

The background of the cover features a microscopic image of cells. The cells are stained, with some showing prominent purple and pink inclusions, likely representing viral particles or specific cellular components. The overall color palette is dominated by shades of teal and green, with a yellow horizontal band across the middle.

# Cytomegalovirus (CMV) and pregnancy

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**DiaSorin**

The Diagnostic Specialist

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# 1. Materno-fetal CMV infection

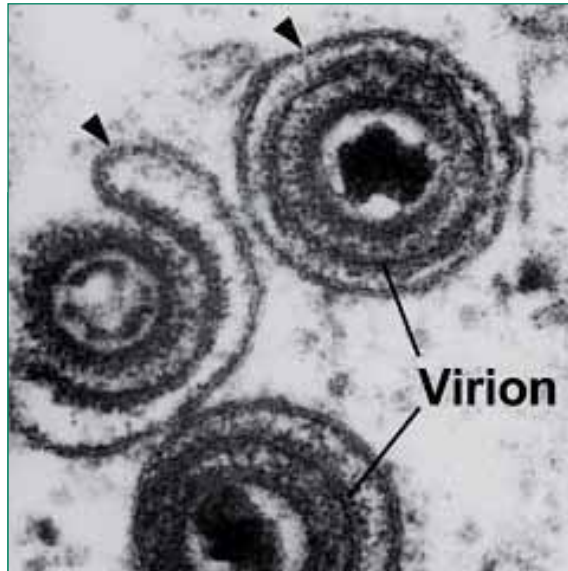
## 1.1 Epidemiology and pathogenesis

CMV is a DNA-enveloped virus belonging to the *Herpesviridae* family (photo 1). Indeed, CMV is the major cause of neurosensory impairment responsible for congenital infection. In fact, CMV infection is said to be the primary cause of congenital infections and that each year about 1% of newborn babies contract CMV infection *in utero*.

Overall, 50% of women of childbearing age in Western Europe are infected by CMV, but prevalence rate varies according to socio-economic level, age, profession, parity and ethnic origin. As a result, 50% of pregnant women are exposed to the risk of primary CMV infection. In addition, cases of congenital CMV infection resulting from maternal secondary infection (reinfection or reactivation) are also reported, in particular within populations with a high prevalence rate of CMV infection<sup>5,7</sup>.

The most exposed subjects are seronegative women working with very little children or women with first children in day-care centres, since the virus spreads extremely fast within groups of very young children. Transmission is only through close interpersonal contact (with urine, oropharyngeal secretions, milk, tears, and genital secretions). Pregnant seronegative women should thus avoid close contact with little children as much as possible and should take certain hygiene measures to significantly reduce the risk of CMV infection during pregnancy. The partners of such women should also take the same precautions.

It is generally accepted that materno-fetal CMV transmission is mostly caused by peri- or post-conceptual maternal primary infection<sup>11</sup>. The incidence of primary infection during pregnancy is estimated at between 0.5% and 2%. Roughly 50% of infected women transmit the virus to their children, but transmission



**Photo 1:** Human cytomegalovirus AD169 strain. Ultra-thin cross-section of human primary fibroblasts; complete virions with intact envelope (arrows) and electron-dense nucleocapsid core; incomplete virions without nucleocapsid.

Graciously provided by: Wade Gibson, PhD, Johns Hopkins University, Department of Pharmacology and Molecular Sciences.

varies depending on the term of pregnancy. According to Bodeus et al., transmission is estimated at 36% in the first quarter, 45% in the second quarter and 77% in the third quarter<sup>1</sup>. To Revello and Gerna, transmission is estimated at 45% in the first and second quarters, and 79% in the third<sup>10</sup>. Transmission to the foetus occurs through transplacental haematogenous spread during maternal viraemia concomitant with primary infection. The foetus can also be infected, but to a lesser extent, following reactivation (reappearance of viral production) or maternal reinfection by a new virus strain. It is estimated that 10% to 30% of women excrete the virus in the course of pregnancy, and that roughly 0.2% of children born to a seropositive mother before

pregnancy are infected at birth<sup>7</sup>. The incidence of materno-fetal CMV transmission after maternal secondary infection is difficult to estimate because differential diagnosis of reinfection or reactivation is practically impossible.

Finally, the neonatal period is highly contagious (through cervicovaginal secretions, maternal milk, close contact), but is not followed by neurological sequelae. Perinatal infection is very frequent; overall 12% to 15% of newborn babies negative at birth are found to be infected after one month.

### 1.2 Clinical picture

The clinical signs of primary CMV infection in

pregnant women are relatively frequent, but mostly non-specific (fatigue, fever, headache) and therefore recognized only *a posteriori*. This is why they are generally insufficient to signal primary CMV infection<sup>12</sup>.

The risk of transmission to the foetus is estimated at 30% to 50% in the course of pregnancy, but it increases as pregnancy progresses. The consequences of fetal infection vary, but are normally more severe after maternal primary infection than after reactivation or reinfection (Figure 1). The risk of sequelae to the foetus reaches a peak (20% to 30%) when maternal primary infection occurs before the twentieth week of gestation and declines thereafter<sup>6,9,13</sup>. Maternal infection during the last quarter of

