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ROLE OF SECOND TRIMESTER ULTRASOUND IN PREDICTION OF NEWBORNS NEUROLOGIC DAMAGE AFTER MATERNAL CYTOMEGALOVIRUS INFECTION

Presentata da: Anna Nunzia Della Gatta

Coordinatore Dottorato

Susi Pelotti

Supervisore

Giuliana Simonazzi

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Introduction

1. Cytomegalovirus infection during pregnancy

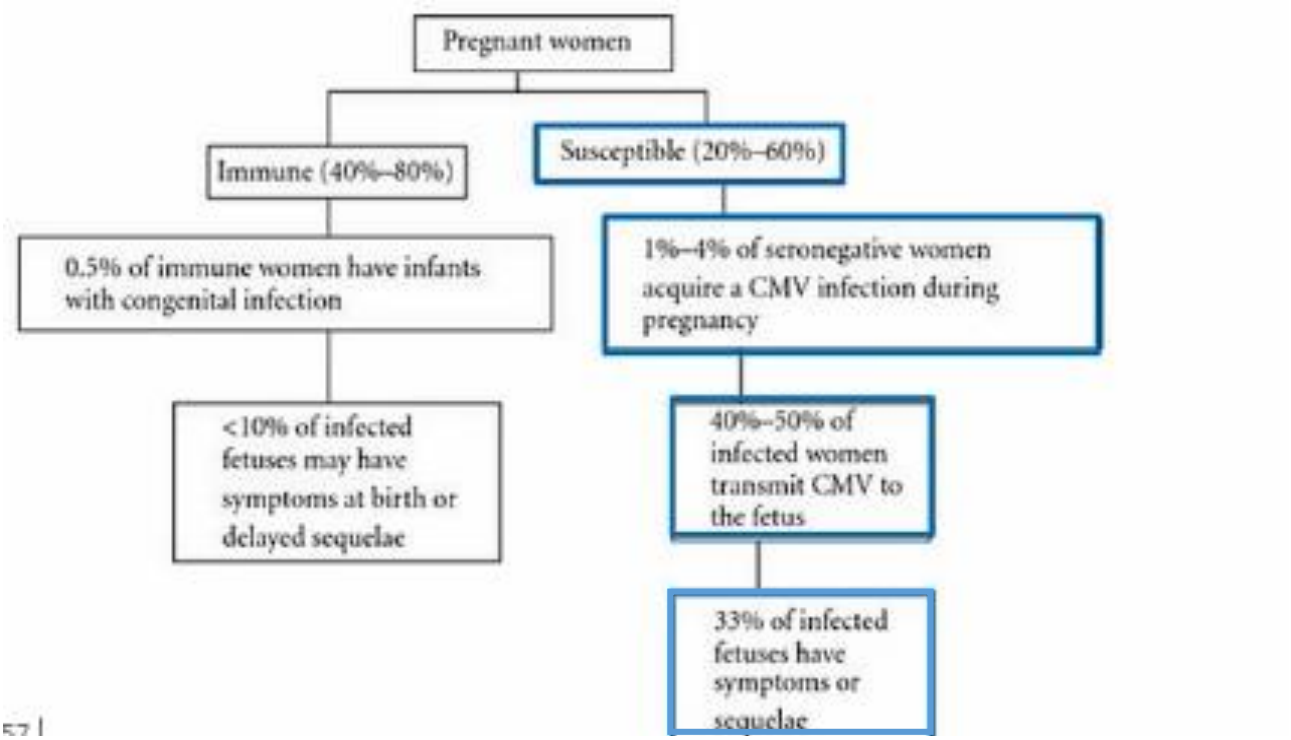
Cytomegalovirus (CMV, Herpesvirus 5) is the largest (220 nm in diameter) and most complex herpesvirus, with a 235,000 double-stranded DNA genome.1 It has a high specificity for humans and this infection is usually acquired by direct contact with infectious body fluids, such as urine, saliva, blood, tears, semen, and breast milk. CMV can be transmitted sexually and through transplanted organs and blood transfusions. Macrophages, endothelial cells and monocytes are the first target during CMV infection but it can actively replicate in liver and spleen. It causes a latent infection in monocytes after the primary infection, thus it can reactivate and replicate in seropositive hosts; these episodes are, usually, asymptomatic in healthy people, but can cause severe disease in immunocompromised people. Despite all, the existence of different types of CMV, can cause reinfection. 2 Usually it is asymptomatic or fever, weakness, myalgia, flu like symptoms are typical mild manifestations; these can be associated with an increase in liver enzymes, lymphocytes and prolonged viral shedding, even during the asymptomatic phases. 34

CMV is the most common cause of intrauterine infection, affecting 0.3 – 2% of live-born infants. Ten to Fifteen percent of congenitally infected infants are symptomatic at birth: intrauterine growth restriction, microcephaly, hepatosplenomegaly, thrombocytopenia, brain parenchymal calcifications, ventricle enlargement, cerebellar hypoplasia, and so forth. 90% of newborns, despite infected, are asymptomatic at birth. About 10 per cent of them will die and up to 15% of the survivors will develop long term sequelae, such as mental retardation, motor/hearing or visual impairment that can manifest later in life, even in asymptomatic neonates. 5 6 In fact, 5 – 15% of asymptomatic newborns are at risk of developing neurological sequelae such as deafness, visual impairment and neurological deficits. Most fetal and neonatal sequelae are associated with first- or second-trimester infections because, during third-trimester, the risk to the fetus and neonate is probably minimal. 7 Transplacental transmission of the virus may happen for disease acquired by the mothers during preconceive time, due to the slow viral replication, with a 25-45% transmission rate. 89

