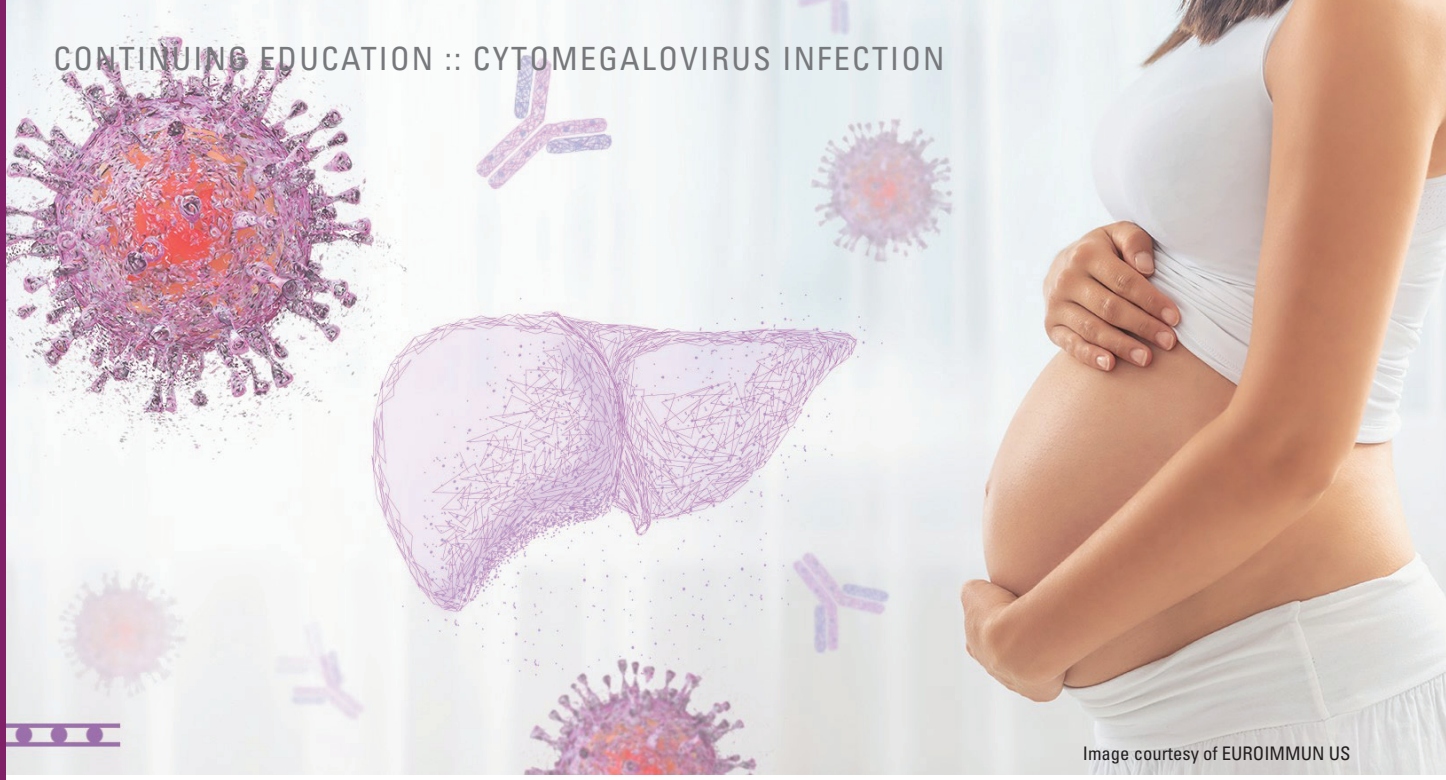

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Cytomegalovirus infection: When and why to detect antibodies

By Ilana Heckler, PhD and Maite Sabalza, PhD

Cytomegalovirus (CMV) is a beta-herpesvirus that causes viral inclusion bodies and enlarges infected cells. It is the largest herpesvirus known to infect humans, with a seroprevalence of 60–90% worldwide.[1,2] Higher prevalence occurs in lower socioeconomic groups in developing countries.[2] In the United States, nearly one-third of children have CMV by age of five, and more than half of adults have it by the age of forty.[3]

CMV is transmitted from person to person by direct contact. The virus is shed in body fluids — with main transmission via saliva and urine of young children to other children or adults. Other forms of transmission include sexual contact, blood transfusions, and organ transplants. In healthy individuals, CMV infection is often asymptomatic, but it may be fatal in immunocompromised patients.[4] Symptoms of CMV in mild cases are described as flu-like and include fever, sore throat, fatigue, and swollen glands. More serious cases, such as those occurring in people with weakened immune systems, exhibit symptoms affecting the eyes, lungs, liver, esophagus, stomach, and intestines.

