Congenital Infection by Cytomegalovirus (CMV) Responsible for Sensorineural Hearing Loss. What Has to be known from the Viewpoint of the Obstetrician, the Neonatologist and the ENT Clinician

Natascha Tessier

Introduction

The Cytomegalovirus (CMV)

The CMV virus is a DNA virus of the β herpes virus family. It is species specific: the HCMV (human CMV only affects humans), whereas the murine MCMV and the GPCMV respectively only affect mice and guinea pigs.

The seroprevalence in the general population is quite important: 60-80% of the population is seropositive. It has been noted that the prevalence depends on the age of the population and the social level. It has been noted that the prevalence of CMV infection is more frequent in low weight and premature babies.

In immunocompetent adults, CMV induces a flu-type syndrome. However, in case of materno-fetal infection, the consequences on the baby may be more severe. It is considered as the first cause of mental and sensorineural hearing loss (SNHL) and therefore a major national healthcare problem.

The congenital infection by CMV concerns 1% of all live births. In 10% of these, the infection may be symptomatic at birth. It may lead to 10% of mortality. Another 60% of these children may develop cerebral lesions with neurological sequelaes, such as microcephaly, seizures, hypotonia, feeding disorders as well as sensorineural sequelae: chorioretinitis and hearing loss. The others may present with growth retardation, jaundice, organomegaly and low platelet count.

In the asymptomatic group of children, it is important to note that 8-15% will have neurological or hearing sequelea.

CMV and hearing loss

Regardless of the etiology, hearing loss concerns 1 to 2/1000 live births. Of these, 50- 60% have a genetic cause, 40- 50% are acquired. Amongst the latter, there may be congenital infections, meningitis, traumas and toxics. It is estimated that CMV concerns 0.2-1.3 out of 1000 live births depending on the studies; therefore, 36% of all sensorineural hearing losses may be due to this virus.

CMV and pathogenesis

Several factors influence the neonatal outcome in case of congenital CMV infection. The consequences of a primary or a recurrent infection during pregnancy are not the same on the child to be. In case of primary infection, there is a high risk of embryofetopathy and hypotrophy. The risk of materno-fetal transmission is

estimated to be of 30-50%. It has also been established that if the infection happens in the six months preceding conception, materno-fetal transmission is still possible but less frequent. However, in case of preconception immunity, the virus remains quiescent in the salivary glands. It does not protect from embryofetopathy; the estimated risk of materno-fetal transmission is of 1.4%. This may be due either to a viral reactivation of the preexisting CMV by a synchronous co-infection (viral or bacterial) or to a new infection by other CMV strains, the immunization being only partial with other viral strains. It has been shown in mice that LPS (a pro-inflammatory cocktail of liposaccharides) reactivates CMV infection and induces an infection similar to a primo-infection.

The viral prevalence depends on several factors. Mother's age influences the risk of materno-fetal infection [1]. In fact, contamination usually appears at the beginning of sexual activity, whereas 50-60% of the population is seropositive. A pregnant woman younger than 20 has three times more risk of having a contaminated child. Contact with toddlers, and in particular young mothers with children in day-care are at high risk of infection. Children younger than two shed CMV in the saliva and urines; this may persist up to the age of 48 months. Depending on the social class, the prevalence varies: 50% of women in middle and upper classes are seronegative.

Finally, the immunological status of the mother is another important element influencing the risk of materno-foetal contamination. HIV positive mother have a higher risk of having a child with a symptomatic CMV infection at birth: 30.8% symptomatic infections (vs 6.3% in HIV-) [2].

Materno-fetal transmission: the importance of neutralizing IgG

Materno-foetal transmission is correlated to the viral titers and inversely to the antibody titers, in particular the titer of neutralizing IgG of great avidity. In fact, the CMV virus bonds to IgG for transplacentary passage by transcytosis [3]; in case of high avidity IgG, the complex is recognized by the macrophages of the chorial villosities. This leads to viral destruction. However, in case of low avidity IgG, the complex is not recognized by the macrophages, thus avoiding them, ant this leads to fetal infection. The materno-fetal transmission may increase in case of synchronous bacterial or viral infection [4]. The risk of fetal transmission is also inversely correlated to gestational age [5].

CMV and placental pathogenesis

Two types of damages on the foetus can be identified in case of congenital infection by the CMV: placental and fetal damages. Fetal hypotrophy seems to be the consequence mainly of the placental insufficiency and not of the fetal infection.

