child care workers may be offered serologic monitoring during pregnancy. Monitoring may also be considered for seronegative pregnant women who have a young child in day care (III-B).

Diagnosis of CMV maternal infection

CMV is the largest of the human herpesvirus family: its transmission occurs by close contact, thought contamination from urine, saliva, blood, semen, and cervical secretions (15). Vertical infection can occur antenatally though the placenta, during delivery though cervical secretions and blood and postnatally though breastfeeding.
Most CMV infections encountered in pregnant women are asymptomatic even during the acute stage. Less than 5% of pregnant women with primary infection are reported to be symptomatic, and an even smaller percentage suffer from a mononucleosis syndrome (16).
Most frequent symptoms include malaise, persistent fever, myalgia, cervical lymphadenopathy, and, less commonly, pneumonia and hepatitis (17).
Laboratory tests may sometimes disclose atypical lymphocytosis and slightly raised transaminase levels.
Laboratory tests (virology and serology) are the best means of establishing diagnosis. The diagnosis of primary CMV infection is straightforward if seroconversion to CMV is detected. However, since documentation of CMV seroconversion is rare, as women are not routinely screened for CMV antibodies prior to gestation, the detection of CMV IgM has been used as a marker of active or recent CMV infection. Different kits can be used; agreement varies from 56% to 75% with a sensitivity between 30% and 88% (18).
When anti-CMV IgM antibodies are detected in a pregnant woman, the diagnosis remains open, because they cannot always be correlated to primary infection. Infact pregnant women can produce IgM during reactivations or reinfections (18). In addition, anti-CMV IgM antibodies have been detected in some pregnant women from six to nine months after the end of the acute phase of primary infection (19), and false positive results are common (18,20) and may arise in subjects with other viral infections (B19Virus, Epstein Barr Virus, etc).
The anti-CMV IgG avidity test is currently the most reliable procedure to identify primary infection in pregnant women (18,21-23). The IgG avidity test is highly specific (100%) and sensitive (94.3%).
The degree of antibody avidity increases progressively and slowly reflecting the maturation of the immune response.
Low avidity indices indicate low avidity IgG antibodies in serum caused by acute or recent primary CMV infection (18). Low avidity indices are encountered 18-20 weeks after the onset of symptoms in immunocompetent subjects.
The determination of anti-CMV IgG avidity, performed before the 16th-18th week of pregnancy, identifies all women who will have an infected fetus/newborn (sensitivity 100%). After 20th weeks' gestation, sensitivity is drastically reduced (62.5%) (24).
A high avidity index during the first 12-16 weeks of gestation could be considered as a good indicator of past infection.
The presence of true IgM combined with low/moderate avidity index has the same diagnostic value as seroconversion (20,23,25).
Virological tests play a secondary role in the diagnosis of primary CMV infection in pregnant women. Virus isolation in urine and/or cervical secretions is a poor indicator of the risk of intrauterine transmission and the severity of fetal/neonatal damage.
CMV can be detected in blood by virus isolation and/or the search for viral components by the antigenaemia tests and poly- merase chain reaction (PCR). Nevertheless, the