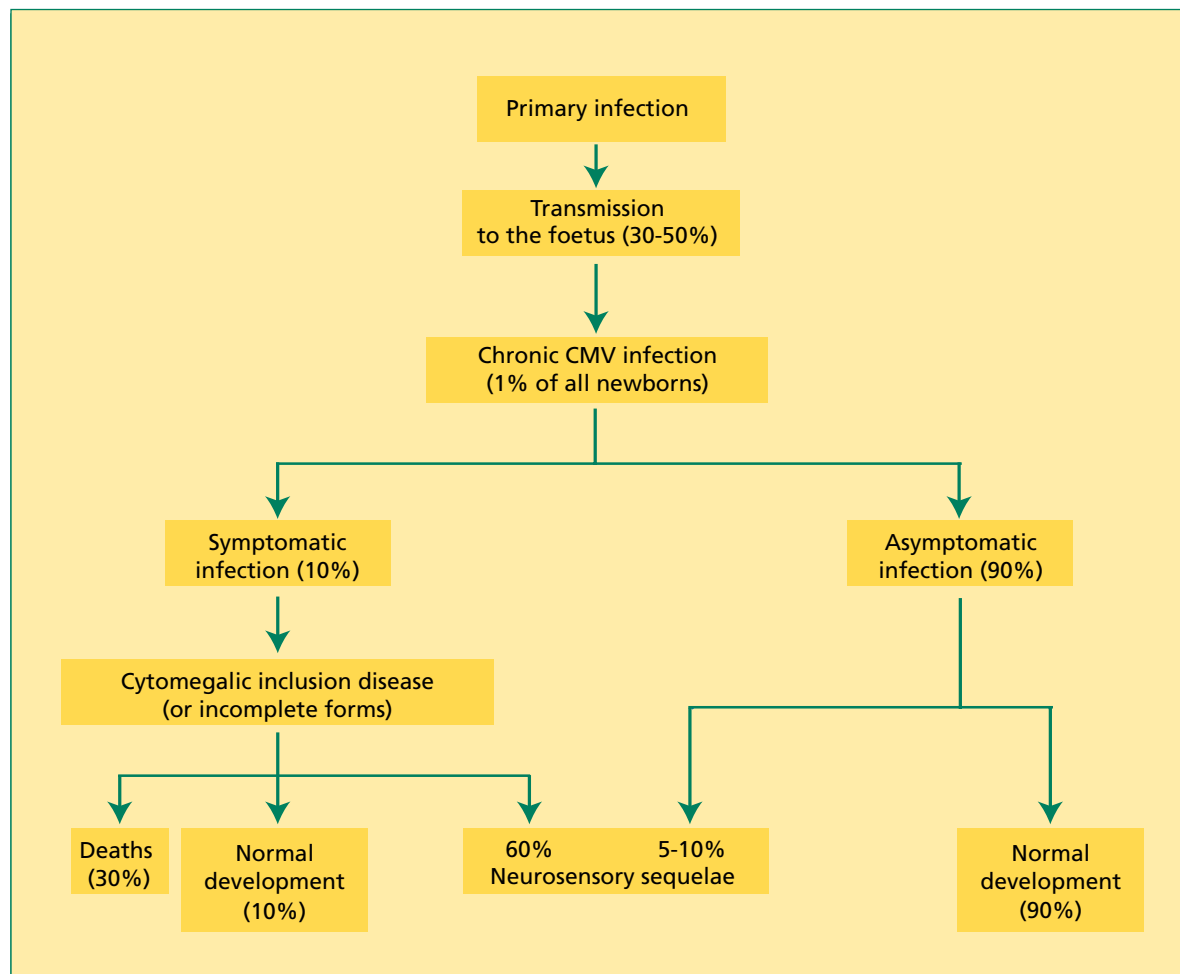


Figure 1: Fetal risk in the event of maternal CMV infection

pregnancy can indeed harm the foetus, causing microcephaly, hearing and minor neurological impairment. However, when maternal primary infection occurs in early pregnancy, more serious effects are observed on the foetus, giving rise to Cytomegalic Inclusion Disease (CID). CID is a consequence of viral replication in vital fetal organs that regresses spontaneously, but causes persistent damage that can lead to serious long-term sequelae. Consequently, severe Central Nervous System (CNS) impairment, intracranial calcification, and myelination abnormalities are normally observed. If pregnancy reaches term, the newborn baby most often presents hypotrophy, hypotonia, hepatosplenomegaly, sometimes respiratory disorders and haemostatic complications. Among children with CID, 10% to 20% die within the first weeks of after birth, and among the survivors, 80% to 90% develop neurosensory sequelae with psychomotor retardation. In France, the figures commonly found in the literature are really overestimated. Indeed, situations where doubts remain over estimates relating to very serious lesions become rare when pregnancy is correctly followed up in specialised centres (which should always be the case when maternal seroconversion or fetal CMV infection is diagnosed). Recent improvement in resolution of Magnetic Resonance Imaging (MRI) has decreased the number of cases where severity of neurological impairment cannot be assessed. Flexibility of abortion time limits in France also decreases significantly the number of newborn babies affected by CID.

This very serious scenario is fortunately increasingly rare: in fact, congenital CMV infection is estimated to be asymptomatic at birth in more than 90% of cases. IntraUterine Growth Restriction (IUGR) is sometimes observed by ultrasound scan. However, clinical follow-up of these children is crucial, since 5% to 10% of initially asymptomatic children eventually develop neurosensory sequelae, most often involving hearing loss. Hearing loss

may be complete or partial and may not be apparent at birth, but may eventually develop within first years of birth.

The clinical consequences of maternal secondary infection on the foetus are not well known. Studies show that pre-existing immunity somehow offers protection during pregnancy. Such protection, however, may be insufficient to prevent CMV transmission to the foetus, and consequently, more or less serious fetal abnormalities<sup>2,4</sup>.

### 1.3 Virological diagnosis

Large-scale screening of CMV infection during pregnancy is not currently recommended in most European countries, but hygiene measures are of paramount importance to curb the incidence of maternal CMV infection. IgG antibodies are sometimes detected in early pregnancy in order to determine maternal serological status, especially when the patient is at risk professionally or relationship wise (day-care personnel, nurses, kindergarten teachers, mothers of infant children). Diagnosis of maternal infection can also be conducted when the mother shows symptoms, but clinical signs are often misleading since they are non-specific. The main consequence of all these factors is that many CMV infections are undiagnosed during pregnancy. However, if ultrasound abnormalities are detected (IUGR, cerebral abnormalities, oligoamnios, hyperechogenic bowel, fetal hydrops), maternal CMV infection should be investigated.

#### 1.3.1 Laboratory diagnosis of maternal primary infection

Viraemia in immunocompetent subjects may indicate recent primary infection, but is not used in clinical practice for diagnostic purposes. On the other hand, maternal viraemia should be detected before establishing prenatal diagnosis in amniotic fluid specimens in order to curb the risk of iatrogenic infection of the foetus. Indeed, the virus can persist in the blood

stream for several weeks, which explains why an infection contracted in the weeks before conception can cause fetal infection.

Maternal viraemia is not useful for diagnosis of primary CMV infection, since it is often positive in case of reactivation, which only very rarely causes symptomatic congenital infections.

Diagnosis of primary CMV infection in pregnant women is therefore based essentially on serological tests (Figure 2).

IgG and IgM antibodies are usually detected using ELISA techniques. The presence of specific IgG confirms contact with the virus, but a single specimen can in no way indicate the time of maternal primary infection. Seroconversion normally confirms primary infection, which is however difficult to identify in the absence of

large-scale screening. Detection of specific IgM does not necessarily indicate recent infection. Indeed, although IgM antibodies are consistently detected in recent primary infections, they may also be observed in consequence of cross-reactions with IgM directed to other viruses (EBV for instance). In addition, the use of more and more sensitive techniques also allows specific IgM to be detected long after the onset of primary infection, during secondary infections, or in case of polyclonal stimulation of the immune system, when a different infection is in progress.