CMV is responsible for vascular damages of the placental villosities, leading to inflammation, fibrosis and necrosis of the chorial villosities. This induces a placental thickening, itself leading to a decrease in blood supply. At last, this process causes fetal hypoxia, and thus growth retardation and low birth weight. Hypoxia itself also has consequences: liver and splenic affection, low platelet count, paraventricular leucomalacia and calcification. CMV may also be responsible for an altered permeability of the placenta [6].

Neurological and hearing damages are however the consequence of the conjunction of specific viral fetal damages and hypoxia.

Susceptibility of CMV and neurological damages depends on gestational age

When analyzing the neural pathogenicity of CMV, it has been shown that the lesions are greater for undifferentiated neural cells, and in particular stem cells in the paraventricular zone [7]. This explains the observed neurodevelopmental lesions. The virus induces the loss of neural stem cells and intermediate cells: mechanically, it leads to a smaller brain size and maturation troubles. Polymicrogyria, brachycephalus, dilated pericerebral spaces are other consequences of the troubled neural differentiation and stem cell migration.

In case of infection of the astroglial cells, the cellular support is also injured, leading to maturation and connectivity problems.

CMV and diagnosis

Viral diagnosis: determining if the foetus is infected

All the difficulty in the management of the CMV infection is determining during pregnancy, if the foetus may be infected in case of maternal infection. Furthermore, in case of proven fetal infection, the chronology of the infection during the pregnancy is another important element. Several exams may help by giving indirect informations concerning the immunological status of the mother. Firstly, the sero-conversion of a previously sero-negative mother gives crucial information. However, the pre-conception status is not always known, since there is no universal consensus on systematic serology during pregnancy. Moreover, the maternal seroconversion does not systematically induce a fetal transmission.

Like in other infections, the existence and the kinetics of the IgM give valuable information concerning the timing of the infection; however, in case of CMV infection, the interpretation may be trickier. The IgM appear during first infection but may reappear during viral reactivation; they may be present for several months. However, if seroconversion is not proven, the combination of IgM anti CMV and the existence of low avidity IgG are in favor of primary infection. In fact, the avidity of the IgG gives information of great importance concerning the chronology of the infection. Avidity of the IgG increases with time: low avidity IgG may persist 20 weeks after infection.

When trying to determine if there is a fetal CMV infection, the CMV DNA may be detected in the amniotic fluid or the fetal blood, either by PCR or viral culture.

In children, the diagnosis may be done early in life but unfortunately rarely after the age of two.

Newborns less than two weeks old may be tested by PCR in their urines (sensitivity 71-100%; specificity 99%); if positive, it accounts for a maternal-fetal infection. Recently it has been demonstrated that retrospective diagnosis could be done on dried blood spots [8]. However, this is a limited in time since dried blood spots are rarely kept for more than two years. Another important element in the neonatal period is the viral load; it is directly correlated to risk of neural sequelae in symptomatic and asymptomatic children.

Prenatal ultrasound: looking for signs of CMV infection in the foetus

Several authors have shown that prenatal ultrasound surveillance may not be sufficient to suspect CMV infection in the fetus in women not monitored for CMV. Abdel-Fattah in 2005 [9] stated that signs seen during gestational ultrasound surveillance such as intra-uterine growth retardation, paraventricular cyst, liver or intestinal echodensities; organomegaly, ascites, hydrops, microcephaly and calcifications are unfortunately usually too late. Benoist (2008) [10] reported that in fetus with a known CMV infection, the US only has a sensitivity of 86% and specificity of 80%. One out of five to one out of seven foetuses have normal US although confirmed foetal infection. [11]

Post-natal CT Scan and MRI : retrospective evaluation of congenital CMV infection

When Ioviono [12] reviewed systematic CT-scan and MRI done in newborns infected by CMV, 52 % of these children had abnormal scan for term, 43% enlarged subarachnoid spaces and 41% myelination anomalies. Other encountered anomalies were: cerebral cysts, calcifications, ventricular enlargement, anomalies of the differentiation white/grey matter, pachygyria, cerebellar hypoplasia. The most frequently encountered anomalies are either delayed myelination and enlarged subarachnoid spaces, or enlarged subarachnoid spaces and lateral ventricles.

For fetuses [13] infected before 26 weeks of gestation (2nd trimester), paraventricular cysts and cerebral lesions may be found on MRI. For infections between 16 and 22 weeks, sulci anomalies are mostly seen.

