Congenital and Other Related Infectious
Diseases of the Newborn
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Diagnosis of Maternal and Congenital Cytomegalovirus Infection

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Introduction

Human cytomegalovirus (CMV) is one of the eight viruses belonging to the *Herpesviridae* family to infect humans. CMV belongs to the *Betaherpesvirinae* subfamily of viruses characterized by a restricted host spectrum, *in vitro* replication in fibroblasts of the natural host species *in vivo*, a slow replication cycle, the induction of intranuclear and intracytoplasmic inclusions and the ability to induce latency mainly in the myeloid cell linage.

Although rarely pathogenic in immunocompetent individuals, the virus poses a significant health threat to immunocompromised individuals. CMV is an important post-transplant pathogen as between 50% and 100% allograft recipients (depending on serological status of donor/recipient and type of immunosuppression) develop CMV infection.

Congenital CMV infection is the leading cause of congenital virus infection in developed countries, occurring in 0.3–2.4% (mean value 1%) of all live births depending on the seroprevalence of the population examined.

Intrauterine primary infections are second only to Down's syndrome as a known cause of mental retardation.

The virus

CMV virions are pleomorphic and measure between 150 and 300 nm in diameter. The CMV genome is made up of a double-stranded DNA and it is the largest of all known human viruses. It contains over 235 kilobase pairs, and has a potential

protein encoding content of approximately 200 open reading frames (ORFs) (Chee et al., 1990).

Sequential genome expression is temporally regulated in productive infection. The first phase of transcription is commonly called the "immediate-early" phase, and immediately follows entry of the virus into the host cell. Immediate-early antigens accumulate in the nucleus. These antigens are non-structural viral proteins, i.e. not part of the virion itself, but instead exert important regulatory functions in the switch to the early phase of CMV infection by transactivating early gene promoters (Mocarski, 2001).

The early phase of CMV infection is defined as occurring before the onset of viral DNA synthesis. This phase starts about 2–4 h after infection, and proceeds until about 24–48 h after infection when viral DNA synthesis starts. About 75% of the viral genome is transcribed during the early phase of infection, and many structural and non-structural proteins are produced. Furthermore, cell rounding occurs during this phase. Finally, late genes are expressed at high levels after DNA replication. They mostly encode structural viral antigens such as the viral capsid proteins, the matrix or tegument proteins, and the envelope glycoproteins. During the late period of infection, more than 90% of the CMV genome is transcribed. This period extends from 24 to 48 h after infection until cell death occurs, and it is the phase in which cytopathology proceeds towards cell lysis, infectious virus is produced and mature virions appear in the culture supernatant (Stinksi, 1999; Mocarski, 2001; Murphy et al., 2003).

Epidemiology

CMV infection is endemic and ubiquitous and not subject to seasonal fluctuations. During their lives, from 40% to 80% of individuals in the industrialized countries and almost all those in the developing world will be infected by CMV. The seroprevalence of CMV infection increases with age in every group that has been studied (Ho, 1990; Britt et al., 1996).

Human beings are the only reservoir for human CMV. The virus is transmitted by direct contact and only indirectly in rare cases. Because of viral fragility towards environmental factors, close contact is required for horizontal propagation of infection.

Sources of infection include: oropharyngeal secretions, urine, cervical and vaginal secretions, sperm, breast milk, tears, faeces and blood. Propagation is fostered by prolonged elimination of the virus and the fact that most infections produce no symptoms.

The infection is generally acquired during childhood, with an incidence of 30–40% during the first year of life, mainly by breast milk, and subsequently by close personal contact in day-care centers and schools (Pass et al., 1984; Handsfield et al., 1985). Among the adult population, especially fertile women, sexual activity and close daily contact with children play a significant role in the spread of CMV infection (Taber et al., 1985; Pass et a., 1987). Blood products, solid organ and

bone marrow transplant can also transmit active as well as latent CMV (Barbara et al., 1987).

In most cases, CMV infection in normal hosts leads to a clinically inapparent infection (primary and non-primary infection). Although rarely pathogenic in immunocompetent individuals, the virus poses a significant health threat to immunocompromised individuals. Infection can occur by reactivation of latent virus, by reinfection in patients who are already infected (non-primary infection) or by primary infection (Pass, 2001). CMV infection in immunocompromised individuals causes different clinical syndromes in different groups of patients and the severity of the infection parallels the degree of the immunosuppression.

The infants may acquire infection from the mother as a result of intrauterine infection (congenital infection), or through contact with infected genital secretions during passage through the birth canal (perinatal infection) or postpartum through breast feeding (postnatal infection).