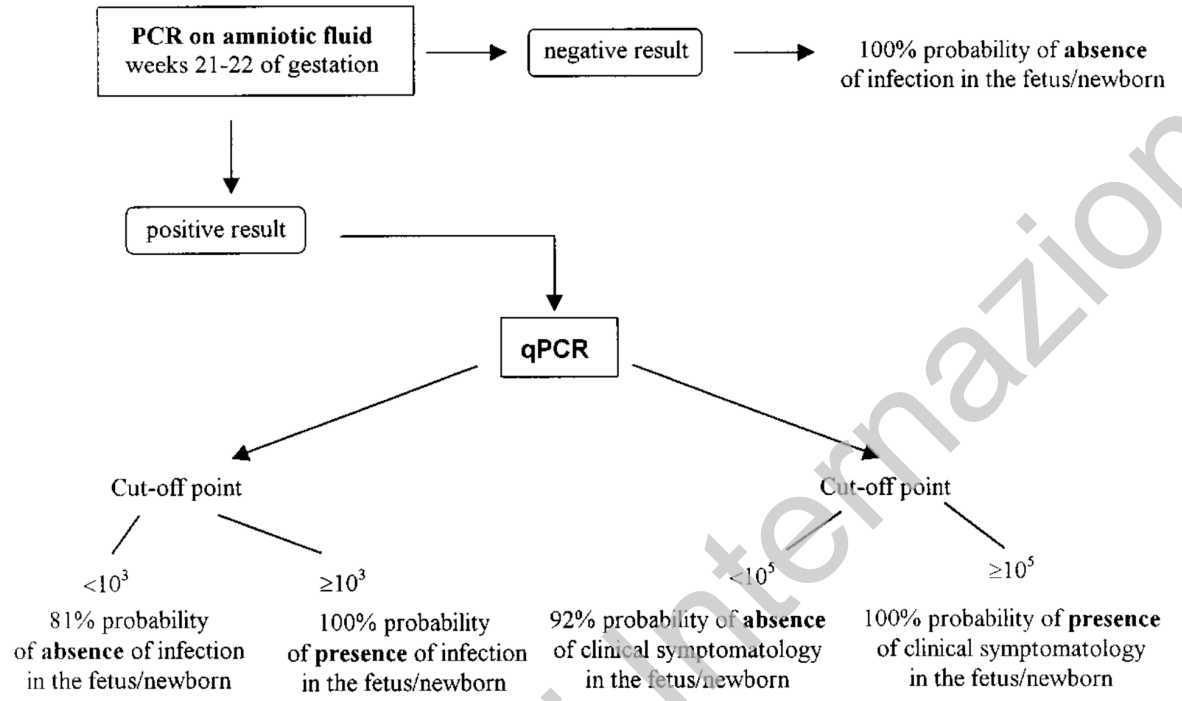
results of these diagnostic tests also fail to correlate with either the clinical course of infection and/or the risk of intrauterine transmission and the severity of fetal/neonatal injury (20,26). Both antigenaemia and PCR tests had a low sensitivity (14.3% and 47.6%, respectively) for detecting vertical CMV transmission in a group of pregnant women who acquired primary CMV infection between four and 30-week-gestation. Specificity and positive and negative prediction rates were also poor (20).
These findings suggest that CMV may or may not be detected in maternal blood in pregnant women undergoing primary infection at the time of diagnosis. Positive viral detection is not associated with a greater risk of infection and/or fetal/neonatal injury (20).
In conclusion diagnosis of primary maternal cytomegalovirus (CMV) infection in pregnancy should be based on de-novo appearance of virus-specific IgG in the serum of a pregnant woman who was previously seronegative, or on detection of specific IgM antibody associated with low IgG avidity (II-2A).
The diagnosis of secondary infection should be based on a significant increase of IgG antibody titre with or without the presence of IgM and high IgG avidity.

Diagnosis of fetal infection

The first step in the prenatal diagnosis of congenital CMV infection is determination of maternal primary and secondary infection by serological testing. In women with proven CMV infection, the second step is to identify fetal infection (10) by non-invasive (ultrasound examination) and invasive (amniocentesis) prenatal tests.
Ultrasound has the advantage of not being invasive and will disclose any structural and/or growth abnormalities caused by CMV infection, but its sensitivity is poor and it correctly identifies no more than 5% of infected babies (11).
Ultrasonographic examinations detect only severely affected fetuses showing obvious ultrasonographic anomalies, and more subtle features are likely to be missed. Although a normal result of fetal anatomic survey can provide some reassurance for patients at risk for fetal infection, it cannot predict a normal outcome.
The most frequently reported sonographic findings of fetal CMV infection include (27-29):
- fetal growth restriction
- cerebral ventriculomegaly
- ascites
- intracranial calcifications
- abnormality of amniotic fluid volume (usually oligohydramnios)
- microcephaly
- hyperechogenic bowel
- hydrops fetalis
- pleural effusion
- liver calcifications