Predictive factors of fetal infection

Several clinical, radiological and biological signs may suggest fetal infection[14]:

- Hypotrophy
- Fetal blood count: if the platelet count is lower than 120000/mm^3 and the γ GT are increased, these elements are of bad prognosis. However, when platelets and US normal, the risk of a severe CMV infection is very low (negative predictive value: NPV = 85%)
- IgG avidity in the mother's serum: the lower the avidity, the less the protection and therefore the greater the risk of foetal contamination.
- CMV PCR in the amniotic fluid: if the number of viral copies are greater than [15]:
- 1000 copies, there is a 100 % risk of foetal transmission
- 5000 copies, the infection will be symptomatic at birth
- However, if CMV is not found by PCR in the amniotic fluid, it is not necessarily proof that the foetus is infection-free. If suspicion is high, the foetal blood should then be tested.

CMV and sensorineural hearing loss

CMV and SNHL

In a prospective 10 yrs study, Foulon [16] identified 74 children with congenital CMV infection out of 14021 live births (0.53%). Out of these, 5.4% had a symptomatic form at birth. Sixty of those with congenital CMV infection had repeated hearing exams during their childhood (evoked potentials, distortion products, and subjective audiometry). SNHL was identified in 21% of asymptomatic

children and 33% symptomatic children. Therefore, 22% of all infected children, may they be symptomatic or asymptomatic at birth, had some degree of SNHL. Some children presented with late onset (5%), progressive (11%) or fluctuating (16%) SNHL. By extrapolating these data, it is estimated that 36% of the SNHL may be related to congenital CMV. This confirms that CMV is therefore the first cause of congenital SNHL.

The serological status of the mother was also analyzed in this study: 4 /26 (15%) maternal seroconversion, 1/14 (7 %) after recurrent infection. This proves that prior immunization does not protect the foetus from hearing sequelaes[17].

The delay after which SNHL usually appears has been calculated to be 33 months in case of symptomatic infection and 44 months when asymptomatic [18]. The SNHL therefore may appear later in case of asymptomatic infection, thus suggesting a prolonged audiological surveillance even for asymptomatic children. It has been noted that 50% of infected children worsen their hearing levels during their childhood [19].

SNHL risk factors

Risk factors for SNHL have been identified:

- A symptomatic infection at birth (OR= 9.3)
- A high viral load at birth: >5000 PFU in urines and >10000 copies/ml in blood
- IUGR
- Low platelets count

Managing CMV

CMV and prevention

Some authors say that the key to managing congenital CMV infection is prevention. Vauloup-Fellous [20] has shown in a prospective study that hygiene education helps decrease seroconversion in seronegative mothers. Therefore, efforts should be made to educate mothers to wash their hands frequently, in particular those around toddlers.

Vaccine is another interesting preventive option [21]. Several trials are now going on. However, knowing that different serotypes exist, questions still remain on the possibility of developing immunity against one or many serotypes. This notion is important since it has been shown that immunized mothers may be reinfected by a new serotypes and their foetus may develop a congenital infection. The true aim of the vaccine is more the decrease of the CMV prevalence, just like rubella, than an authentic maternal protection.

Prenatal management: antiviral drugs

Several antiviral drugs are available (**Table 1**) however, most have severe side effects or have not been tested in pregnant women. Ganciclovir and valaciclovir are the molecules that are most frequently used prenatally. Jacquemard [22] administered 8 g/day of valaciclovir starting at 30 weeks of pregnancy during 7 weeks in 20 pregnant women. The antiviral drug concentration in the amniotic fluid was satisfactory. This led to a decrease in the viral charge and a decrease in foetal lesions, although this was not significant when compared to the controlled group.

Table 1. Antiviral drugs available

	Advantages	**Inconveniences**	**Effects on congenital CMV infection**
Ganciclovir	Well tolerated in newborns Good CNS penetration No teratogenesis Good concentration in amniotic fluid and serum	No effect on urinary and salivary excretion Bone marrow toxicity Spermatogenesis and mutagen effect	Better evoked potential results Benefits on neurological disorders For severe infections
Valganciclovir	Good oral absorption In pills, liquid		prospective trials
Valaciclovir	Good oral absorption Little 2r effects	Not authorized for children	Good concentration in amniotic fluid
Foscarnet	- No bone marrow toxicity	Appearance of viral resistance Kidney toxicity Bone and dental toxicity	No data for newborns
Cidofovir		Kidney and bone marrow toxicity Teratogenesis, spermatogenesis and mutagen effect	No data for newborns

Prenatal management, a novel option : hyperimmune serum

Hyperimmune serum has been proposed for preventive or therapeutic treatment in women followed for CMV seroconversion. Nigro [23] showed that hyperimmune serum led to a decrease in the ventricular dilation and the organomegaly observed in the foetuses. It also seems to decrease the placental thickening. The intra-uterine growth retardation receded and the foetuses showed weight gain.