The risk of vertical transmission for maternal infection acquired in the 3 months before the conception is around 8%, while the risk is comparable to the first trimester acquired infection when mothers have the infection during the 4 weeks after the last period. (30.8%)10

Not only after primary CMV maternal infection may occur vertical transmission , but also after reactivation or secondary infection, through transplacenta transmission or during labor. There are remarkable differences between a primary and non primary infection in term of transmission rate and neonatal sequelae.

1.2 Epidemiology of congenital CMV infection



Adler et al, Cytomegalovirus during pregnancy Current Opinion in Obstetrics and Gynecology 2011, 23:123–128

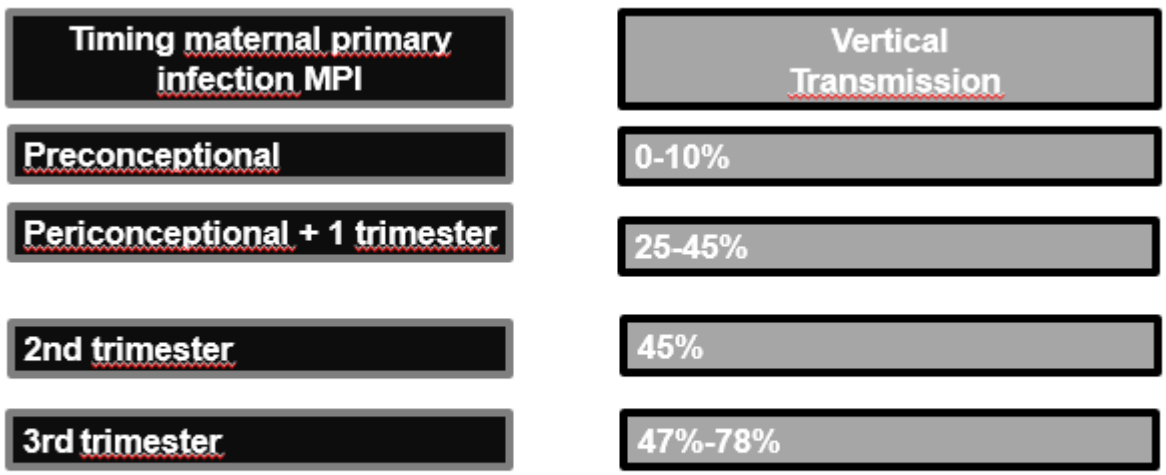
CMV is endemic, with no differences between the seasons. The serum prevalence of this virus in women in reproductive age is globally 86% (95% CI: 83-89), with the highest prevalence in the Mediterranean area (92%). 11 Neonatal CMV infection has a prevalence around 1% and the main risk factor seems to be young and to have at least one child. 12

The main prognostic factors are the gestational age when the infection is acquired and the presence of symptoms at birth. The risk of vertical transmission increase with the

progression of gestational age, from 5% for infections acquired in the weeks before the conception to 60% for those acquired in the third trimester. Fig 1 The most severe consequences of the infections come usually when it is acquired in the 4 weeks before the conception (29%) and in the first trimester (19%) with a drop to 1% after the second trimester.13 Different consequences of the infection secondary to different time of the gestation when it is acquired, are easily explained by the process of development of placenta and fetal brain.

Placenta is the first target of viral particles who spread through cytotrophoblasts, with the risk of infection higher as they are more differentiated. This is the biological explanation to the increase of the risk of vertical transmission with the gestational age. 13 On the other side, the chance to develop fetal damages is higher as earlier the CMV infection is acquired. The development of human brain starts at 4 weeks of gestational age, with a tiny layer of neuroepitelial cells who rapidly replicate to form the neural tube.9 CMV inhibits the cellular proliferation and differentiation, increasing the apoptosis of young neural cells. The severity of neural damage depends on when and in which phase the neural development is interrupted: the neural damage is around 70%for preconceptional infection,20% for infection acquired in the first trimester, 5% in the second trimester and unremarkable in the third timester . fig 2 14

Fig 1



Y Ville et al, Timing of primary maternal cytomegalovirus infection and rates of vertical trasmission and fetal consequences AJOG 2021; 30: 1213–1216