The congenital CMV infection in the developed countries occurs with an incidence between 0.3% and 2.4% of all live births (Alford et al., 1990; Peckham, 1991). Only 10–15% of congenitally infected babies present symptoms of infection at birth and these infants have a perinatal mortality rate of around 30% with 70–80% of surviving babies presenting major neurological sequelae (Boppana et al., 1992). Despite infection, 85–90% of babies have no symptoms at birth, but 8–15% of them will suffer delayed injury (Boppana et al., 1992; Fowler et al., 1992).

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%) (Alford et al., 1990; Fowler et al., 1992). Cases of CMV transmission due to non-primary infection have been reported in 1–2.2% of cases, i.e. at a much lower rate than those resulting from primary infection (Fowler et al., 1992). Nevertheless, increasing evidence shows that the outcome of non-primary maternal infection may be symptomatic and severe (Boppana et al., 1999; Gaytant et al., 2003). Recently, the possibility that recurrences and unfavourable outcome might be related to reinfection by a new viral strain has been suggested (Boppana et al., 2001). Congenital CMV infection is strongly dependent on maternal serological status.

Fowler et al. (2003) reported that the older maternal age ($\geqslant$25 years) and gravidity (>2) were associated with decreased risk of congenital CMV infection. The presence of maternal antibody at the previous delivery was highly protective against delivering a future newborn with congenital CMV infection (RR, 0.32; 95% CI, 0.17–0.62).

Since the prevalence of maternal antibody increases rapidly with age in young women, it is likely that a greater proportion of younger women would have been seronegative near the time of their first pregnancy. It has been gauged that about 30–40% of the young population ($<$ 25 years) in northern Italy is seronegative for CMV making the same proportion of fertile women at risk of contracting CMV infection during pregnancy (unpublished data).

Clinical manifestations in symptomatic newborns range from severe multiorgan involvement with jaundice (with high direct bilirubin levels), thrombocytopenic

purpura, hepatomegaly, splenomegaly, pneumonia and encephalitis. Mild clinical manifestations usually include liver problems with hepatosplenomegaly (60% of cases) and thrombocytopenia (53–77% of cases), and around half the babies present delayed intrauterine growth with low birth weight (Ramsay et al., 1991; Boppana et al., 1992). Structural abnormalities mainly affect the central nervous system (ventriculomegaly, intracranial calcifications and cerebral atrophy), whereas other organs are seldom involved. Associated visual impairment and hearing loss have also been reported and CMV has been implicated in non-immunological hydrops (Inoue et al., 2001).

In addition 8–15% of asymptomatic newborns develop long-term sequelae, namely psychomotor delay and hearing loss (Fowler et al., 1997). CMV is the leading non-genetic cause of deafness in children: more than half the babies born with symptomatic infection and 10% of asymptomatic newborns will develop mild-to-severe neurosensory hearing loss which is progressive in 50% of cases (Stagno et al., 1986; Dahle et al., 2000). Hearing loss is bilateral in 50% of cases leading to language impairment and learning delay whose severity is directly proportional to the delay in diagnosis precluding prompt rehabilitation (Kimberlin et al., 2003).

In summary, around 30–40% of congenitally CMV infected newborns will present problems varying in severity at birth and/or throughout life.

Transmission of CMV from mother to foetus occurs with the same frequency throughout all three trimesters of pregnancy (Stagno et al., 1986). More recently, Bodeus reported an overall rate of transmission of 57.5% with maternal seroconversion during pregnancy; however, the transmission rate was lower, 36% with first-trimester infection than with third-trimester infection, 77.6% (Bodeus et al., 1999).

CMV can also be transmitted to the foetus when primary maternal infection occurs before conception. Revello et al. (2002b) showed that preconception primary CMV infection (3 months before the last menstrual period) carries a low risk of intrauterine transmission, one (9.1%) infected newborn out of 12 examined. In the periconceptional CMV infection (4 weeks after the last menstrual period) the virus was transmitted to 4 newborns (30.8%) of 13 pregnancies. The authors conclude that periconceptional primary CMV infection seems to bear a higher risk of unfavourable outcome than preconceptional infection, and counselling should be adjusted accordingly.

The extent of foetal–newborn injury, namely severe brain damage, is correlated to the gestational epoch in which vertical transmission occurs: the most severe is correlated to primary maternal infected contracted in the first 2 months of pregnancy (Stagno et al., 1986). A report by Pass et al. (2006) demonstrated that children with congenital CMV infection following first-trimester maternal infection are more likely to have CNS sequelae, especially sensorineural hearing loss, than are those whose mothers were infected later in pregnancy. However, some degree of CNS impairment can follow even late gestational infection .

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 (Pass et al., 1999). Even in rare cases with symptoms, the manifestations are non-specific and mild such as persistent low fever, muscle ache and gland enlargement. Laboratory tests may sometimes disclose atypical lymphocytosis and slightly raised transaminase levels.