**It is therefore apparent that the detection of specific IgM is insufficient to diagnose primary infection, and that its presence can be difficult**

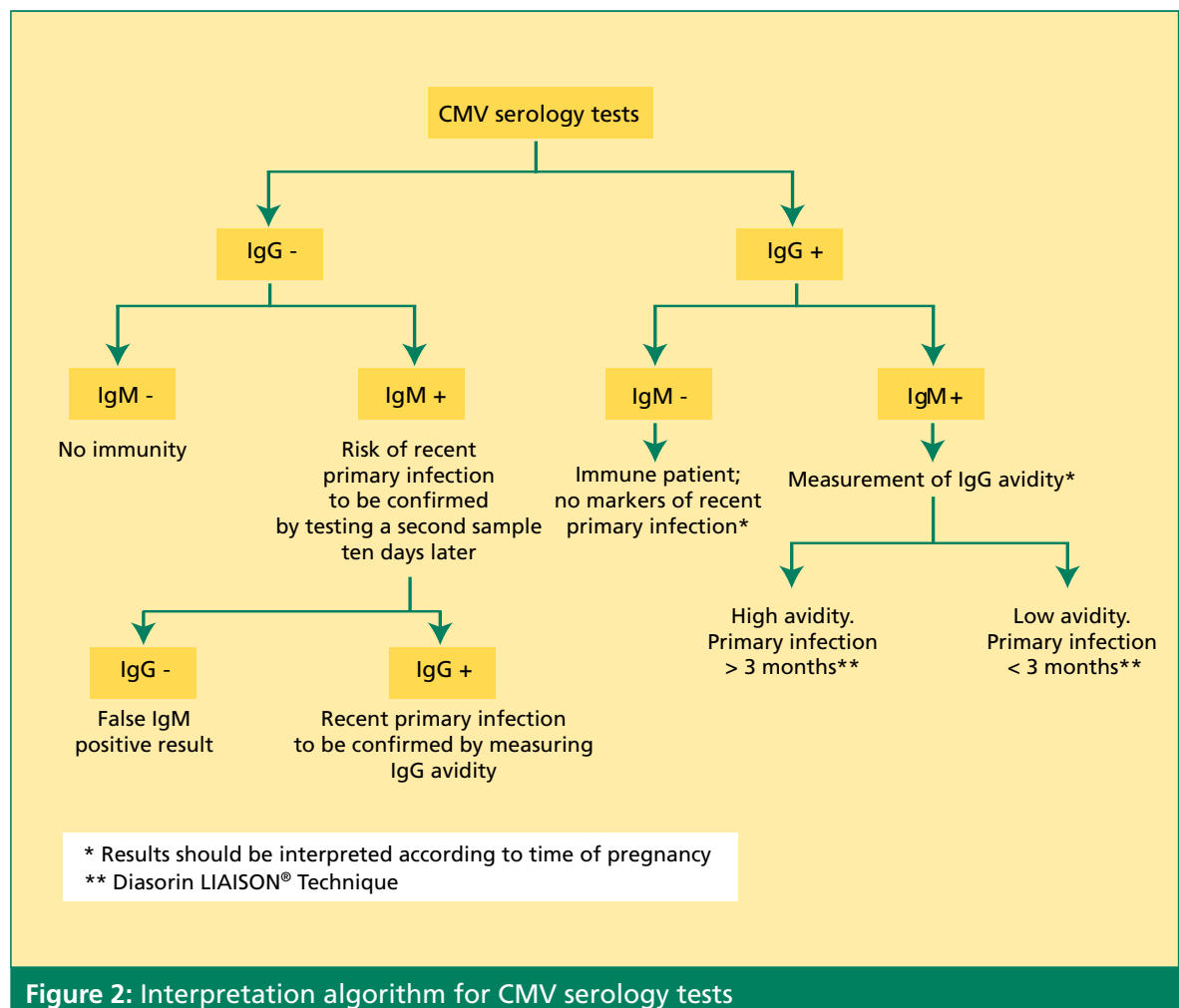


Figure 2: Interpretation algorithm for CMV serology tests

to interpret. For these reasons, it is often necessary to measure specific IgG avidity and to test in parallel serum samples collected at different times during pregnancy in order to determine whether the infection is post-conceptual in nature (Figure 2). IgG avidity index indicates the strength of the bond between multivalent antigens (contained in the reagent) and polyclonal IgG antibodies present in the patient serum. This technique is based on the fact that specific IgG avidity increases with the time of the immune response. At the onset of infection, IgG avidity index is low, and the more time elapses from the onset of infection, the greater the index becomes. Most often, measurement of avidity index is based on the use of denaturing agents (in general, elevated urea concentration) in ELISA tests. The denaturing agent is then either contained in specimen diluting solution to prevent the formation of antigen/antibody complexes (principle of dilution) or it is added to wash solution after the formation of antigen/antibody complexes (principle of elution). It is then possible to obtain absorbance (Abs.) values in the absence and in the presence of the denaturing agent for the same sample. The avidity index is then calculated as follows:

$$\frac{\text{Abs. in the presence of denaturing agent}}{\text{Abs. in the absence of denaturing agent}} \times 100$$

The avidity index is a function of the time of infection and in general allows exclusion or confirmation that a recent primary infection is less than three months old<sup>12</sup>. The avidity results should then be interpreted depending on the term of pregnancy. Beyond the first quarter of pregnancy, high avidity means that post-conceptual infection cannot be excluded. The avidity index also depends on the subjects tested and the techniques used. Indeed high avidity generally excludes primary infection contracted less than three

months before; however, the cut-off values for exclusion vary according to the technique used and comparison is not always feasible. It should also be noted that the avidity index may be incorrect in the presence of low IgG titre and therefore not always easy to interpret (Figure 3).