Primary infection occurs in those who have never been infected before. As with other herpes viruses, CMV remains latent in the host after the first infection and may reactivate at a later period. Reinfection occurs when a person is infected with a different viral strain.

CMV is the leading viral cause of congenital defects. CMV can cross the placenta and infect the fetus after primary infection, reactivation, or reinfection of the mother. The transmission is most likely in women with a primary CMV infection, and the risk of transmission increases throughout the third trimester.[5,6] Infection occurs in 0.5% to 2.5% of neonates, and most babies with symptoms at birth (5%) have long-term effects including sensorineural hearing loss, microcephaly, chorioretinitis, and motor disabilities.[7] A large percentage of asymptomatic newborns (15%) subsequently suffer impairments, most often hearing loss.[7,8] Furthermore, CMV infection is the most prevalent and dangerous opportunistic infection following solid organ transplantation (SOT) or hematopoietic stem cell transplantation (SCT) and in HIV patients.[2,9] CMV infection has also been linked with atherosclerosis, glioblastoma, and other diseases.[10,11]

There is no vaccine available to prevent CMV infection but there are antiviral drugs to treat immunocompromised individuals. Antiviral medication may improve hearing and developmental outcomes in infants with congenital cytomega-

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LEARNING OBJECTIVES

Upon completion of this article, the reader will be able to:

1. Discuss the viral family, prevalence, and transmission route of cytomegalovirus (CMV).
2. List the vulnerable populations and complications of CMV transmission.
3. Describe CMV testing currently used in prenatal, fetal, and newborn testing.
4. Discuss future suggestions for the screening of newborns for CMV and its utility worldwide.

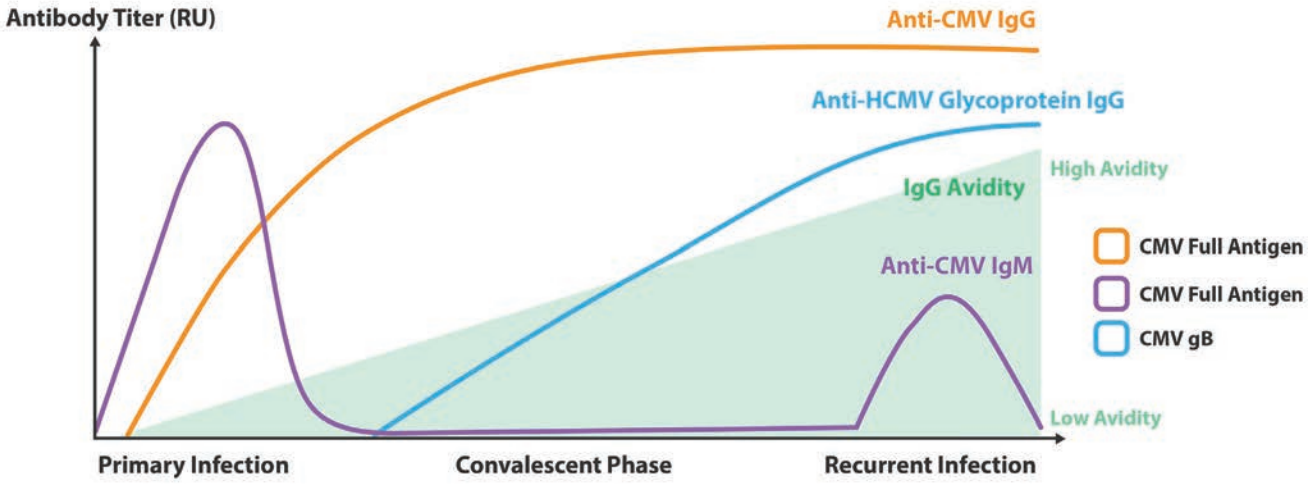

Figure 1: Antibody kinetics in CMV infection.