Pathogenesis of congenital infection

Congenital infection is transmitted through the placenta. The virus in maternal leucocytes infects the placenta and replicates until it comes into contact with the foetal circulation (Fisher et al., 2000). *In vitro* studies have clearly demonstrated how CMV productively infects the placental trophoblasts (Halwachs-Baumann et al., 1998; Hemmings et al., 1998). It has also been shown how inflammation triggers the expression of adhesion molecules (namely ICAM-1) on the trophoblast membranes, thereby enhancing the adhesion of maternal blood cells (Xiao et al., 1997).

The placenta acts as a portal of entry for the virus, but it also acts as a barrier because even during maternal primary infection, transmission occurs in only 40% of cases. Twin pregnancies represent an interesting model because different foetuses are simultaneously exposed to the same maternal influences and had a completely different outcome. In our recent studies, we described three cases of CMV-infected twin pregnancies. Only six of seven newborns were infected and three of whom were symptomatic (Lazzarotto et al., 2003; Gabrielli et al., 2003).

During primary infection of the mother, leucocytes carrying infectious virus may transmit CMV infection to trophoblasts and from these cells the virus seems to spread cell-to-cell into the stroma replicating in fibroblasts and then reaching foetal endothelial cells. Recent studies indicate a complete but limited CMV replication in trophoblast cells and a subsequent high virus replication within the stromal cells (Fig. 1). Therefore, even if a low viraemia phase occurs in the mother a congenital infection might occur as a result of virus amplification at placental level (Gabrielli et al., 2001).

After an initial foetal viraemic stage (dissemination stage), the virus can invade and productively replicate in target organs like the central nervous system, liver, inner ear, spinal cord, kidney, duct epithelium and vascular epithelium, etc. In particular, the tubular epithelium within the kidney appears to be a major site of viral replication. Cleared by foetal diuresis into the amniotic fluid (AF), the virus can be newly ingested by the foetus and replicate in the oropharyngeal epithelium giving rise to more extensive dissemination via the blood (Fig. 2).

Diagnosis of maternal infection

Since pregnancy generally does not affect the clinical course of infection, usually symptom-free in immmunocompetent subjects, laboratory tests (virology and serology) are the best means of establishing diagnosis. It is up to the physician to decide on the basis of maternal status prior to conception whether to test for CMV

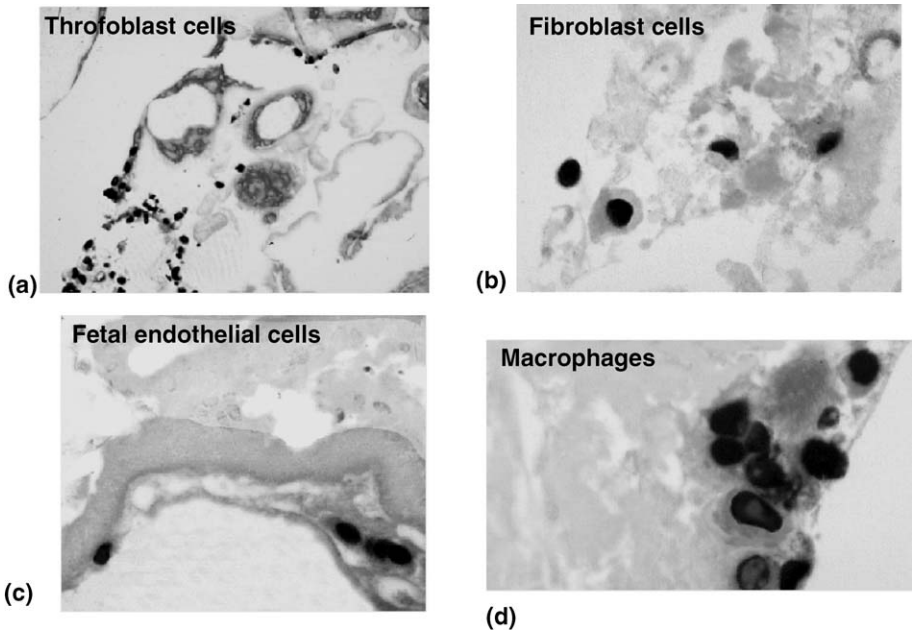

Fig. 1 Immunohistochemical double detection of viral and cellular in CMV-infected placenta explants. (A) In blue, the cytokeratin marker showing the trophoblast layer and in brown CMV-early antigen (× 200); (B) In blue, the vimentin-positive cells showing fibroblast cells and in brown CMV-immediate early antigen (× 400); (C) In blue, the endothelial cell marker and in brown CMV-early antigen (× 400); (D) In blue, the CD68 marker showing macrophages and in brown CMV-early antigen (× 500). From Gabrielli et al. (2001) (for color version: see color section on page 261).