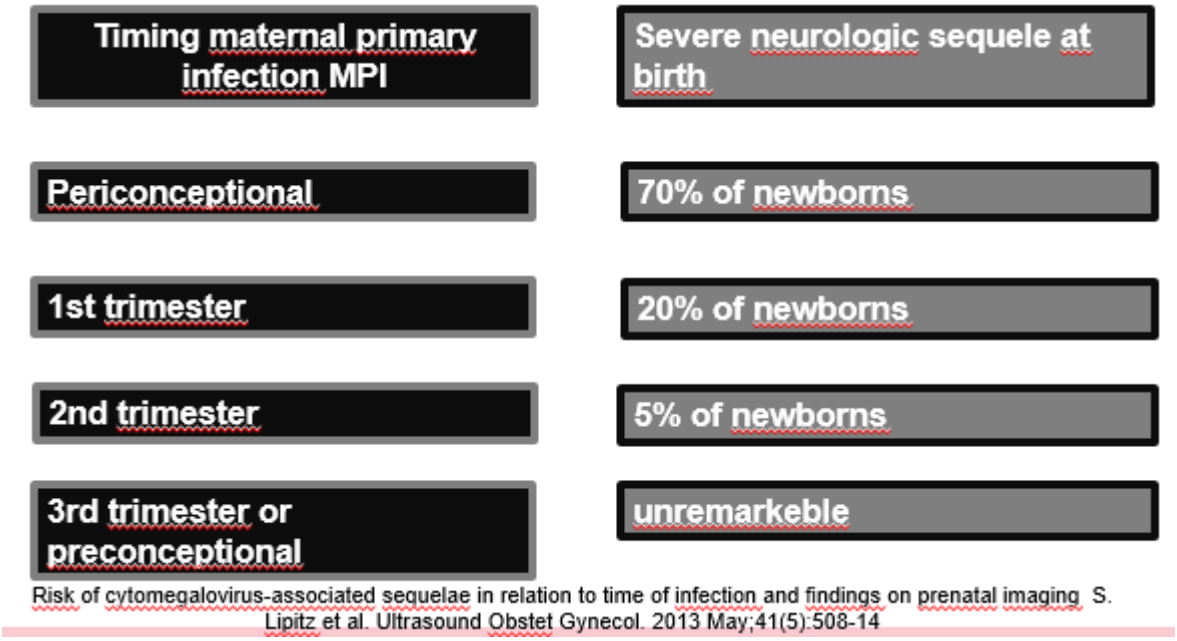


Fig 2

2 Diagnosis

Not every European countries test pregnant women for cytomegalovirus (CMV) during the first trimester of pregnancy, but these tests are usually performed in the presence of a suspect of infection at ultrasound. In Italy, according the National Sanitary System (SNN) guidelines for pregnancy (LEA) it is not mandatory to test routinely pregnant women for CMV. 15 Despite national recommendations, in several Italian regions, pregnant women are advised to undergo screening for IgG and IgM anti-CMV before 10 weeks' gestation: those who test seronegative are checked a second time at the end of the fourth month of pregnancy. Women with test results showing seroconversion or IgM positivity are sent to a referral center for further diagnostic investigation. The screening is performed with commercially available kits for both anti-CMV IgG and IgM.16

Soon after the first contact with the virus, human body usually produces IgM against CMV and, almost suddenly, IgG specific for the virus. After the initial acute phase, IgM usually disappear in 6-9 months, while IgG will test positive all life long. 16 Pregnant women who test positive for IgG and negative for IgM at their first control during pregnancy, even if they are at risk of reactivation or reinfection, are not required to undergo to further tests: the risk of a fetal infection is minimal. Women with positive IgM and negative IgG should undergo to a confirmatory test to check the seroconversion of IgG wich is confirmatory of primary infection with the second detection of IgM positive. If IgM are still positive and IgG negative at the second check, it could be a false positive test due to a cross reaction to other infections

(Parvovirus, Toxoplasma gondii, Virus Epstein-Barr etc) or autoimmune diseases. Pregnant women who test positive to IgG and IgM anti CMV with unknown prepregnancy sierologic state, could be of tricky evaluation and should undergo to further tests. 17

2.1Maternal primary infection (MPI)

Diagnosis of primary CMV infection is relatively straightforward if seroconversion is detected: that means positive IgM, before negative and positive IgG after 2-3weeks. When at first serologic test we find IgG and IgM positive for CMV it may be a recent infection or a state of antibody persistence, possible for 6 to 9 months after the acute phase. IgM may be persistent even in non primary infection. There are a lot of further tests that can be performed to distinguish among these cases:

- **IgG avidity test**. The IgG avidity test may be of great help in clarifying the clinical significance of IgM antibody. IgG avidity is indicative of the low functional affinity of the IgG class antibody. Early after primary infection, antibodies show a low avidity for the antigen, but progressively mature acquiring higher avidity. This characteristic is used at diagnostic level to discriminate recent primary infections in several viral diseases. 18 As antibody maturity is completed in 18-20 weeks from the contact, the positive predictive value of the test is reduced later in pregnancy, so it is used within 20 weeks of gestational age. Until 12-16 week of gestational age: low avidity of IgG is representative of primary infection; high avidity index are representative of a non primary infection. Independently from the gestational age, low avidity is always representative of a primary infection.
- **Western Blotting** is the elective test able to confirm IgM, differentiating primary from non primary infection on the basis of the different antibody profile against the viral proteins. 19 CMV IgM immunoblot tests are conceived for detection of CMV-specific IgM in human sera. The blots contain structural viral proteins purified from viral particles and recombinant structural and nonstructural proteins obtained by molecular biology. The sensitivity of this assay in detection of CMV infection is 100%, the specificity 98.6%, the positive predictive value 96.9%, and the negative predictive value 100%.20
- **Virologic tests**. In addition to serologic diagnosis, it is possible to test the presence of viral DNA in biologic fluids, like blood, saliva, urine, with a secondary role in the diagnosis of primary CMV infection. Blood samples of pregnant women with a known

serologic primary infection may test negative for CMV DNA, with no correlation with the risk of intrauterine transmission or fetal and neonatal deterioration. 21

