Congenital and perinatal cytomegalovirus (CMV): has diagnosis improved in 30 years?

Jonathan Howard, Beverley Hall and William Rawlinson

Virology Division,
Dept of Microbiology,
South Eastern Area Laboratory Services
Prince of Wales Hospital,
Randwick NSW 2031

School of Medical Sciences and
Biotechnology and Biomolecular Sciences,
University of New South Wales
Sydney NSW 2052
Tel (02) 9382 9113
Fax (02) 9398 4275

The implication of a diagnosis of cytomegalovirus (CMV) during pregnancy or in the neonatal period remains uncertain despite our increased understanding of the pathophysiology of the disease. Current tests for CMV include serological tests (usually EIA IgG, IgM, avidity) and nucleic acid testing (NAT). When used together, these tests offer improved reliability in diagnosis of CMV in pregnant women and infants.

Diagnosis in pregnant women

Congenital CMV infections may be the result of either a primary or recurrent infection. Less than 5% of pregnant women with primary CMV infection are reported to be symptomatic [1] and most CMV infections are asymptomatic during the acute stage [2].

There are many diagnostic procedures for the detection of CMV [3, 4]; however, there is no adequate single test. The primary concern to most pregnant women and their medical advisors is a diagnostic test that predictively defines the clinical outcome for the baby.

Virological and serological testing of the mother is necessary to establish a diagnosis. Documented seroconversion of the mother from IgG seronegative to IgG seropositive is the definitive method of determining a primary CMV infection. CMV IgM positivity is used extensively as a marker of active or recent infection, but IgM positivity does not always correlate with primary infection. Indeed, older studies suggest IgM antibody may not be detected until 6-9 months after the acute phase of a primary infection in a small number of women [5]. IgM detected using modern sensitive enzyme immunoassay (EIA) methods may persist for years post-primary infection in a proportion (5-10%) of infected women [6, 7]. During reactivations or reinfections, pregnant women may also test IgM positive and excrete CMV in their urine [6].

Improvements in serological testing include the CMV IgG avidity test. Avidity results (a numerical value) are informative despite lack of standardisation between the various manufacturers of the tests [eg. VIDAS, BioMerieux]. Low, intermediate and high values offer broadly useful information in defining recent (<3 months, low avidity) or past infection (>3 months, high avidity) [6, 8]. However, not all past infections show high IgG avidity and not all recent infections show low avidity. Careful interpretation of avidity testing in conjunction with IgM and NAT testing of peripheral blood may identify primary CMV infection and offer a guide to timing of the infection (Table 1).

The advent of NAT has certainly improved the detection of clinical infectious pathogens and is now increasingly being adopted by diagnostic laboratories. Polymerase chain reaction (PCR) is more sensitive, specific, cost-effective, less laborious and provides accurate and rapid diagnosis compared to conventional culture methods. Some laboratories can now screen clinical samples for CMV in blood, urine, amniotic fluid, newborn screening cards (NBSC) and autopsy materials. In addition, PCR can be adapted to test for various causative agents in one PCR reaction (multiplex PCR) [9].

Prenatal diagnosis

Routine antenatal screening for CMV during pregnancy is not performed in Australia but is performed as a matter of clinical judgment. Fetal abnormalities, if detected on ultrasound, may lead to maternal investigation with subsequent diagnosis of CMV. However, ultrasound is an insensitive method for detecting congenital CMV [10] and this technique has poor sensitivity. It has been claimed that ultrasound detects less than 5% of infected babies [11] and does not detect the subset of infected neonates with sensorineural hearing loss and other subtle late complications of congenital CMV. Nonetheless, ultrasound has the advantages of being non-invasive and can show structural and/or growth abnormalities.

After diagnosis of maternal infection, some pregnant women may desire fetal diagnosis (Table 2). Invasive techniques for diagnosing CMV include cordocentesis and amniocentesis. Cordocentesis is used in some centres for detection of CMV IgM antibody status, CMV PCR, liver enzymes, hematocrit and platelet count; however, analysis of amniotic fluid is probably the most appropriate for prenatal diagnosis [12, 13]. Sampling of amniotic fluid for CMV testing is usually done between 21-22 weeks' gestation [14, 15]. This gestation has been selected as it may take 9 weeks for CMV to be excreted from the fetal kidneys and be detectable in the amniotic fluid.