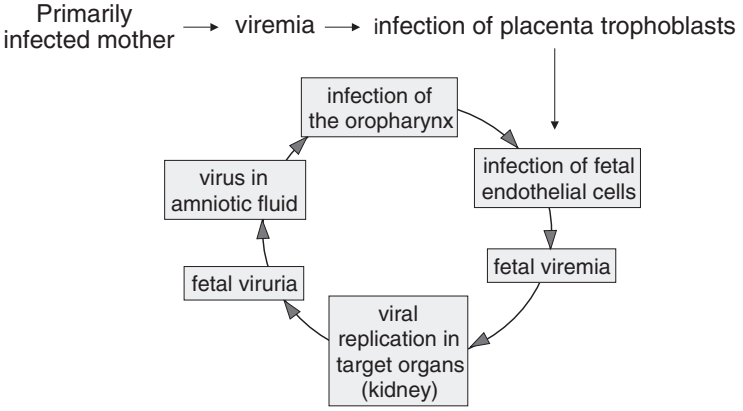

Fig. 2 Transmission of CMV through the placenta barrier and infection of the foetus.

in pregnancy and which tests to prescribe and then to interpret the results accurately.

Women seropositive for CMV before conception

IgG-specific antibodies in serum disclosed at the first test in pregnancy (8–10th week) is indicative of past infection and no further investigations are required. There is tacit agreement among the international community that no laboratory testing for CMV needs to be carried out, unless indicated by particular clinical conditions such as abnormal ultrasonographic findings. Even though it does not offer full protection, acquired immunity will defend the mother from primary infection in pregnancy which carries a much greater risk of foetal damage. Any reinfection or reactivation of infection carries the same risk as pregnancy itself.

For these reasons, the information on maternal status prior to conception precludes the need for screening during pregnancy.

Women seronegative for CMV within the 6 months before conception

Non-immune pregnant women are therefore at risk of acquiring primary infection. First and foremost they must be informed of hygiene and behaviour measures (avoiding direct contact with organic materials, close contact with pre-school children and frequent thorough hand-washing) to reduce the chance of infection (Cannon et al., 2005). A recent study reported that pregnant women who received an intervention involving hygienic practices were significantly less likely to acquire CMV infection than were non-pregnant women (Adler et al., 2004).

Seronegative pregnant women must also undergo periodic serological testing. Although there are no universally accepted guidelines, monthly testing until the 18–20th week of pregnancy is reasonable to implement foetal investigations in case of seroconversion. If the mother continues to be seronegative, serological follow-up testing can be limited or confined to one more test at 35–37 weeks to select newborns at risk of congenital infection in the case of late seroconversion (Guerra et al., 2000).

Women whose pre-pregnancy serological status is unknown

Diagnosis of CMV infection is more complex in women unaware of their serological status before pregnancy. As most infections are asymptomatic, the only way to disclose primary infection is to implement specific testing as early in pregnancy as possible.

Serological findings

Testing for anti-CMV IgM antibodies is the most widely used procedure for screening pregnant women. However, there is currently some concern over the fact

that different commercially available kits frequently yield discordant results, limiting their diagnostic value. Agreement between kits varies from 56% to 75% with a sensitivity between 30% and 88% (Lazzarotto et al., 1997a).

When anti-CMV IgM antibodies are detected in a pregnant woman the diagnosis remains open.

Anti-CMV IgM antibodies are a good indicator of acute or recent infection, but cannot always be correlated to primary infection. Findings indicate that fewer than 10% of IgM-positive women congenitally infect their foetus/newborn (Lazzarotto et al., 2004). This is because pregnant women can produce IgM during reactivations or reinfections (Lazzarotto et al., 1997b). In addition, anti-CMV IgM antibodies have been detected in some pregnant women 6–9 months after the end of the acute phase of primary infection (Stagno et al., 1986) and false-positive results are common (Lazzarotto et al., 1997a, 2004).

Hence, the detection of IgM in the serum of pregnant women may simply be a starting point for further diagnostic investigation.

The anti-CMV IgG avidity test is currently the most reliable procedure to identify primary infection in pregnant women (Grangeot-Keros et al., 1997; Lazzarotto et al., 1997b; Eggers et al., 2000; Mace et al., 2004). Antibody avidity indicates the strength with which a multivalent antibody binds to a multivalent antigen. The antibodies produced during the primary response have a much lower antigen avidity than the antibodies produced during the non-primary response. For this reason, low-avidity antibodies are found after primary antigenic stimulation. 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, whereas high-avidity indices (high-avidity serum IgG) indicate no current or recent primary infection (Lazzarotto et al., 1997b). Low-avidity anti-CMV IgG are found in more than 90% of primary infections in both immunocompetent and immunocompromised subjects, whereas they are never detected in non-primary infection (Lazzarotto et al., 1997b).