### 1.3.2 Laboratory diagnosis of maternal secondary infection

Currently, it is practically impossible to establish a diagnosis of CMV reinfection or reactivation in a pregnant woman. Indeed it is widely accepted that increased specific IgG (whether in the presence of specific IgM or not) in a woman infected before her pregnancy (or most often at early pregnancy) indicates secondary infection. This clinical condition can also be observed much more frequently in non-specific polyclonal stimulation of the immune system. Measurement of IgG avidity is of no use in this

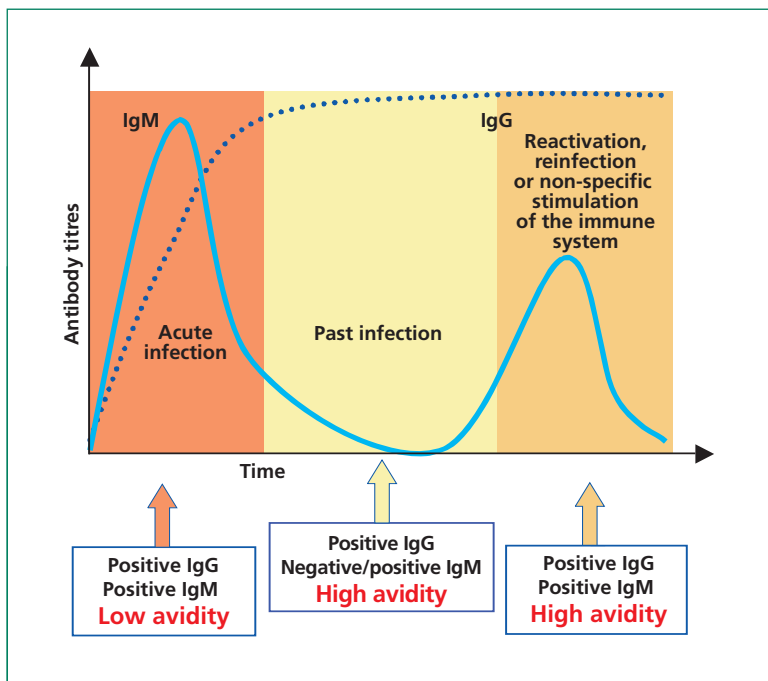
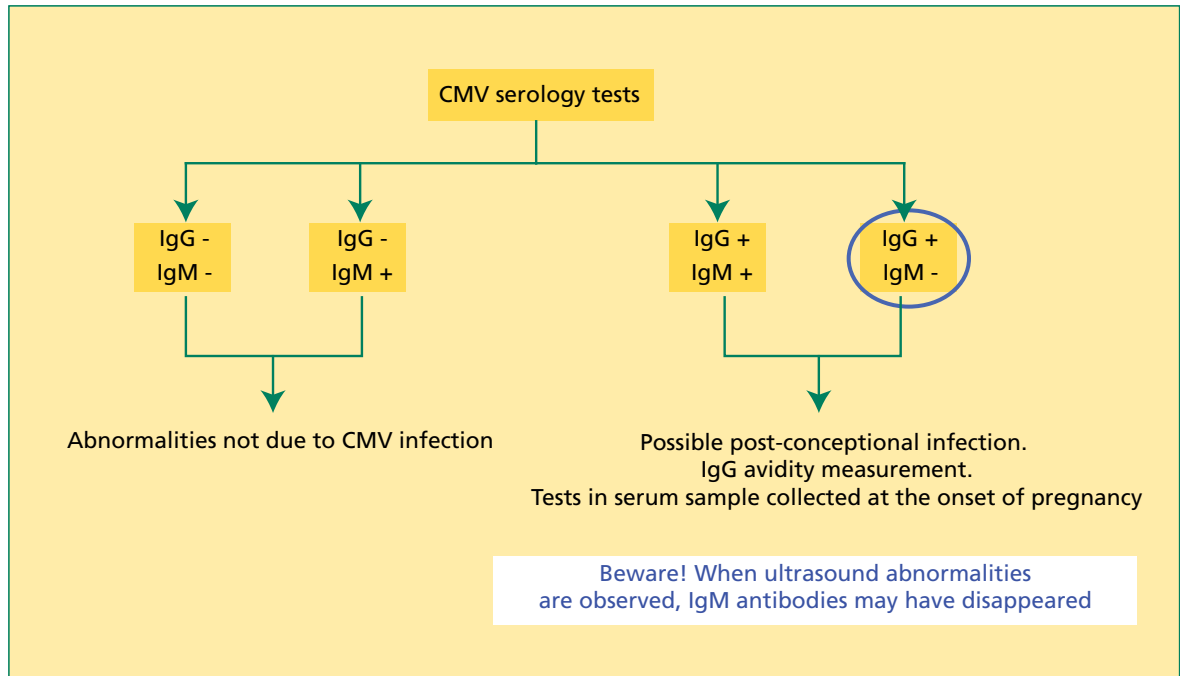


Figure 3: Diagram for kinetics of CMV IgG and IgM antibodies and IgG avidity index



**Figure 4:** Interpretation of maternal serology tests when ultrasound abnormalities are observed, suggestive of CMV infection

case, since it is high in both situations. At most the infection can be retrospectively presumed as secondary when a child is born infected to a mother positive for CMV before her pregnancy. In practice the diagnosis of maternal secondary infection is difficult to achieve and is only rarely correctly established.

### 1.3.3 Laboratory diagnosis in case of ultrasound abnormalities suggestive of maternal infection

When ultrasound abnormalities suggestive of CMV infection are observed, maternal infection should be investigated. When IgG titre is negative (regardless of IgM titre), CMV can be excluded as the causative agent. When IgG titre is positive (irrespective of IgM titre), the infection should be dated by

IgG avidity index measurement as well as by IgG detection in serum specimens collected at the onset of pregnancy for other screening purposes, like trisomy 21 or  $\beta$ -hCG assay. Indeed as seen previously, the presence of IgM does not automatically indicate primary infection.

Furthermore, by the time ultrasound abnormalities are observed, IgM may have already disappeared. However, if results obtained in a serum sample collected at the onset of pregnancy are not suggestive of primary CMV infection (IgG+/IgM- or IgG+/IgM-/high IgG avidity), prenatal diagnosis is strongly recommended, since IgM may disappear, and infection occurring in the weeks before conception or secondary infection may be the cause of fetal infection (Figure 4).



### Clinical case No. 1

Date	IgG	IgM	Avidity index
	cut-off: 0.6 IU/mL GZ*: 0.4-0.6 IU/mL	cut-off: 30 AU/mL GZ: 15-30 AU/mL	%
31-07-07	6.9	72.2	7 (very low avidity)
17-08-07	6.6	57.2	13 (low avidity)

\* ZG: grey zone

The first serum sample is collected from Mrs A. on 31<sup>st</sup> July 2007 and shows an IgG concentration of 6.9 IU/mL, which remains unchanged seventeen days later (6.6 IU/mL). Specific IgM is detected in both samples. IgG avidity index is compatible with recent primary infection (less than three months before).