2.2 Fetal CMV infection diagnosis

Gold standard for prenatal diagnosis is the detection of viral DNA in amniotic fluid as fetuses eliminate the virus through urines. The perfect timing for amniocentesis should be at least 8 weeks after the primary infection and after 17 weeks of gestational age. 22 The amniotic fluid analysis should be analyzed by shell vial method, in other words direct research of the virus or PCR to quantify viral genome. The diagnosis of congenital infection should be confirmed by analysis performed with two different methods (two different types of PCR or viral isolation). A negative result do not exclude a false negative one. 23

2.3 Neonatal CMV infection diagnosis

There is no universal screening ongoing in the developed countries for CMV detection in newborns. Neonatal diagnosis is recommended in the presence of a known primary maternal infection, in the presence of neonatal symptoms suggestive for CMV infection and in those who do not undergo the universal newborn hearing screening, despite the low sensitivity of this in identifying neonatal hearing loss CMV related. 24 PCR for the indentification of CMV DNA in saliva and urine within 3 weeks from birth is the diagnostic gold standard. 25

2.4 Congenital infection

Most neonates with congenital CMV never show signs or have health problems or they may just experience laboratory alterations like high levels of transaminases, jaundice with low level of bilirubin, purpura or low platelet levels. However, some babies have health problems at birth or that develop later as:

- Rash
- Jaundice (yellowing of the skin or whites of the eyes)
- Low birth weight
- Hepatosplenomegaly (enlarged liver and spleen)
- Seizures
- Retinitis (damaged eye retina)

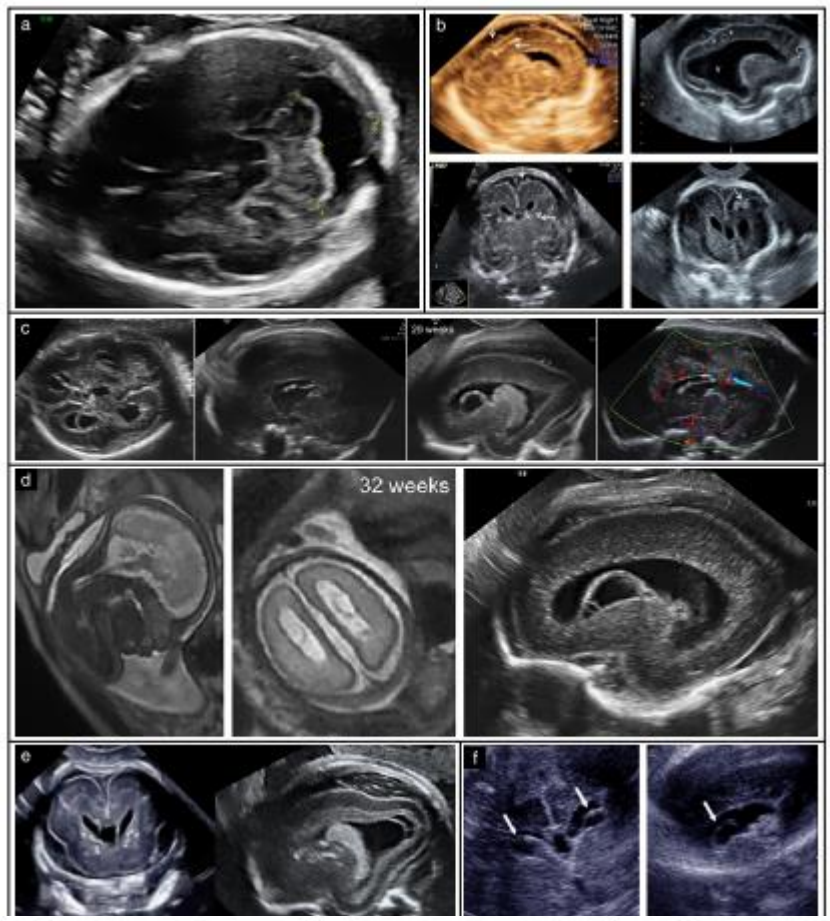
Some babies with signs of congenital CMV infection at birth can have long-term health problems, such as:

- Hearing loss: around 7% of asymptomatic newborns will experience it
- Developmental and motor delay
- Vision loss
- Microcephaly (small head) that is the most predictive factor of mental retardation, especially when associated with intrauterine growth restriction26
- Seizures