Serology in adults and infants older than 12 months

Initial infection leads to the production of CMV-specific immunoglobulin M (IgM) antibodies that persist in the blood for a short period of time and later to the production of IgG antibodies that can persist forever **(Figure 1)**.[13,14] The main challenge with IgM antibody detection is that some individuals can have persistent IgM levels for over a year.[15] IgM can be also detected in reactivation or reinfection. Therefore, the detection of IgM does not confirm an active primary infection. Furthermore, IgM antibodies are not highly specific and false positive results may occur.[16] A primary CMV infection can be distinguished from a past infection by measuring immunoglobulin G (IgG) seroconversion (follow-up collection samples required) and/or IgG avidity. IgG avidity tests evaluate the binding strength of IgG antibodies to the virus. Low-binding strength (low avidity) IgG antibodies are produced in response to initial CMV infection, and over the course of 2–4 months, develop into high binding strength (high avidity) **(Figure 1)**.[17] Therefore, high avidity IgG would indicate a past infection while low avidity IgG would indicate a primary infection. Due to the potential complications of CMV infection, particularly in pregnant women, is important to distinguish between primary infection, past infection, reactivation, and reinfection.

CMV testing in pregnancy

In the United States, routine screening for CMV infection during pregnancy is not recommended by the Centers for Disease infection after maternal primary infection. These estimates are based on data from the MFMU Network Randomized Trial to Prevent Congenital Cytomegalovirus.[23] This CMV calculator predicts cCMV infection in the context of primary maternal CMV infection and no ultrasonographic indications of congenital infection. Having this information may aid in patient counseling and decision making.[24] This calculator considers the results of an IgM antibody test, IgG avidity, and presence or absence of virus in maternal plasma.

Serological assays using glycoprotein B (gB) as an antigen for IgG antibody detection in pregnant women are included in some guidelines for screening pregnant women in Europe.[25-27] In general, the IgG antibody response to CMV gB is delayed by up to 100 days **(Figure 1; Table 1)**.[28] Therefore, IgG antibodies against gB indicate a past infection and a recent or primary infection can be excluded. These results are comparable to the finding of high avidity IgG antibodies. However, only 82% of CMV-infected individuals produce IgG antibodies against gB.[29] Consequently, a negative result can be a false-negative. Therefore, it is highly recommended to look at a combination of IgM, IgG seroconversion, IgG avidity, and antibodies against gB.

Fetal population testing

When an active infection is detected in a pregnant woman, the next step is to check fetal infection. There are two prenatal tests that can be used: non-invasive (ultrasound examination) and invasive (amniocentesis). CMV isolation from amniotic fluid

Detection parameter	Information gained	Primary infection detection	Discrimination between active primary vs. reactivation/reinfection
IgM	Active infection Past infection: Persistant IgM is possible.	Not possible IgM can be detected during primary, recurrent, or reinfection	Not possible IgM can be detecetd during primary, recurrent, or reinfection
IgG	Past infection: Indicates past CMV infection but does not indicate when infection occurred.	Possible IgG seroconversion	Possible Past infection by detecting IgG antibodies against late stage markers (anti-gB IgG) (only 82% of the population)
IgG avidity	Primary infection: Low avidity IgG antibodies Past infection: High avidity IgG antibodies	Possible Detected low avidity IgG antibodies	Possible High vs. low IgG avidity antibodies

Table 1: Serological testing in CMV infection.

Population	Molecular testing	Serology			
		IgM	IgG	Seroconversion	Avidity
Pregnant woman	Detection of active infection	The combination of all serology parameters will aid in determining if the infection is primary, secondary, or reinfection.			
	Not possible to differentiate primary infection from reactivation/reinfection.				
Fetus	Amniocentesis: Detection of active infection	Not relevant			
Newborn	Detection cCMV transmitted by mother within the first 2–3 weeks after birth.	Not relevant			
Transplant donor/recipient	Detection of active infection before and after transplantation	Not relevant	IgG detection important for risk assessment	Not relevant	Not relevant

Table 2: Relevant laboratory testing for different at-risk populations for CMV infection.