### Clinical case No. 2

The first serum sample is collected from Mrs B. on 2<sup>nd</sup> February 2007 and shows an IgG concentration of 1.6 IU/mL which remains unchanged twenty days later (1.7 IU/mL). Specific IgM is detected in both samples. IgG avidity index is compatible with primary infection older than three months.

Date	IgG	IgM	Avidity index
	cut-off: 0.6 IU/mL GZ*: 0.4-0.6 IU/mL	cut-off: 30 AU/mL GZ: 15-30 AU/mL	%
02-02-07	1.6	36	86 (high avidity)
22-02-07	1.7	40	89 (high avidity)

### Comments on clinical cases Nos. 1 and 2

The observation of stable specific IgG concentrations is reassuring for many biologists and clinicians. Indeed, IgG can plateau several weeks after the onset of infection depending on the subjects tested and the techniques used. Stable IgG concentrations do not therefore indicate past infection. It should be underscored that the term past infection is not to be used since past infection is synonymous with pre-conceptual infection for most clinicians, regardless of the term of pregnancy at the time the serology tests are performed!

**Clinical case No. 3**

CMV serology tests routinely prescribed to Mrs C. on 11<sup>th</sup> October 2004 are negative. Five weeks later specific IgG and IgM are detected. During the final serology tests performed on 4<sup>th</sup> April 2005, roughly five months after the first positive tests, high IgM concentrations are still present. Furthermore, a small but regular decrease in IgM levels is observed from November 2004 to March 2005. IgG concentrations remain stable for the two following months after a significant increase is observed from November 2004 to January 2005.

Date	IgG	IgM
	cut-off: 0.6 IU/mL GZ*: 0.4-0.6 IU/mL	cut-off: 30 AU/mL GZ: 15-30 AU/mL
11-10-04	< 0.2	< 8
<b>15-11-04</b>	1.1	<b>188</b>
07-01-05	2.9	156
07-02-05	2.9	130
07-03-05	3.2	115
<b>04-04-05</b>	4.4	<b>125</b>

**Comments**

Low concentrations of long-lasting CMV IgM commonly persist for months after seroconversion. However, high IgM levels may be detected for longer than six months as in case No. 3.

#### Clinical case No. 4

CMV seroconversion was detected using two different techniques (A and B) from December 2000 to January 2001 for Mrs D. On 22<sup>nd</sup> January, low IgM concentrations are detected with both techniques. At that time, IgG titres are borderline with technique A and positive with technique B. One month later, IgM titres are borderline with technique A (signal-to-cut-off ratio: 0.83) and very slightly positive (signal-to-cut-off ratio: 0.56) with technique B. By March, two months after the onset of seroconversion, IgM titres are no longer detected with technique A and are borderline with technique B. CMV IgG titres stabilised very soon after seroconversion, regardless of the technique used.

Date	IgG		IgM	
	A	B	A	B
	cut-off: 0.6 IU/mL GZ: 0.4-0.6 IU/mL	cut-off: 15 AU/mL	signal/cut-off: 0.9 GZ: 0.7-0.9	signal/cut-off: 0.5 GZ: 0.4-0.5
06-10-00	< 0.4	/	/	/
22-12-00	< 0.4	0	/	0.23
<b>22-01-01</b>	4	77	<b>0.98</b>	<b>0.64</b>
<b>20-02-01</b>	10	103	<b>0.83</b>	<b>0.56</b>
26-03-01	13	123	0.54	0.47
18-04-01	9	90	0.42	0.41

#### Comments

Case No. 4 shows that the presence of specific IgM may be transient, albeit rarely. Consequently, the absence of IgM does not allow recent primary infection during pregnancy to be ruled out unless serology tests are performed soon after conception. Laboratory biologists therefore strongly recommend storing samples positive for  $\beta$ -hCG, which in general are the earliest samples collected after conception.

**Clinical case No. 5**

In the presence of symptomatology suggestive of toxoplasmosis, CMV, or EBV infection, serology tests were performed. As indicated in the following table, Toxoplasma, CMV and VCA IgM were detected at the time clinical signs were observed, in July 2005. Primary EBV infection was ruled out because of the presence of EBNA antibodies. A sample collected two months later was not conclusive: specific IgM to all three agents investigated had disappeared. It is interesting to note that EBNA IgG and VCA IgG increased significantly from July to September.

Date	Toxoplasmosis	CMV	EBV
	IgG cut-off: 6 IU/mL IgM cut-off: 1	IgG cut-off: 0.6 IU/mL IgM cut-off: 30 AU/mL	EBNA IgG cut-off: 20 U/mL VCA IgG cut-off: 20 U/mL VCA IgM cut-off: 40 U/mL
28-07-05	IgG: 60 <b>IgM: 2.17</b>	IgG: > 22 <b>IgM: 213</b>	EBNA IgG: 140 VCA IgG: 202 <b>VCA IgM: &gt; 160</b>
21-09-05	IgG: 85.5 <b>IgM: 0.73</b>	IgG: > 22 <b>IgM: 15.3</b>	EBNA IgG: 315 VCA IgG: 392 VCA IgM: 14.8

Avidity of Toxoplasma and CMV IgG measured in a serum sample collected in July helped rule out recent toxoplasmosis and CMV infection...

Anamnesis and additional tests helped confirm the presence of Lyme disease.

Date	Avidity of Toxoplasma IgG	Avidity of CMV IgG
	IgG avidity: > 30% Infection older than 4 months	IgG avidity: > 30% Infection older than 3 months
28-07-05	56%	61%

**Comments**

The presence of IgM as well as increased specific IgG titres can be observed in primary infection as well as in non-specific polyclonal stimulation of the immune system, as is the case here, where the patient is indeed affected by Lyme disease.

### Clinical case No. 6

In the presence of symptomatology suggestive of mononucleosis or CMV infection, serology tests were performed on 14<sup>th</sup> April, at the time clinical signs were observed. CMV IgM and VCA IgM titres were borderline and positive respectively, whereas CMV IgG and VCA IgG titres were negative and borderline respectively. Nine days later, CMV IgG titres remained negative, whereas VCA IgG titres became positive. EBNA IgG titres were consistently negative. These results showed recent EBV infection.