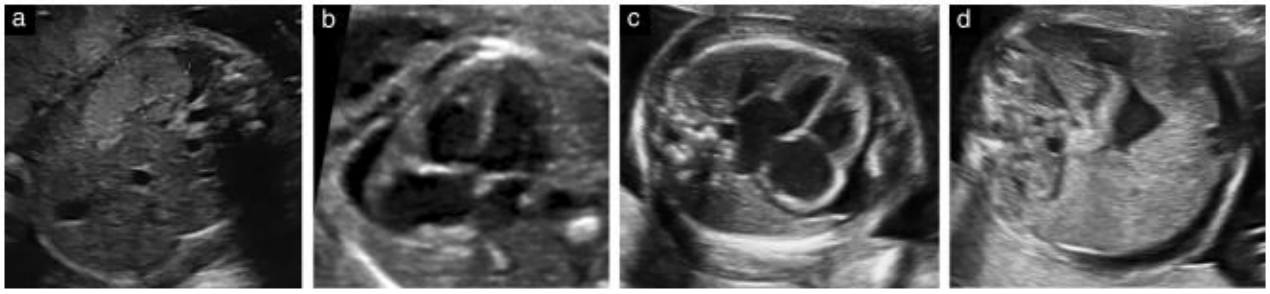
Some babies can have hearing loss at birth or can develop it later, even babies who passed the newborn hearing test or didn't have any other sings at birth. CMV is the most common infectious cause of birth defects in the United States. About 1 out of 200 babies is born with congenital CMV.26

Pseudo cysts, calcifications, ventriculomegaly, cerebral atrophy, cerebellar hypoplasia and calcifications, abnormalities in the development of white matter are usually found in more than 70% of symptomatic newborns. 27 The presence of cerebral abnormalities within first month after birth is the most predictive factor of psychomotor delays.28 More than 50% of newborns with symptomatic infection and 10% of asymptomatic, will develop neurosensory hearing loss. Less frequently, but retinitis and chorioretinitis, cataract and optic atrophy have been described. 29

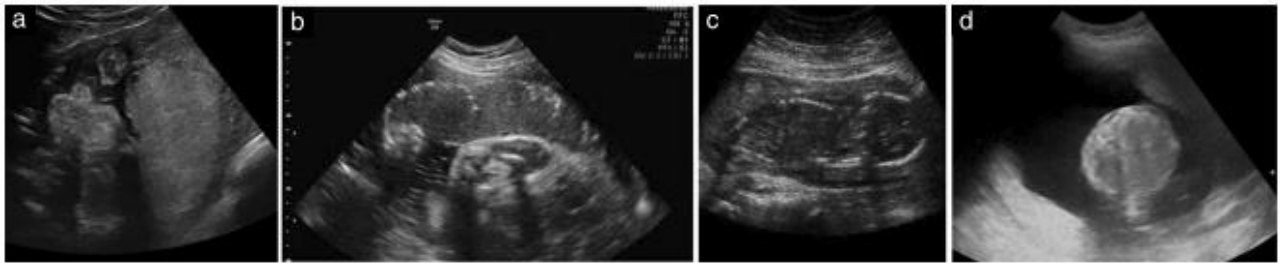
In the absence of a routine antenatal screening program for CMV, the most common circumstance in which CMV is diagnosed prenatally is following the discovery during a routine scan of an abnormal ultrasonographic finding suggestive of possible CMV infection. As a result of this non-systematic mode of discovery, severe ultrasound abnormalities are described more often than are subtle findings. Ultrasound findings can be categorized as fetal cranial, fetal extracranial and placental/amniotic fluid abnormality, as summarized in the description of the following tables 1,2,3. 30 31



1 Ultrasonographic and MRI cranial features typical of CMV infection vary, and include: megacisterna magna (a), intracranial calcifications (b), ventriculomegaly, germinolytic cysts, agenesis of corpus callosum and intraventricular adhesions (c,d), periventricular cystic changes (c,f), lissencephaly (d), cerebral calcifications and periventricular cysts (e) and subependymal cysts (f).



2 Ultrasonographic extracranial features typical of CMV infection vary, and include: splenomegaly (a), cardiomegaly (b,c), pericardial effusion (b,c), hydrops (c) and ascites (d).



3 Ultrasonographic placental/amniotic fluid abnormalities typical of CMV infection vary, and include: placentomegaly (a), placental calcifications (b), oligohydramnios (c) and polyhydramnios (d).

Congenital cytomegalovirus (CMV) infection can cause, as said, a wide range of brain anomalies: these changes have been well described postnatally, but descriptions of their in

utero evolution are scarce, so its antenatal diagnosis and prevention is a major challenge in perinatology. 32It is important to improve the clinicians' ability to assess and predict the outcome of congenital CMV infection in order to provide the most accurate counseling to worried couples. This area is innovative with very limited information in literature: there are only few studies on quantitative MR imaging in CMV 33, there are no studies on ultrasound 3D assessment of fetal brain with this infection and none had a follow-up or tried to examine a prospective correlation between the ultrasound findings and future outcomes. With these ideas in mind, the beginning project of this PhD Programme was the prospective analysis of the sonographic spectrum of intracranial abnormalities in fetuses with proved CMV infection and to determine characteristic patterns of this infection. In details, the project aimed to consider the specific effects of the infection on fetal cortical development. As described, CMV replication in the developing fetal brain, stops the neuronal migration, during the second trimester of pregnancy, to the cortical area, resulting in abnormal cortical development. In utero, it is usually diagnosed following the development of microcephaly and /or abnormal sulcation and gyration by using magnetic resonance imaging (RMI) after 30 weeks of gestation 34. Despite these evidences, to our knowledge, no studies have ever systematically assessed cortical development by using ultrasound scan, in particular fetal neurosonography,that is the methodology of choice for evaluating the fetal brain anatomy, insults and malformations. 35 36 The aim was to analyze, in pregnant women with primary human cytomegalovirus infection,by fetal neurosonography, the fetal cortical development in pregnancies with primary CMV (**Group A**, pregnant women with primary human cytomegalovirus infection, HCMV).