Low-avidity indices are encountered 18–20 weeks after the onset of symptoms in immunocompetent subjects. The test is reliable and 100% sensitive before the 16–18th week of pregnancy after which sensitivity is drastically reduced (62.5%) (Lazzarotto et al., 2000b).

Immunoblot is the gold standard test to confirm the presence of IgM antibodies in serum (Lazzarotto et al., 1998). In addition, analysis of the virus-specific IgM response to individual structural and non-structural CMV proteins will disclose fairly typical reactive profiles to distinguish primary from non-primary infection. Moreover, counting the number of bands recognized by IgM present in sera from CMV-infected women, we observed that serum IgM from women who transmit CMV infection reacts with a higher number of bands than does serum IgM from those who do not transmit the infection ($P<0.0001$) (Lazzarotto et al., 1998).

Figure 3 shows some examples of IgM-reactive profiles against viral and recombinant CMV proteins in serum samples from pregnant women IgM-positive and IgM-negative for CMV.

A management scheme for CMV serology in pregnant women is proposed in Fig. 4.

Virological findings

Virological tests play a secondary role in the diagnosis of primary CMV infection in pregnant women. During and after pregnancy CMV is commonly cleared in

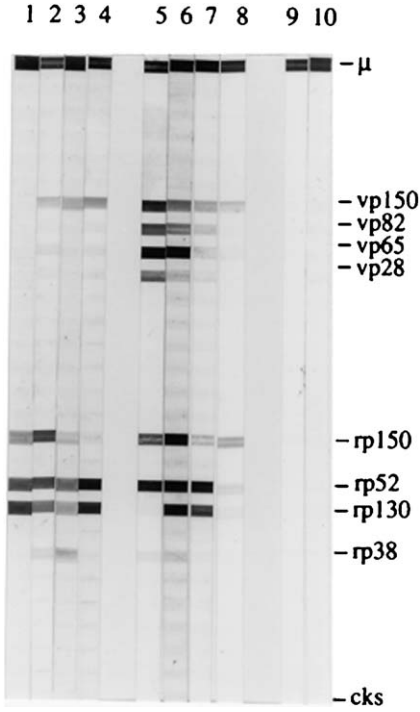

Fig. 3 The new immunoblot. Individual suspensions of each of the four purified viral proteins (vp150, vp82, vp65 and vp28) and the four purified recombinant proteins (rp150, rp52, rp130 and rp38) were deposited onto the nitrocellulose strip. Furthermore, two additional control proteins were added: the CKS protein (negative control) and human μ chain (IgM) (positive control). Representative examples of serum reactivity with the immunoblot. Viral and recombinant proteins are identified on the right. CKS is the negative control, and μ is the IgM heavy chain and represents the positive control. Lanes: 1–8, IgM-positive sera from pregnant women; 9 and 10, IgM-negative sera from pregnant women. Sera 1–4 preferentially reacted with recombinant proteins, while sera 5–8 reacted with both viral and recombinant proteins. Sera 5 and 6 were from pregnant women who transmitted the infection. From Lazzarotto et al. (1998).