Date	CMV	EBV
	IgG cut-off: 0.6 IU/mL IgM cut-off: 30 AU/mL GZ: 15-30 AU/mL	EBNA IgG cut-off: 30 U/mL VCA IgG cut-off: 20 U/mL - GZ: 10-20 U/mL VCA IgM cut-off: 40 U/mL
14-04-07	IgG: < 0.2 <b>IgM: 27</b>	EBNA IgG: < 3 VCA IgG: 10.1 <b>VCA IgM: 52.2</b>
23-04-07	IgG: < 0.2 <b>IgM: 52.2</b>	EBNA IgG: < 3 VCA IgG: <b>25</b> VCA IgM: > <b>160</b>

#### Comments

Anti-CMV IgM presence was probably due to a cross-reaction between CMV and EBV IgM, given that both viruses are members of the *Herpesviridae* family. In the present case, the presence of anti-CMV IgM is not related to non-specific polyclonal stimulation of the immune system, because of the absence of anti-CMV IgG. From our experiment, cross-reactions are more often observed with IgMs, but this remains to be confirmed.

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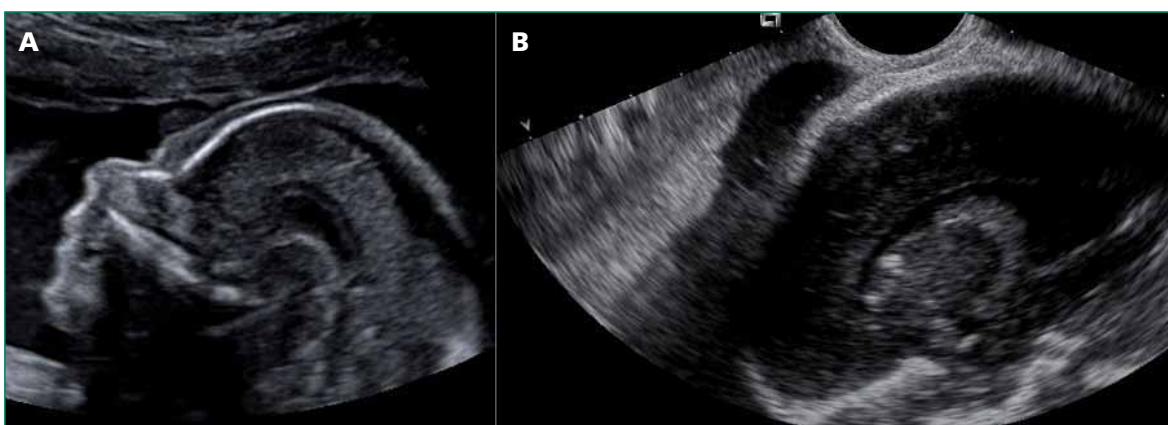
## 2. Diagnosis of congenital CMV infection

### 2.1 Diagnosis of fetal infection

#### 2.1.1 Fetal imaging

Congenital infection is contracted during maternal viraemia both primarily and through reinfection or reactivation. The infection is initially placental, then it may be transmitted to the foetus via the infected placenta<sup>1</sup>. Dissemination in the foetus then occurs via the blood stream and all fetal cells can be infected; potential infection severity is related to fetal brain damage. A vast majority (70% to 80%) of children who are infected *in utero* present no long-term sequelae to CMV infection, 10% to 15% present moderate sequelae, notably unilateral or bilateral hearing loss, and the remaining 10% to 15% present severe neurosensory sequelae with psychomotor retardation<sup>2</sup>. The real challenge in prenatal follow-up of CMV infection is therefore to identify severely affected foetuses at risk of serious sequelae with high sensitivity and specificity. Fetal impairment is detected via ultrasound scan either coincidentally with a

routine pregnancy ultrasound scan, or as a result of frequent ultrasound follow-ups performed because the mother is affected by primary infection. The ultrasound scan can detect impairment of one or more fetal organs that may or may not be associated with systemic impairment. Systemic impairment involves fetal hepatosplenomegaly with the potential complication of ascites, revealing the presence of cholestatic hepatitis or hepatic insufficiency<sup>3</sup>. Less frequently, generalised oedema associated with ascites suggests hydrops linked to the combined effect of hepatic insufficiency and anaemia due to medullary impairment. CMV enterocolitis involves hyperechogenic fetal bowel<sup>4</sup>, and fetal kidney impairment can be detected via renal hyperechogenicity or oligohydramnios<sup>5</sup>. IntraUterine Growth Restriction (IUGR) can result from fetal or placental impairment. In the event of proven fetal infection, fetal brain damage must be investigated until the end of pregnancy using a combination of ultrasound scan and Magnetic Resonance Imaging (MRI) (photo 2)<sup>6</sup>. The most obvious cerebral signs are



**Photo 2:** A. Sagittal section of the fetal profile and brain in a normal quantity of amniotic fluid. B. Sagittal section of the fetal brain at the same age, showing ventricular dilation and periventricular calcification in CMV infection. Amniotic fluid is absent due to infection.

Pr. Yves Ville, Poissy Maternity.

microcephaly, unilateral or bilateral dilation of the cerebral ventricles and cerebral microcalcifications. More subtle abnormalities of myelination or gyration of the fetal brain must be investigated with fetal MRI. The presence of clear cerebral abnormalities on ultrasound scan, notably microcephaly, is a sign of very poor prognosis with risk of severe neurosensory sequelae close to 100%<sup>7</sup>. The predictive value of extra-cerebral ultrasound abnormalities has not been evaluated.

### 2.1.2 Biological diagnosis of fetal infection

The reference method for diagnosing fetal infection is CMV detection by PCR test in amniotic fluid collected by amniocentesis<sup>5</sup>. Improved sensitivity of PCR test compared with viral culture is clearly demonstrated in the literature<sup>8</sup>. Until very recently in-house CMV PCR tests have been used in most virology laboratories: traditional, multiplex or real-time PCR tests. Recently, CE-marked kits have become available for CMV PCR test, and some are validated for prenatal diagnosis<sup>9</sup>.

The choice of a sensitive CMV PCR test is crucial for reliable prenatal diagnosis; such test should amplify all CMV strains and include an internal control in order to detect possible PCR inhibitors. Furthermore, it is of paramount importance to properly schedule the time of amniocentesis after 21 weeks of amenorrhoea (when fetal urinary system is mature) and at least seven weeks after maternal primary infection<sup>10,11</sup>. Under optimal conditions of sample collection and technique, sensitivity of prenatal diagnosis is above 90%. False negative results are however described, with prenatal negative diagnosis and positive diagnosis at birth; these cases are generally related to late virus transmission to the foetus, which is not yet infected when prenatal diagnosis is conducted<sup>12</sup>.