Nigro [24] also suggested that hyperimmune serum had a protective effect ($p<0.001$). He demonstrated a significant decrease in the risk of congenital infection ($p=0.04$).

The supposed mechanism of the immunomodulation effect of the Ig for treated women passes by the increase in the avidity of the IgG and other specific anti-CMV antibodies. A decrease in the number of lymphocytes Natural Killers leads to a decrease in foetal damage [25].

The increase in circulating antibodies leads to a decrease in the viral load.

Placental inflammation seems to diminish [26]: chorionic villi vessels regenerate thus allowing an increase in the foetal blood flow. This then leads to a better nutrition and foetal oxygenation. However, these enthusiastic results have yet to be confirmed by a controlled trial.

Post-natal treatment

Kimberlin [27] suggested that six weeks of ganciclovir IV at 12 mg/kg/day protected against hearing loss for at least the first two years of life (21% vs 68% of progressive hearing loss at one year ($p<0.01$)); he also stated that it seems to have a positive effect of neurological development as well. However, the drug does have severe side effects such as bone marrow toxicity and mutagenicity. Nigro [28] tested oral and intravenous ganciclovir on children with neonatal symptomatic neurological infection. He showed that oral treatment was beneficial on neurological development, although not so on the audiological outcome. Prolonged treatment had better results. Lackner [29] tested 23 children with asymptomatic and symptomatic neonatal infection with intravenous ganciclovir during 10 mg/kg during 21 days. He noted no SNHL in the treated group.

Conclusion

Managing CMV

The diagnostic management of the CMV infection depends on several factors. The first important element is to determine if the infection occurs in a context of primary infection or recurrence. There is a risk of SNHL in both cases although greater in case of primary infection. The existence of a late onset and progressive hearing loss in children seems to indicate the persistence of active chronic infection during childhood or a prolonged inflammatory process.

The second step for a proper management is to determine if the infection is an isolated placental infection or if the foetus is itself infected. The research for the existence of CMV must be done in the amniotic fluid, or better still, in the foetal blood. The prenatal ultrasound surveillance is another important element although it may underestimate the foetal infections. Lastly, the avidity of the IgG gives important information to determine the moment of the infection.

The therapeutic management of the CMV is another delicate subject. Several questions are still open: when (during the pregnancy or after birth?), for how long? The place of vaccine is not yet determined. Will it lead to eradication of the virus or a decrease in the prevalence in the general population?

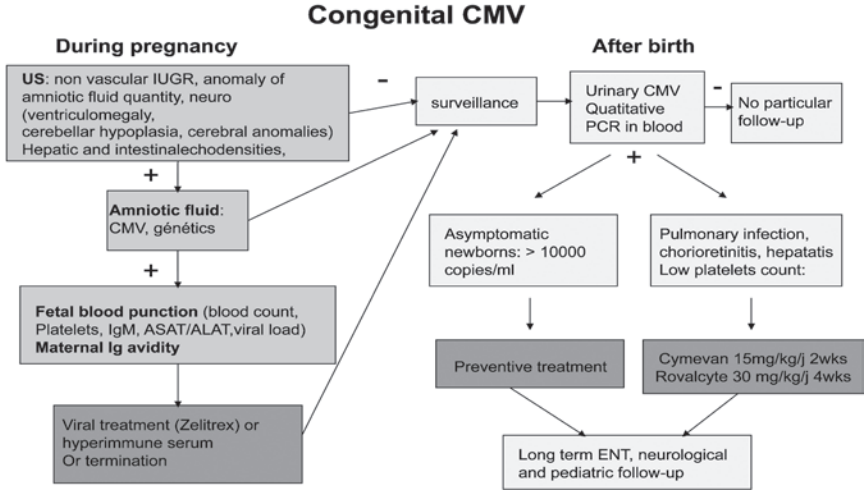
The modalities of the antiviral treatment are not clearly codified. Protocols vary ot the drug used, the dose, the duration and the timing of the treatment. New perspectives open with the hyperimmune serum, but controlled trials are still lacking.

And finally, in case of severe foetal infection, termination of the pregnancy may be discussed, depending on the country.

SNHL management

Considering the high risk of SNHL in children infected congenitally by the CMV, universal neonatal hearing screening should be proposed (**Algorithm 1**). However, knowing the possibility of the existence of progressive and late onset SNHL, audiological assessment should be done every 6 months to a year up to the age of 10 years at least. In case of SNHL, hearing aids should be prescribed. If hearing loss is profound, cochlear implants are an interesting option. They offer good results. However, these results may be compromised if there are associated neurological and psychiatric problems related to CMV.

Algorithm 1. Decision tree of the management of the CMV during pregnancy and after



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