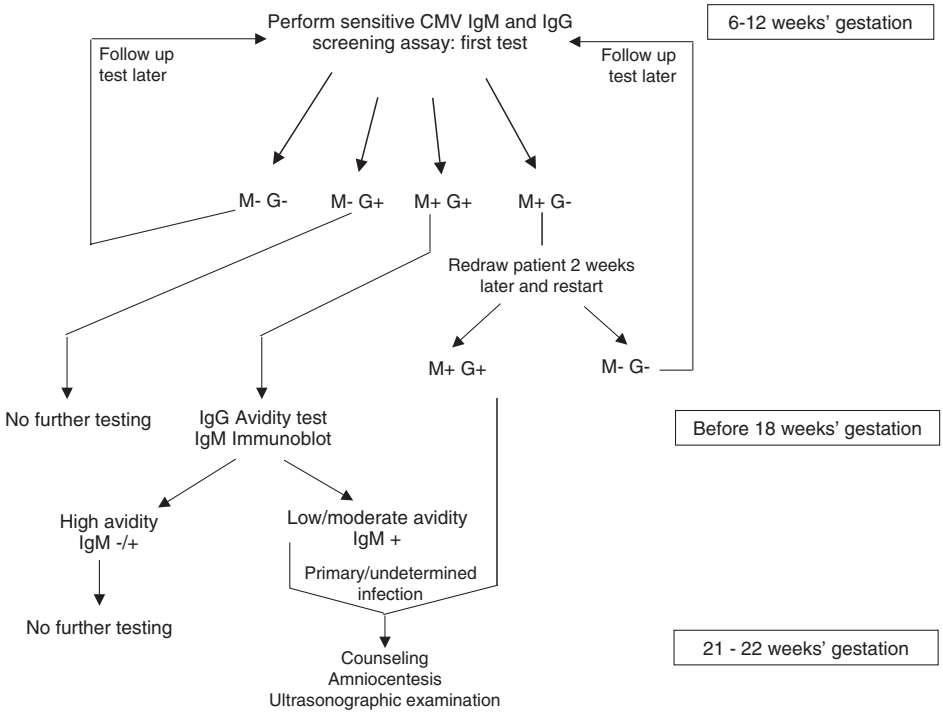

Fig. 4 A management scheme for CMV serology in pregnant women. M, CMV-specific IgM; G, CMV-specific IgG; – negative for antibody; + positive for antibody.

organic secretion so that virus isolation in urine and/or cervical secretions is a poor indicator of the risk of intrauterine transmission and the severity of foetal/neonatal damage. We found low positive prediction rates for congenital CMV infection and foetal injury when the mother shed virus in the saliva and/or urine during the first two trimesters of pregnancy (29.2% and 57.1%, respectively) (Table 1).

The viraemic phase is much shorter in immunocompetent subjects with respect to immunodepressed patients. CMV can be detected in blood by virus isolation and/or the search for viral components by the antigenaemia tests and qualitative and quantitative polymerase chain reaction (PCR). Findings demonstrated that CMV may be found in the blood of pregnant women during acute or recent primary infection (Revello et al., 1998b).

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 foetal/neonatal injury (Lazzarotto et al., 2004), confirming literature reports (Revello et al., 1998b). Both antigenaemia and qualitative PCR tests undertaken in a group of pregnant women infected between 4 and 30 weeks' gestation with primary CMV had a low sensitivity (equal to 14.3% and 47.6%, respectively) with respect to the number of cases of mother–foetus viral transmission.

Table 1

CMV detection in the urine and/or saliva of primarily infected women in relation to congenital CMV infection. Urine samples were obtained from pregnant women during the first and second trimester (range 7–24 weeks' gestation) at the time of diagnosis of primary infection with serologic methods

		CI yes	CI no	Total	Sens (%)	Spec (%)	PPV (%)	NPV (%)
Urine and/or saliva[a]	pos	7	17	24	36.8	75	29.2	80.9
	neg	12	51	63				
Total		19	68	87				

Abbrevations: CI, congenital infection; pos, positive; neg, negative; SENS, sensitivity; SPEC, specificity; PPV and NPV, positive and negative predictive value.
[a]Virus isolation (shell vial).

Specificity and positive and negative prediction rates were also poor (Lazzarotto et al., 2004).

The outcome of studies designed to identify and quantify the viral genome and/or viral components in maternal blood in relation to symptomatic infection in the foetus/newborn also yielded disappointing results from a diagnostic standpoint.

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 foetal/neonatal injury (Lazzarotto et al., 2004).

Immunological findings

Revello et al. (2006) investigated the CMV-specific T cell response in pregnant women and, for comparison, in immunocompetent non-pregnant individuals experiencing primary CMV infection. The authors reported that the cellular immune response to CMV did not differ significantly between pregnant and non-pregnant patients with symptomatic primary CMV infection. In addition, the study indicate that a sustained deficit in the cellular immune response, as evaluated by the lymphoproliferative response analysis, appears to be significantly associated with intrauterine transmission of the virus, confirming literature reports. This work is still at the investigational stage and because exceptions were observed in the study, as well as in previous studies, the authors indicate that, at the moment, the lymphoproliferative response analysis cannot be used to reliably predict intrauterine transmission in individual cases (Revello et al., 2006).

Diagnosis of foetal infection

The foetal compartment can be studied by invasive prenatal diagnostic investigation and ultrasound. The risks linked to invasive testing are counterbalanced by

certain diagnosis of foetal infection. 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 not more than 5% of infected babies (Ville, 1998). In addition, a structural abnormality may be disclosed, a long time, after with initial negative tests and borderline structural changes detected early in pregnancy could be temporary.

In other words, normal ultrasound findings are reassuring for the pregnant woman, but poorly predictive of a normal newborn. Frankly pathological findings (e.g. multiple cerebral calcifications, severe ventriculomegaly, hydrocephalus, hydrothorax, ascites, etc.) carry a definitely unfavourable prognosis. On the other hand, borderline ultrasound findings (e.g. mild ventriculomegaly) can sometimes regress spontaneously and should not have a major effect on counselling.