A positive CMV PCR of the amniotic fluid indicates fetal infection, although the association between infection and disease remains an area of uncertainty. Qualitative analysis of CMV in amniotic fluid is sensitive (92-98%) and specific (90-98%) [16]. Some authors correlate worse outcomes with higher viral load in the amniotic fluid [17]. A negative PCR result, generally an indicator of absence of CMV, may also be a false-negative result if the procedure is performed less than 6-9 weeks after maternal infection and/or before 21 weeks' gestation.

Diagnosis in the newborn

The reference standard for diagnosing congenital CMV infection remains isolation of the virus from urine, saliva or plasma in the first 3 weeks of life. IgM positivity is indicative of congenital infection, but IgM antibodies are present in about 70% of infected babies [18]. Beyond 3 weeks of life, tests can no longer differentiate congenital from perinatal infection.

Infants suspected of CMV beyond the early neonatal period (especially for infants presenting with deafness) may have blood tested for CMV if blood has been routinely collected at birth and dried on a NBSC as part of a newborn screening programme. Retrospective diagnosis of congenital infection in infants presenting with later clinical illness and not to genetic causes is routinely performed by some laboratories [19, 20]. Detection of CMV in blood at birth has been claimed to be as sensitive and specific as detection in urine [21, 22].

NAT is used to detect the presence of CMV in maternal and infant urine, plasma and serum, along with fresh placenta, fresh umbilical cord and paraffin-embedded tissue, using CMV PCR. In addition, using *in situ* PCR, placenta and fetal tissue from CMV PCR positive liveborn and stillborn babies may be

Table 1. Determining time of CMV infection.

CMV IgM	CMV IgG avidity
• May remain positive for >2 years	• If low infection, usually <3 months previous
• Different assays with different sensitivities	• May be high in recent infection (uncommon)
• Some laboratories utilise two assays	• If low in trimester 1 infection, may be pre-pregnancy
• May inverse in recurrent infection	• Retesting in 2-3 weeks may indicate kinetics of antibodies

Table 2. Diagnosis of congenital CMV during pregnancy (fetal infection) and in infants.

Fetal infection	Neonate and older infection
Serology	
CMV IgG pre-pregnancy + pregnancy scan if possible	CMV IgG
IgM	IgM
IgG avidity	IgG avidity
Amniocentesis	
Amniocentesis of >21 weeks' gestational age (CMV viral load in amniotic fluid (high value suggests increased risk fetal infection)	
Molecular	
Maternal peripheral blood (NAT)	Peripheral blood and urine (NAT)
Placenta at birth (NAT)	>21 days old, request testing of NBSC (NAT)

examined. This allows the determination of several factors: the anatomical localisation of CMV in the placenta, addressing cellular tropism *in vivo;* the co-localisation of virus and tissue inflammation, informing if tissue damage is from viral mediated or immune mediated (possibly cytokines); and the sensitivity of histopathology for detecting viral infection. *In situ* PCR is a specialised method that is not available to most pathology labs; however, it is a sensitive method of addressing these issues whilst providing details about viral transcription [23]. These data are significant for further understanding the pathophysiology of CMV, providing correct diagnosis, directing therapies, and improving the prognosis of congenitally ill babies.

Ultimately, further tests and research are required to develop the diagnostic test that predictively defines the outcome of a CMV infection during a woman's pregnancy. In the meantime, use of multiple tests, with careful consideration of the results, allows us to provide useful information to mothers with infection during pregnancy and to parents of infected children.