It is furthermore recommended to check that no maternal viraemia is present before

amniocentesis is carried out. Indeed, there is theoretical risk of iatrogenic contamination of the foetus during amniocentesis in the event that the virus circulates in maternal blood, even though a recent study shows that this risk is exceptionally low<sup>12</sup>. The technique of choice to rule out the existence of maternal viraemia is PCR test in a blood sample; indeed, PP65 antigenemia test is significantly less sensitive than the PCR technique and false negative results may occur with PP65 antigenemia test in cases of low viraemia<sup>13</sup>.

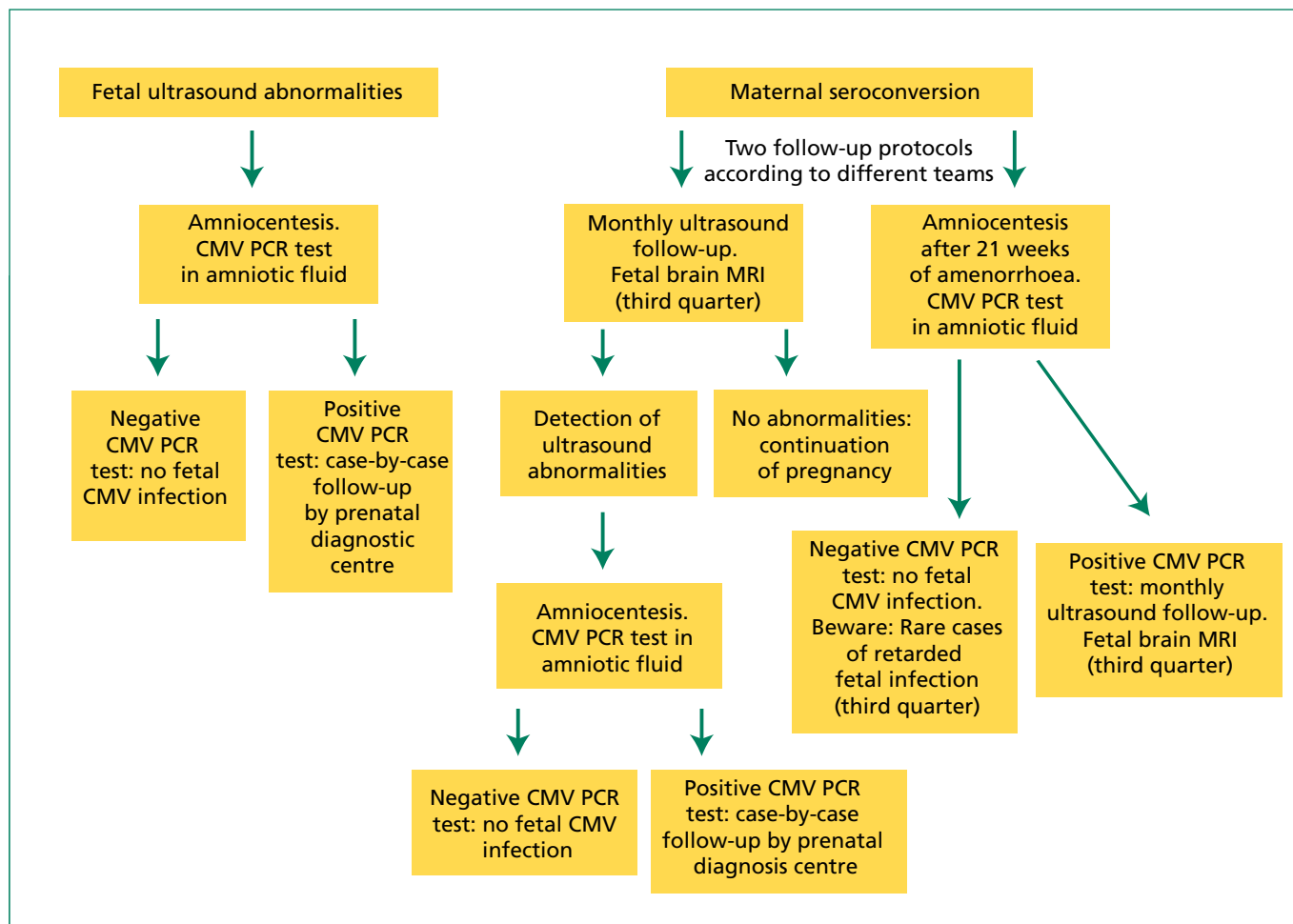
Specificity of PCR test in amniotic fluid specimens ranges between 90% and 100% according to different studies<sup>14,15</sup> with reported false positive results probably related to PCR test contamination. Risk of false positive results should be curbed by the generalised use of automated extraction techniques and real-time PCR.

Viral load in amniotic fluid does not seem to indicate infection severity, notably because viral DNA accumulates in the amniotic fluid over time<sup>16</sup>. When diagnosis of fetal infection is confirmed by a positive PCR test in amniotic fluid, some teams collect a fetal blood sample that helps measure fetal DNA by quantitative PCR tests and fetal platelet levels. While the prognostic value of fetal thrombopaenia now appears to be established<sup>17</sup>, the prognostic value of viral load in fetal blood is not yet demonstrated.

## 2.2 Diagnosis of congenital CMV infection in newborn babies

The reference method for diagnosing congenital infection in newborn babies is detection of the virus or viral DNA via culture or PCR test in urine samples collected during the first two weeks of life<sup>18</sup>. Detection of viral DNA in saliva samples collected in the first days of life has been proposed as an alternative to the use of urine samples, which is a cumbersome procedure, but there is currently limited literature about sensitivity





**Figure 5:** Algorithm for prenatal follow-up of materno-fetal CMV infection

and specificity of this technique<sup>19</sup>. Detection of DNA in blood seems to be able to identify 80% of congenitally infected newborn babies<sup>20</sup>. However, measuring viral load in blood at birth may be of prognostic value since asymptomatic newborn babies with higher viral load are at greater risk of developing hearing loss<sup>20, 21</sup>.

### 2.3 Retrospective diagnosis of congenital CMV infection in children

Retrospective diagnosis of congenital infection may be established by PCR test using dry blood spots on Guthrie cards<sup>22, 23</sup> whenever neonatal diagnosis has not been established and a small child shows clinical

signs consistent with congenital CMV infection (neurological signs or hearing loss). Specificity and sensitivity of this diagnostic method are currently under evaluation.