Invasive prenatal diagnosis is currently established by amniocentesis. Recent studies have demonstrated that the AF is the most appropriate material (Donner et al., 1994; Lipitz et al., 1997; Revello et al., 1998a; Enders et al., 2001; Grangeot-Keros and Cointe, 2001), obviating the need for cordocentesis, an invasive technique with a two-fold higher risk to the foetus (1–2% vs. 0.5–1%) (Weiner, 1988).

Studies of prenatal diagnosis of foetal CMV infection such as detection of virus or virus components in foetal blood were found to be of poor sensitivity to significantly improve prenatal diagnosis of intrauterine transmission of the virus. The statistical analysis of the data of different test for diagnosis of congenital infection on foetal blood showed that the sensitivity of antigenaemia was 57.9%; of viraemia, 55.5%; and of leukoDNAemia, 82.3% (Revello et al., 1999b).

Tests performed on foetal blood may provide prognostic information, in particularly when performed quantitatively. In retrospective studies (Revello et al., 1999b; Enders et al., 2001), it was observed that the presence and the level of CMV-specific IgM in foetal blood identified foetuses with abnormal findings with high probability. Moreover, low CMV load in foetal blood at 20–24 weeks of gestation may have a more favourable outcome (Revello and Gerna, 2002a).

Given the high risk of mother–foetus transmission and foetal damage, prenatal diagnosis is recommended to women with primary and undetermined CMV infection contracted in the first half of pregnancy (documented by antibody seroconversion or advanced serological tests) and in case of foetal abnormalities suggestive of infection (Guerra et al., 2000; Lazzarotto et al., 2000a).

Invasive prenatal diagnosis

Amniocentesis entails sampling the AF under ultrasound control and is undertaken exclusively between the 21st and 22nd weeks of gestation. This period has been chosen for the following reasons: (1) CMV is a slow replication virus and 6–9 weeks are required after maternal infection for the virus to be eliminated in the foetus's urine in amounts large enough to be detected in the AF (Ruellan-Eugene et al., 1996) and (2) foetal disease is more severe if the infection is contracted in the first 12–16 weeks of gestation (Pass et al., 2006). In addition, false-negative results are

common when amniocentesis is carried out earlier in pregnancy and some viruses are shed by the foetal kidney, the elective site of replication, due to limited diuresis early on.

The AF is subject to direct search for CMV virus in culture and for the viral genome by qualitative PCR. Viral isolation from the AF is an indicative of congenital infection, but the procedure is not sensitive (70–80%). False-negative results are partly due to transporting and maintaining the AF in optimal conditions as the viral particles must be infective to be detected in culture.

The qualitative search for CMV DNA in AF has a good sensitivity and specificity (90–98% and 92–98%, respectively) with respect to viral transmission from mother to foetus (Ruellan-Eugene et al., 1996; Lipitz et al., 1997; Revello et al., 1998a; Guerra et al., 2000; Enders et al., 2001).

If both techniques are negative, foetal infection can be ruled out with a high degree of certainty. If results are positive, investigation is completed by DNA quantification by quantitative PCR (Guerra et al., 2000; Lazzarotto et al., 2000a; Gouarin et al., 2002). There is a low risk of symptomatic infection in the presence of viral loads $<10^3$ copies/ml (Guerra et al., 2000; Lazzarotto et al., 2000a). We recently observed that among 81 positive samples of AF from mothers who transmitted the virus to their babies, 18 had a result below 1000 copies. These 18 congenitally infected babies were asymptomatic at birth and subsequent monitoring in 16 of them confirmed normal development and the absence of late-onset sequelae. Twelve of 16 infected infants were followed up for at least 12 months and the remaining four infants for at least 6 months.

In agreement with other literature reports (Gouarin et al., 2002; Revello and Gerna, 2002a), these findings suggest that low viral loads in the AF, sampled at the appropriate times (at 20–22 weeks gestation and the time interval between onset of maternal infection is $\geqslant$6–8 weeks) may be a good indicator ruling out foetal damage at birth and/or subsequent exacerbation of infection with the onset of sequelae like hearing loss and/or delayed psychomotor development.

In conclusion, 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 at high risk of mother–foetus transmission and reassures the mother in continuing her pregnancy. Reassuring results are also obtained when minimal amounts or traces of the virus are found in the AF since the newborns are infected but asymptomatic at birth and subsequent follow-up checks.

Diagnosis of infection in newborns

At birth, it is essential to use appropriate tests for the diagnosis of CMV congenital infection. 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 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 (Revello et al., 1999a).

After 2 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 (Revello and Gerna, 2002a; Ross et al., 2005; Lanari et al., 2006).