References

1. Pass, R.F. *et al.* (2006) Congenital cytomegalovirus infection following first trimester maternal infection: symptoms at birth and outcome. *J. Clin. Virol.* 35, 216-220.
2. Munro, S.C. *et al.* (2005) Diagnosis of and screening for cytomegalovirus infection in pregnant women. *J. Clin.Microbiol.* 43, 4713-4718.
3. Rawlinson, W.D. (1999) Diagnosis of human cytomegalovirus infection and disease. *Pathology* 31, 109-115.
4. Trincado, D.E. and Rawlinson, W.D. (2001) Congenital and perinatal infections with cytomegalovirus. *J. Paediatr. Child Health* 37, 187-192.
5. Stagno, S. *et al.* (1986) Primary cytomegalovirus infection in pregnancy. *J. Am. Med. Assoc.* 256, 1904-1908.
6. Lazzarotto, T. *et al.* (1997) Search for cytomegalovirus-specific immunoglobulin M: comparison between a new western blot, conventional western blot, and nine commercially available assays. *Clin. Diag. Lab. Immunol.* 4, 483-486.
7. Lazzarotto, T. *et al.* (2004) Congenital cytomegalovirus infection: recent advances in the diagnosis of maternal infection. *Human Immunol.* 65, 410-415.
8. Munro, S.C. *et al.* (2005) Symptomatic infant characteristics of congenital cytomegalovirus disease in Australia. *J. Paediatr. Child Health* 41, 449-452.
9. McIver, C.J. *et al.* (2005) Development of multiplex PCRs for detection of common viral pathogens and agents of congenital infections. *J. Clin. Microbiol.* 43, 5102-5110.
10. Liesnard, C. *et al.* (2000) Prenatal diagnosis of congenital cytomegalovirus infection: prospective study of 237 pregnancies at risk. *Obstet. Gynecol.* 95, 881-888.
11. Ville, Y. (1998) The megalovirus. *Ultrasound Obstet. Gynecol.* 12, 151-153.
12. Lazzarotto, T. *et al.* (2000) Prenatal indicators of congenital cytomegalovirus infection. *J. Pediatr.* 137, 90-95.
13. Revello, M.G. *et al.* (1999) Quantification of human cytomegalovirus DNA in amniotic fluid of mothers of congenitally infected fetuses. *J. Clin. Microbiol.* 37, 3350-3352.
14. Azam, A.Z. *et al.* (2001) Prenatal diagnosis of congenital cytomegalovirus infection. *Obstet. Gynecol.* 97, 443-448.
15. Gouarin, S., *et al.* (2001) CMV gB genotypes and outcome of vertical transmission: study on dried blood spots of congenitally infected babies. *J. Clin. Virol.* 21, 75-79.
16. Enders, G. *et al.* (2001) Prenatal diagnosis of congenital cytomegalovirus infection in 189 pregnancies with known outcome. *Prenat. Diagn.* 21, 362-377
17. Lanari, M. *et al.* (2006) Neonatal cytomegalovirus blood load and risk of sequelae in symptomatic and asymptomatic congenitally infected newborns. *Pediatr.* 117, e76-e83.
18. Revello, M.G. *et al.* (1999) Diagnostic and prognostic value of human cytomegalovirus load and IgM antibody in blood of congenitally infected newborns. *J. Clin. Virol.* 14, 57-66.
19. Barbi, M. *et al.* (2006) Neonatal screening for congenital cytomegalovirus infection and hearing loss. *J. Clin. Virol.* 35, 206-209.
20. Barbi, M. *et al.* (2000) Cytomegalovirus DNA detection in Guthrie cards: a powerful tool for diagnosing congenital infection. *J. Clin. Virol.* 17, 159-165.
21. Revello, M.G. and Gerna, G. (2002) Diagnosis and management of human cytomegalovirus infection in the mother, fetus and newborn infant. *Clin. Microbiol. Rev.* 15, 680-715.
22. Ross, S.A. and Boppana, S.B. (2005) Congenital cytomegalovirus infection: outcome and diagnosis. *Sem. Pediatr. Infect. Dis.* 16, 44-49.
23. Trincado, D.E. *et al.* (2005) Highly sensitive detection and localization of maternally acquired human cytomegalovirus in placental tissue by in situ polymerase chain reaction. *J. Infect. Dis.* 192, 650-657.

Dr Jonathan Howard is a postdoctoral research officer at the Virology Division at SEALS and a conjoint lecturer at UNSW. He has worked on stillbirth and congenital infections for over 2 years. His interests are in the detection of pathogens associated with stillbirth and congenital infections.

Beverley Hall is a registered nurse and midwife and works as a research nurse for the congenital infection study at the Virology Division at SEALS. Her interests are maternal, infant and fetal health.

Professor William D Rawlinson – See Bio on page 172.