Following a diagnosis of fetal CMV infection, serial ultrasound examinations should be performed every 2 to 4 weeks to detect sonographic abnormalities, which may aid in determining the prognosis of the fetus, although it is important to be aware that the absence of sonographic findings does not guarantee a normal outcome (II-2B).
Fetal MRI may improve the prognostic evaluation, especially when brain abnormalities are detected by ultrasound. However, the role of fetal MRI in providing useful information in fetuses with CMV still needs to be determined (30,31).
Because of its high sensitivity and specificity, CMV isolation from amniotic fluid has been recognized as the gold standard for prenatal diagnosis of fetal CMV infection (26,32-37).
Given the high risk of mother-fetus transmission and fetal damage, prenatal diagnosis is recommended to women with primary and undefined CMV infection contracted in the first half of pregnancy and in case of fetal abnormalities suggestive of infection (10,38).
In cases of proven secondary infection, amniocentesis may be considered, but the risk-benefit ratio is different because of the low transmission rate (III-C) (17,39).
The prenatal diagnosis of fetal CMV infection should be based on amniocentesis, which should be done at least 7 weeks after presumed time of maternal infection and after 21 weeks of gestation. This interval is important because it takes 5 to 7 weeks following fetal infection and subsequent replication of the virus in the kidney for a detectable quantity of the virus to be secreted to the amniotic fluid (II-2A).
It has been repeatedly reported that prenatal diagnosis procedures performed too close to the onset of maternal infection carry a substantial risk of false negative results (34,40-41).
The diagnosis of fetal CMV infection should be based on the results of culture and PCR testing of amniotic fluid samples. CMV isolation can be done by conventional culture on fibroblasts or by the shell vial technique, which uses monoclonal antibodies to the major immediate early protein p72 and enables detection of the virus 16 to 24 hours after amniotic fluid collection (42-44).
Diagnosis of fetal infection by testing for fetal IgM is not recommended not only because of the risk associated with cordocentesis but also because many fetuses infected by CMV do not develop specific IgM until late in pregnancy, resulting in poor sensitivity.(34,45).
The risk of CMV transmission during antenatal diagnostic procedures performed in the presence of maternal DNAemia does not seem to be major, although it cannot be excluded.
The amniotic fluid is subjected to direct search for CMV virus in culture and for the viral genome by PCR.
Viral isolation from the amniotic fluid is indicative of congenital infection, but the procedure is not sensitive (70-80%). False negative results are partly due to transporting and maintaining the amniotic fluid in optimal conditions, as the viral particles must be infective to be detected in culture.
The qualitative search for CMV DNA in amniotic fluid has a good sensitivity (90-98%) and specificity (92-98%) (26,34,38,36,46).
If both techniques are negative, fetal infection can be ruled out with a high degree of certainty.
If results are positive, investigation is completed by DNA quantification by quantitative PCR (10,38,47).
Quantitative determination of CMV DNA in the amniotic fluid may assist in predicting the fetal outcome (Tab 1). There is a low risk of symptomatic infection in the presence of viral loads $<10^3$ copies/mL (10,37,38,47). Low viral loads in the may be a good indicator for ruling out fetal damage at birth and/or development of sequelae like hearing loss and/or delayed psychomotor development.

Table 1 - Flow chart for prenatal diagnosis of congenital CMV infection. qPCR, Quantitative PCR (modified from Guerra B, Lazzarotto T, Quarta S, Lanari M, Bovicelli L, Nicolosi A, et al. Prenatal diagnosis of symptomatic congenital cytomegalovirus infection. Am J Obstet Gynecol 2000;183:476-82).