Interesting findings recently emerged from viral genome research using PCR on blood adsorbed on Guthrie cards, collected at birth for neonatal screening (Barbi et al., 2000). However, these virological tests are made highly delicate by the complexity of the extraction and purification phase of viral DNA. This test only sometimes offers retrospective confirmation of congenital infection in selected cases with a strong clinical suspicion, and cannot be utilized as a diagnostic test for congenital CMV infection (Barbi et al., 2006).

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 classified as symptomatic or asymptomatic (Boppana et al., 1992). If viral isolation is negative, the baby is considered uninfected and no further tests are warranted (Fig. 5).

The mortality rate among symptomatic newborns is high and 70–80% of surviving babies are at high risk of developing major neurological sequelae (Boppana et al., 1992). Most infected babies (85–90%) are asymptomatic at birth. The

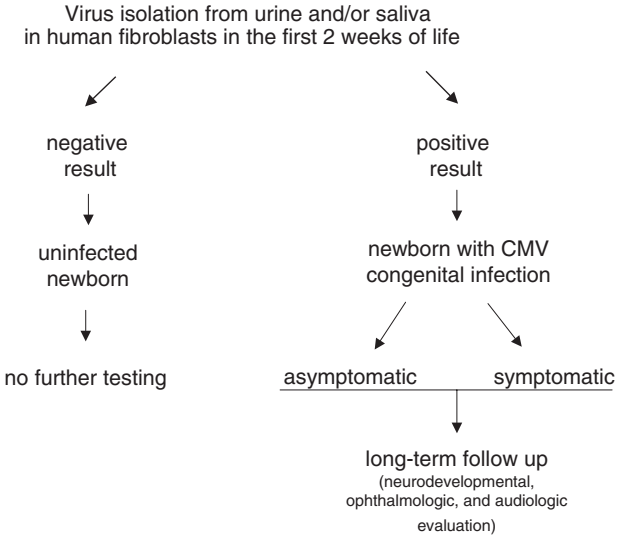

Fig. 5 A management scheme for diagnosis of congenital CMV infection in the newborn.

majority of them will develop normally, but 8% will develop progressive hearing loss (Fowler et al., 1997).

For these reasons, all infected babies undergo follow-up monitoring at 1, 3, 6 and 12 months of life and annually thereafter 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.

In recent years, one of the most widely discussed topics in the management of congenital CMV infection has been the possibility to predict the long-term outcome of infection more accurately in the neonatal period. This would offer a series of advantages including appropriate parent counselling and the implementation of prompt interventions for babies at high risk of handicap. Pinpointing reliable prognostic markers of favourable outcome would attenuate parental anxiety. Patients could also be stratified in terms of varying risk of neurological sequelae to devise more accurate treatments such as antiviral therapies to improve the prognosis.

A report by Rivera et al. (2002) demonstrated that a high viral load in early infancy, expressed by a high amount of virus in urine, is highly predictive of audiological impairment.

Bradford et al. (2005) observed that viraemic infants (presence of CMV DNA in baseline serum sample) were more likely to have (1) hearing loss both at enrollment ($P = 0.045$) and at 6-month follow-up testing ($P = 0.035$) and (2) other indicators of active CMV disease, including elevated levels of alanine aminotransferase, petechial rash and organomegaly.

Finally, we recently obtained encouraging results in this direction studying the role of CMV DNA load in infant blood measured on polymorphonuclear leucocyte (PMNLs) samples taken in the first month of life as a possible prognostic indicator of sequelae. Low neonatal viral load detected by pp65 antigaenemia test and quantitative PCR (qPCR) was highly predictive or absence of sequelae: DNAemia <1000 copies per 10^5 PMNLs has a negative predictive factor of 95%. Different viraemia value ranges are correlated to a different risk of sequelae: ~70% sequelae were found in newborns with a qPCR higher than 10,000 copies per 10^5 PMNLs (Lanari et al., 2006).

Conclusions

Although the diagnosis of congenital CMV infection is still complex, important goals have been achieved in recent years, among which are: the availability of more reliable IgM tests for screening pregnant women whose pre-pregnancy serological status for CMV is unknown (Maine et al., 2001); 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 AF. We are confident that the risk of terminating potentially normal babies would be largely contained by a comprehensive diagnostic approach to CMV infection in

pregnancy. Furthermore, a fully standardized diagnostic algorithm should lessen the anxiety felt by pregnant women, which is mainly due to concerns about mismanagement. We believe that it is time to rethink current strategies, recommendations and attitudes among health authorities, clinicians and pregnant women in order to control congenital CMV infection. Possible future advances in our understanding of the natural history of intrauterine CMV infection and antiviral chemotherapy may allow the prenatal identification of affected foetus to lead to the treatment of the mother with an anti-CMV agent that crosses the placenta and thus provides pre-emptive foetal therapy.

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