It was planned to perform three consecutive transabdominal scans at **19–21**, **26–28** and **30–34 weeks of gestation** (in conformity to our protocol for patients with primary HCMV infection) and a RMI at 30 weeks of gestation. 37

As planned, the protocol for the study was written and, after the evaluation by the ethical committee of Policlinico di S Orsola, Bologna, approved with number 593/2020/Oss/AouBo on the 10th 6 2020.

Despite all the efforts, the drop in cases of CMV infected patients, due to COVID and to a new therapeutic strategy to treat these fetuses, make impossible to reach the sample size of 30 patients per group.

The first drop was due to the COVID infection, that stopped our lives for two of the three years of this PhD Programme.

In 2021 I followed a Course of Statistic applied to clinical queries at University of Modena and Reggio Emilia. For the final exam, I presented a project with title "**Has pandemic changed the incidence of infectious diseases during pregnancy?"** analyzing data regarding the pregnant women who accessed to our outpatient service for infectious diseases acquired during pregnancy.

3. **Abstract: "Has pandemic changed the incidence of infectious diseases during pregnancy?"**

Background. The Coronavirus Disease pandemic represents a unique event during which the medical care systems in all over the world had to cope with a hard challenge. On one hand, healthcare workers had to face the emergency of thousands of people infected by an unknown virus, with limited resources, on the other hand they had to continue to ensure assistance and standard cares. Obstetric services were delivered at the same pace before pandemic, as most of these cares cannot be postponed. Every year we follow up around 400 women with a diagnosis of an infection acquired during pregnancy (TORCH, Toxoplasmosis, Rubeola, Cytomegalic Virus and Herpes Virus) or with a history of a chronic infection (Tuberculosis, Hepatitis).

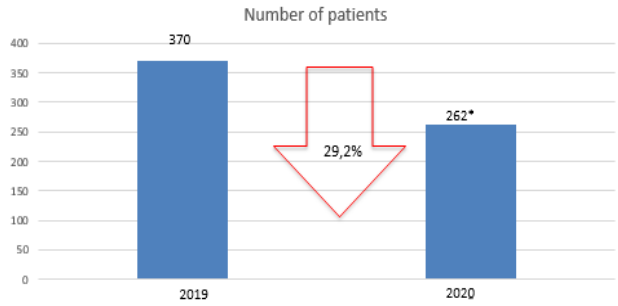


Fig 3

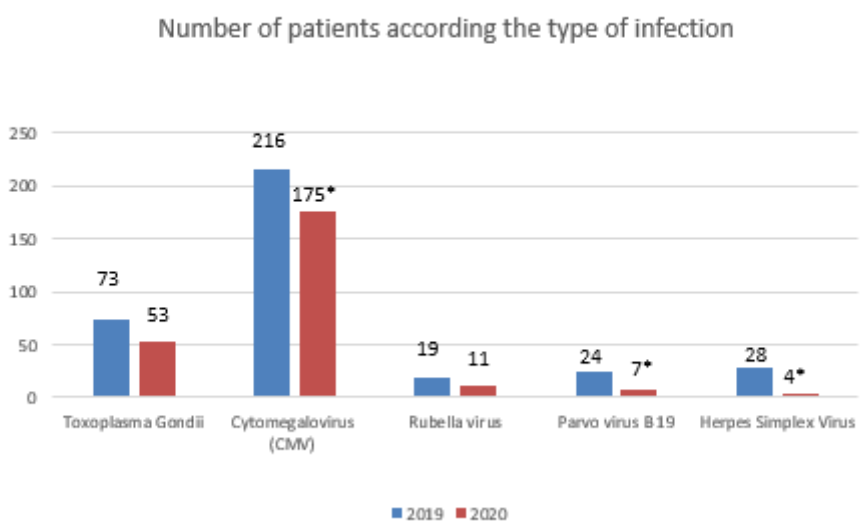
Objectives. - COVID 19 modified the number of women with an infectious disease during pregnancy, as our services were delivered despite the general lockdown? -Where there any differences among the recorded infections, according their way of transmission?

Methods. Retrospective registry based study was led at St. Orsola University Hospital, regional referee center for infectious diseases during pregnancy. Data regarding 2020 and 2019 were collected from registers were all the followed-up patients are usually recorded. The patients were divided according the reason for accessing our service. Differences between 2020 and 2019 were assessed with t-test. Differences in data were expressed as percentages.