A quantitative PCR count of $\geq 10^3$ copies/mL of amniotic fluid is a certain sign of congenital infection and predict mother child infection with 100% probability. This is an important finding, because a positive prenatal diagnosis may greatly influence the mother's decision regarding whether to terminate the pregnancy.
A finding of $\geq 10^5$ GE/mL of amniotic fluid discriminates fetuses that will have symptoms, whereas a value of $<10^5$ GE/mL can exclude symptomatic infection with an acceptable margin of error.
Negative results of invasive prenatal diagnosis can rule out CMV infection in almost 100% of cases. This discourages parents from seeking pregnancy termination on the grounds of primary infection with high risk of mother–fetus transmission. Reassuring results are also obtained when minimal amounts or traces of the virus are found in the amniotic fluid since the newborns are infected but asymptomatic at birth and subsequent follow-up checks.
In conclusion, the quantitative determination of CMV DNA in the amniotic fluid may assist in predicting the fetal outcome (II3B), and a good performance of diagnostic and confirmatory tests and correct interpretation and communication of test results to pregnant women may significantly reduce the rate of unnecessary abortions (48).

Diagnosis of infection in newborns

The gold standard for the diagnosis of congenital CMV infection in newborns remains viral isolation in the urine and/or saliva within the first 2-3 weeks of life. Detection of specific IgM in neonatal serum also discloses congenital infection, but IgM antibodies are only present in 70% of infected babies (49).
After 2-3 weeks of life, virological and serological tests will no longer distinguish pre from perinatal CMV infection and the diagnosis of congenital infection can only be suspected on clinical grounds. The determination of DNA in blood by PCR at birth seems to be as sensitive and specific as recovery from urine for diagnosis of congenital CMV infection (37,50,51).
If urine is positive for viral isolation, various clinical, laboratory and instrumental findings are monitored in the infected babies for subsequent weeks and the newborns are classified as symptomatic or asymptomatic (52). If viral isolation is negative, the baby is considered uninfected and no further tests are warranted.

Possible symptom in the infected neonates:
- central nervous system abnormalities (intracranial calcifications, ventriculomegaly, microcephaly);
- prematurity (<38 weeks' gestation),
- small size for gestational age,
- petechiae,
- jaundice,
- hepatosplenomegaly,
- purpura,

Possible laboratory findings:
- high alanine aminotransferase levels (>80 U/L),
- thrombocytopenia (<100,000 cells/mm^3),
- conjugated hyperbilirubinemia (>2 mg/dL).

All infected babies undergo follow-up monitoring at 1,3, 6 and 12 months of life and thereafter annually until school

age. Monitoring includes physical, neurological and anthropometric evaluation; neurodevelopmental evaluation; auditory brainstem responses; *fundus oculi*; blood sampling for laboratory tests (complete blood count, platelet count, transaminase level, bilirubin levels-direct and indirect); and urine sampling for virus isolation.
Interesting findings recently emerged from viral genome research using PCR on blood adsorbed on Guthrie cards, collected at birth for neonatal screening for metabolic and hereditary diseases. This test seems to be a sensitive and specific test for late diagnosis of congenital CMV infection in cases of strong clinical suspicion and for neonatal screening of congenital CMV infection (53).