### 2.4 Prevention of congenital CMV infection

Congenital infection is prevented with simple hygiene measures. Indeed, home-care for a child under the age of six is one of the main risk factors for maternal primary infection<sup>24</sup>. Hygiene measures like: washing one's hands after changing nappies, feeding, bathing, blowing the child's nose, not sharing spoons, cleaning toys are effective to curb transmission, but are of difficult practical application<sup>25</sup>. In France, the 2004 ANAES

report (National Agency for Health Accreditation and Evaluation) recommends that pregnant women be trained in universal hygiene measures that should be heeded during pregnancy<sup>26</sup>. In fact, in the future only a vaccine will probably provide effective prevention of materno-fetal CMV infection.

## 2.5 Treatment of congenital CMV infection

There is no general agreement for treatment of congenital CMV infection during pregnancy. However, some antiviral drugs, notably valaciclovir, are under study. The three molecules commonly used for treatment of CMV infection in immune-depressed patients – ganciclovir, foscarnet or cidofovir – cannot be used during pregnancy because of their toxicity. Valaciclovir is well tolerated and has good bioavailability in pregnant women and in the foetus. A recent study shows that treatment of pregnant women (whose foetus is infected) with valaciclovir, can significantly lower viral load in fetal blood<sup>27</sup>. A randomised study on effectiveness of valaciclovir in materno-fetal infection is in progress (Cymeval study: treatment *in utero* of congenital cytomegalovirus (CMV) infection with valaciclovir - Multicentre perspective randomised trial versus placebo). In addition, another recent study shows the effectiveness of hyperimmune immunoglobulin administration on virus transmission to the foetus as well as on severity of fetal impairment<sup>28</sup>, but a randomised study is necessary to confirm these results.

The possible benefit of antiviral neonatal therapy with ganciclovir for sensory impairment or psychomotor retardation is hardly known. However, a randomised phase II study reports the beneficial effect on progression of hearing loss of a six-week ganciclovir treatment administered intravenously to children severely affected by central nervous system impairment<sup>29</sup>. In this study, hearing was improved or stabilised after six months in 81% (21/25) of children treated, but in only 59% (10/17) of the control group. Because ganciclovir is toxic by intravenous administration, it is now commonly agreed that it should be used only in newborn babies with central nervous system impairment. Valganciclovir is a ganciclovir precursor, whose bioavailability via oral administration is equivalent to that of ganciclovir via intravenous administration in adults and newborn babies. In addition, valganciclovir is well tolerated haematologically in more than 60% of treated newborn babies<sup>30</sup>. A case involving prolonged six-month treatment with valganciclovir of a newborn affected by severe CMV infection is reported in the literature<sup>31</sup>. In this case, valganciclovir, which proved effective in curbing and then eliminating viral load in urine samples, did not cause side effects and the child had normal hearing at 18 months. A randomised study comparing treatment efficacy with valganciclovir over six weeks and over six months in symptomatic newborn babies is in progress in the U.S. Other therapies may become available in the near future with the launch on the market of new effective and less toxic anti-CMV molecules such as, notably, maribavir.

### Clinical case No. 1

A pregnant woman after 30 weeks of amenorrhoea is referred to a prenatal diagnostic centre for fetal ultrasound abnormalities. The ultrasound scan shows a 20 mm dilation of the cerebral ventricles associated with uterine growth restriction of -3 standard deviations and with hyper-echogenic bowel. Maternal CMV serology tests performed by her medical practitioner after 29 weeks of amenorrhoea show positive IgG concentrations of 8 IU/mL and negative IgM concentrations.

**1) Do maternal serology tests mean that primary infection during pregnancy may be ruled out?**

*Answer:* No. The absence of IgM after 29 weeks of amenorrhoea means that primary infection may not be ruled out during the periconceptual period or at the onset of pregnancy.

**2) What laboratory tests should be carried out in order to rule out or confirm fetal CMV infection?**

*Answer:* The reference technique for diagnosis of prenatal CMV infection is detection of CMV-DNA with PCR test in amniotic fluid collected with amniocentesis.

### Clinical case No. 2

A pregnant woman after seven weeks of amenorrhoea shows CMV serological tests suggestive of recent primary infection. The woman and the doctors at the prenatal diagnostic centre decide to carry out amniocentesis in order to diagnose whether the foetus is infected.

**1) At what stage of pregnancy can this procedure be performed and why?**

*Answer:* After 21 weeks of amenorrhoea, when the fetal urogenital system is mature and more than eight weeks have elapsed from maternal primary infection and amniocentesis. If amniocentesis is carried out too soon, risks exist of false negative prenatal diagnosis.

**2) Which maternal virological test must be carried out before performing amniocentesis?**

*Answer:* The absence of maternal viraemia must be checked (negative PCR test in maternal blood) before amniocentesis.

**3) Amniocentesis is performed after 23 weeks of amenorrhoea while the fetal ultrasound scan shows no abnormalities. What is the probability that the foetus is infected?**

*Answer:* The probability of materno-fetal transmission is 50% in the presence of maternal primary infection.

**Clinical case No. 3**

A pregnant woman after 29 weeks of amenorrhoea is referred to a prenatal diagnostic centre because ultrasound scan detected fetal abnormalities with fetal ascites associated with hyper-echogenic bowel. Two years before, during a previous pregnancy, this woman had positive CMV IgG concentrations of 12 IU/mL in the absence of IgM. The recent tests show that CMV IgG concentrations are still positive at 11 IU/mL in the absence of IgM.

**1) Does a two-year old positive CMV serology test mean that fetal CMV infection can be ruled out?**

*Answer:* No. Fetal infection can be secondary to maternal primary or secondary infection. Only maternal CMV serology profile with negative IgG and IgM allows for fetal CMV infection to be ruled out.

**Clinical case No. 4**

A newborn is affected by intrauterine growth restriction and hepatosplenomegaly; the clinical picture is compatible with materno-fetal CMV infection.

**1) CMV serology profile is prescribed to this newborn and IgG is positive at 18 IU/mL in the absence of IgM. Does the result of this test allow for a diagnosis of materno-fetal CMV infection to be ruled out?**

*Answer:* No. Neonatal serology tests are not reliable to diagnose materno-fetal CMV infection. Indeed, IgM is positive in only 30% of cases.

**2) What is the reference test allowing diagnosis of congenital CMV infection in newborn babies?**

*Answer:* The reference test is CMV detection by viral culture or PCR test in urine samples.

**3) When should this test be prescribed?**

*Answer:* As soon as possible after birth, at the latest on the tenth day of birth. Indeed, if a later test is positive, it will be impossible to discriminate between congenital infection and CMV infection contracted at an early post-natal stage (during birth, breast-feeding).

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