Results and conclusion. Data showed a reduction in the total amount of patients referred to this service with a statistical significative difference between 2019 and 2020 (p=0,000, Reduction= 29,2%) figure3 Data showed that, over the studied months, there is a significative difference, between pregnant women with CMV, Parvovirus and Herpes infection. This can be explained by their way of transmission. Toxo, Rubella and other types of infection did not show any differences . **fig 4, 5**

Type of infection	2019	2020	P value	Reduction (%)
Toxoplasma Gondii	73	53	0.075	27.4%
Cytomegalovirus (CMV)	216	175	0.038	18.9%
Rubella virus	19	11	0.144	42.1%
Parvo virus B 19	24	7	0.002	70.8%
Herpes Simplex Virus	28	4	0.000	85.7%
MISCELLANEA (HepatitisC Virus, Treponema pallidum, Mumps virus, Lyme disease)	22	12	0.086	45.5%

Figure 5



4. Congenital CMV prevention

4.1 Hygiene based measures

There is currently no licensed vaccine for CMV. The main strategy to reduce the risk of infection is behavior modification in order to limit direct contact with saliva or urine of young children who may be excreting CMV particles in these fluids. Simple hygiene-based measures to reduce the risk of CMV acquisition include avoiding sharing utensils, drinks or food with young children, not kissing young children directly on the lips and handwashing after contact with their urine or saliva.38

4.2 Hyperimmune globulin (HIG)

The CHIP Study Group 39 was the first study reporting that CMV HIG therapy was associated with a significantly lower risk of congenital CMV infection, especially symptomatic infection. Further studies reported that, after a primary maternal CMV infection in the first trimester, biweekly HIG administration at a dose of 200 IU/kg prevented maternal–fetal transmission up to 20 weeks' gestation40. Unfortunately, the potential efficacy of HIG was not borne out in a phase-II randomized, placebo-controlled, double-blind study41, which found no significant improvement in the risk of transmission, levels of virus-specific antibodies, T-cell mediated immune response, viral DNA in the blood or clinical outcome at birth. Given these conflicting findings, HIG is currently not recommended routinely for the treatment of women with primary CMV infection in pregnancy. A trial assessing HIG in pregnancy was expected to finish in 2018, but this study was stopped for futility before completion. 42

4.3 Antiviral Drugs

A number of antiviral drugs are active against CMV and are being used in the treatment of immunocompromised individuals. Ganciclovir and aciclovir (ACV) have proven to be effective in preventing CMV infection in kidney transplantation 43 and Ganciclovir has been used successfully in treatment of neonates,44 45 but its known teratogenic and haematopoietic adverse effects contraindicate its use during pregnancy. Treatment of CMV infection with antiviral drugs, like Aciclovir ACV, valganciclovir or valaciclovir VACV significantly reduced the risks of CMV disease and associated mortality in recipients of solid-organ transplants, by reducing viraemia. 46 There are no randomized controlled studies on the use of Valaciclovir during pregnancy. Two multicentre, open-label, phase II studies demonstrated that giving high dose of valaciclovir for a median of 89 days to pregnant

women with a diagnosis of CMV infection (carrying a moderately infected fetus with extracerebral ultrasound signs of infection) was associated with a significantly greater proportion of neonates born asymptomatic (82%) compared with a historical cohort (43%). This study also provided reassuring safety data for the use of valaciclovir in pregnancy: maternal clinical and laboratory tolerances to this high-dose regimen were excellent, and no adverse neonatal effects were observed. 4748

On 2020 the Italian Society of Infectious Diseases and the Italian Society of Perinatal Medicine, required to AIFA, the Italian Agency of Drugs, to enlist Valaciclovir among the drugs to be used off label, to prevent CMV infection and to treat fetuses infected by CMV, according the law 649/96. Valaciclovir should be administered at the dose of 2 g every six hours, to a total of 8 g per day to pregnant women with primary CMV infection, diagnosed in the first 24 weeks of gestation or within 4 week before the conception. Eligible women should undergo the treatment as soon as possible and it could be suspended if the PCR for CMV on amniotic fluid results negative. If the pregnant woman do not want to perform an amniocentesis, treatment should be continued regardless until 26 weeks of gestational age, 49 For those women with a positive test for CMV DNA in amniotic fluid or with fetal moderate disease, treatment should be continued until the end of gestation. Exclusion criteria were abnormal amniotic fluid, fetal growth restriction, ascites, hydrops, liver calcifications, fetal anemia diagnosed by ultrasound Doppler of middle cerebral artery, ventriculomegaly, lissencefaly, periventricular cysts, abnormal corpus callosum.

5. Use of Valaciclovir in CMV infected fetuses. MEGAL-ITALI Trial.

Background.

Our outpatient clinic in Policlinic S Orsola is a regional referral center for the treatment of pregnancies complicated by infectious diseases and in 2021 it took an active part in the MEGAL-ITALI a multicenter observational study about the use of Valaciclovir as therapy for pregnant women with a primary CMV infection.

Objectives.

To test safety and efficacy of Valaciclovir administered to pregnant women with primary CMV infection by evaluating the transmission rate from mothers to fetuses considering positive amniocentesis for CMV DNA. The comparison group was a cohort of women with same diagnosis, followed up in our clinic during the year before the approval of Valaciclovir as a safe treatment during pregnancy.