Consideration about the therapy in pregnancy, postnatal therapy and prevention

Despite advances in the diagnosis of fetal CMV infection, there is no effective therapy.
Some promising data exist on the use of CMV immunoglobulin(HIG) in women with proven CMV infection (54).
The immunoglobulin probably acts by reducing placental inflammation, neutralizing virus with hight avidity antibodies, and perhaps by reducing cytochine mediated cellular immune responses (55).
In this multicentre prospective cohort study (54) of 157 pregnant women with confirmed primary CMV infection evaluated the use of CMV-specific hyperimmune globulin for the treatment and prevention of fetal CMV infection.
The therapy group comprised women whose amniotic fluid contained either CMV or CMV DNA and who were offered intravenous CMV hyperimmune globulin at a dose of 200 U per kilogram of maternal weight. A prevention group, consisting of women with a recent primary infection before 21 weeks' gestation or who declined amniocentesis, was offered monthly hyperimmune globulin (100 U per kilogram intravenously).
Forty-five women had a primary infection more than 6 weeks before enrolment, underwent amniocentesis, and had CMV detected in the amniotic fluid. Thirtyone of these women elected to receive intravenous treatment with CMV-hyperimmune globulin. Fourteen women declined treatment with hyperimmune globulin, and 7 of them had infants who were symptomatic at delivery. In contrast, only 1 of the 31 treated women had an infant with clinical CMV disease at birth although 15 of them were carrying fetuses with ultrasonographic evidence ofCMVdisease. In the prevention group, 37 received hyperimmune globulin, 6 (16%) of whom had infants with congenital CMV infection, compared with 19 of 47 women (40%) who did not receive hyperimmune globulin. No adverse effects of hyperimmune globulin were observed.
Off-label use of HIG during pregnancy should be considered, particularly if there is sonographic evidence of fetal injury, as a possible alternative to pregnancy termination (56).
HIG should be considered when there is a serologically confirmed primary CMV infection after conception and the maternal IgG avidity to CMV is low or amniotic fluid contains CMV or CMV DNA. If no amniocentesis is done and the maternal IgG avidity is low, monthly HIG infusion until delivery should be considered (55). If maternal IgG avidity to CMV>50%, HIG treatment should be unnecessary(54).
Pregnancies with confirmed fetal CMV infection could be treat with oral Valaciclovir (8g/day). Maternal oral administration of Valaciclovir leads to therapeutic concentrations in the maternal and fetal compartments, with a decrease in viral load in the fetal blood (57).
Recent case report have focused on the safe administration of oral Ganciclovir to mathers of CMV-infected fetus (58-60), but the actual efficacy of Ganciclovir remains to be defined in controlled trials.
In conclusion HIG, Valaciclovir and Ganciclovir could be valid treatment, but randomised controlled trials to study these options further is indicated.
Regarding postnatal therapy, there is some evidence suggesting a limited beneficial role for Ganciclovir treatment of neonates with symptomatic congenital CMV infection. A few studies have demonstrated some hearing improvement and less hearing deterioration in infants treated with Ganciclovir (61,62). Potential adverse effect of ganciclovir in neonates includes transiet neutropenia, which may necessitate dose adjustment or interruption of terapy (63-64).
Until an effective vaccine is available, recommendations for seronegative pregnant women with respect to CMV-infection include practising good personal hygiene such as avoiding intimate contact with salivary secretions and urine from young children and careful hand washing after changing diapers and wiping secretions (61). Despite our assumption that changing protective behaviours prevents child to mother transmission of CMV during pregnancy, Adler et al. did not show any benefit for such intervention (68).
However, their data also demonstrated that intervention is more effective during pregnancy than before pregnancy, because pregnant women are more motivated to adhere to recommendations than non-pregnant women (62).

Conclusion

Cytomegalovirus infection is the most prevalent congenital infection in the world and is the leading infectious cause of mental retardation and sensorineural deafness.
Currently, routine serologic testing of all pregnant women is not recommended, and use of serologic testing should be used only in pregnant women who develop influenza-like illness or following detection of sonographic findings suggestive of CMV infection.
Once primary maternal CMV infection has been diagnosed, fetal infection can be accurately determined by amniocentesis.
Prenatal counselling in case of fetal infection is difficult because of our limited ability to predict outcome.
Quantitative PCR determination of amniotic fluid viral load should predict both the infectious and the clinical outcomes of maternal CMV infection in fetuses and neonates. These findings might help clinicians to counsel pregnant women infected by CMV about the likely outcome for the offspring and enable the women themselves to decide the future of the pregnancy on a more informed basis.
HIG, Valaciclovir and Ganciclovir could be valid treatment, but randomised controlled trials to study these options further is indicated.

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