(amniocentesis) has been established as the gold standard due to its high sensitivity and specificity. However, amniocentesis has risks for the pregnant woman and fetus.[30] On the other hand, when fetal abnormalities are detected by ultrasound and the pregnant woman has low IgG avidity antibodies, the fetus has a higher risk of being infected. Therefore, the newborn will need to be monitored to confirm or rule out cCMV infection.[22]

Newborn testing

Molecular testing, such as quantitative polymerase chain reaction (qPCR), is the gold standard for cCMV detection in newborns within the first 2–3 weeks of life to distinguish congenital from a postnatal infection acquired during or after delivery **(Tables 1 and 2)**.[22,31] Saliva and urine are the preferred sample types for testing because they contain high viral loads of CMV. However, blood can also be used. The CDC recommends first testing saliva and then confirming positive samples with urine because CMV is also shed in breast milk. Therefore, confirmation with urine will help to rule-out false positives from breast milk.[17]

If the newborn is negative, the baby is considered uninfected, and no further tests are warranted. If a newborn is infected as indicated through a positive result from molecular testing, the newborn will be monitored for hearing loss or other sequela, thus increasing opportunities for early intervention.[32]

Serological testing for newborns within the first 2–3 weeks is not recommended because IgM antibodies are only present in 70% of infected newborns.[16] Additionally, newborn IgG antibodies mainly come from the mother and transfer through the placenta to the fetus.[33] As with molecular testing, serological tests will not distinguish prenatal from perinatal CMV infection after 2–3 weeks of life.[16]

As mentioned above, a large percentage of infected newborns are asymptomatic at birth but develop symptoms later.[7,8] Therefore, it can be helpful to screen newborns at birth. Several studies support the need of neonatal screening to identify earlier infected infants at risk to develop neurological sequelae and provide the appropriate treatment to reduce and treat CMV diseases.[34] In the United States, universal screening is not included in routine newborn screening. The CDC is investigating dried blood spot (DBS) to be used for this purpose.[17] This is important because DBS are collected from all newborns for metabolic screening and sometimes for detection of newborn disorders.[22,35] In fact, there are already commercially available assays to detect cCMV in newborns through DBS.[36] Interestingly, some states have already implemented universal screening. In February 2022, Minnesota become the first state in the nation to screen every newborn for cCMV.[37]

Transplantation population testing

Another population that is at risk of developing complications from CMV infection are recipients of organ or hematopoietic stem cell transplantation.

Direct detection of CMV by molecular testing is suggested for detecting and monitoring current infections in transplant recipients **(Table 2)**. Transplant donors must be also tested for CMV active infection prior to donation.[22,38] On the other hand, serological testing is recommended for transplant donors and recipients to reduce the risk of a primary infection and reactivation.[38,39]

Conclusion

CMV is a common virus that can infect people of all ages. As does herpes virus, CMV remains latent in the human body. Therefore, the virus can be reactivated after primary infection and induce an active infection. Immunocompromised individuals are the main population at risk to develop complications from CMV primary infection, reactivation, and reinfection. These include pregnant women, newborns, and transplant recipients. Depending on the at-risk population, either serology or molecular testing are performed to detect an active infection or differentiate a primary infection from reactivation or reinfection.

A combination of molecular testing and serology provides the most accurate diagnosis of CMV infection. Due to the complications associated with a primary infection in pregnant women, it is important to raise awareness about CMV infection and implement initiatives to reduce the risk of transmitting the virus to the fetus. Furthermore, monitoring newborns is essential for identifying the infection quickly and administering the appropriate treatment. However, in some countries, prenatal or universal newborn screening is not recommended. One of the factors influencing that decision is the cost associated with testing. Nonetheless, it would be interesting to investigate the long-term consequences and costs of not screening these two populations.

Vaccine candidates are now being evaluated in clinical studies.[40] The approval of a vaccine to prevent CMV infection will have a significant impact on the groups at risk. In addition, there may be a shift in the role of serology in terms of monitoring the immune system's response to vaccines. While waiting for a vaccine, those at risk should adopt proper hygiene practices to avoid CMV infection. For instance, frequent handwashing and avoiding touch with another person's saliva, especially avoiding contact with the saliva and urine of small children.

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