Material and Methods

Prospective Observational study of pregnant women with primary CMV infection referred to our outpatient service in IRCSS-Policlinico Sant'Orsola between March and November 2021 who gave their informed consent to treatment with Valaciclovir. The figure below (5) summarize the details of the enrollment.

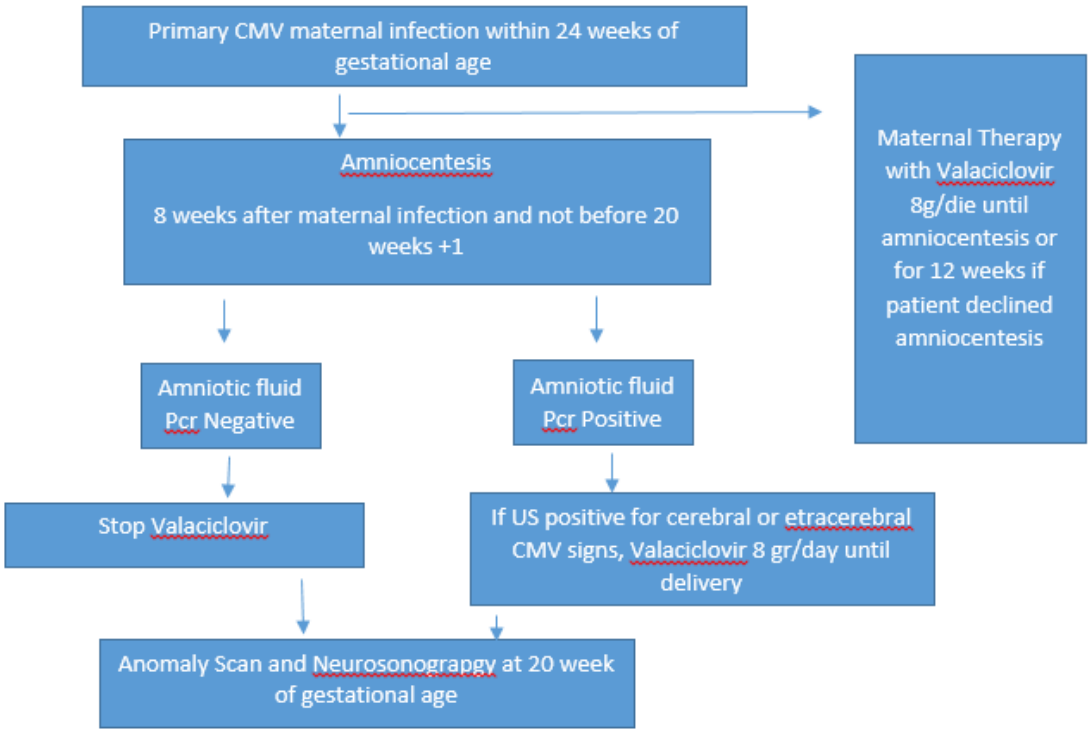


Figure 5

Results

Numeric variables were summerized as media +- standard deviation [range interquartile (IQR)]; categoric variables as percentages and comparisons between the two groups were lead by using t-test. A logistic regression was performed to test the correlation between the primary maternal infection, and gestational age at amniocentesis fig 6 . Valaciclovir (VACV) efficacy was tested with a two way table and standard logistic regression with pericnceptional infection and gestational age of amniocentesis as covariates. All analysis were performed using Stata software,16 version (StataCorp. 2019. Software statistico Stata: Release 16. College Station, TX: StataCorp LP). Significance level was established at 0.05.

	TREATED (20)	CONTROLS (71)	P (<0.05)
GESTATIONAL AGE AMNIOCENTESIS	108 days	107 days	0.91
GESTATIONAL AGE INFECTION	90.2 days	90.4 days	0.98
VERTICAL TRANSMISSION RATE AFTER MPI			
	0 (15)*	14(53)	0.000

*Data based upon amniocentesis

Figure 6

Between January 2019 and November 2021 94 patients with primary CMV infection were referred to our Centre. 68 of these underwent amniocentesis 8 weeks after maternal infection. The drug, approved at the end of 2020, was proposed as sperimental therapy to 23 women, but only 20 accepted to be enrolled in the study (87%). Medium time lapse **between diagnosis and therapy was 17.2+-14 days. Medium time of administration of**

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VERTICAL TRANSMISSION RATE AFTER MPI			
	0 (15)*	14(53)	0.000

*Data based upon amniocentesis

VARIABLE	ODDS RATIO (95%IC)	P (<0.05)	IC
VALACICLOVIR TREATMENT	1	0.65	0.90/1.50
PERICONCEPTIONAL INFECTION	0.2	0.044	0.05/0.95
GESTATIONAL AGE AT AMNIOCENTESIS	1	0.55	0.85/1.34

	POSITIVE AMNIOCENTESIS	NEGATIVE AMNIOCENTESIS	
TREATED WITH VALACICLOVIR	0/15	15/15	TREATED GROUP **EER= 0**
CONTROLS	14/53	39/53	CONTROL GROUP **CER=0.26**
TOTAL NUMBER OF PATIENTS	14	53	

EER RISK FOR THE EVENT IN THE TREATED

CER RISK FOR THE EVENT IN THE CONTROLS

FIGURE 7

The therapy was 107.6+-18 days. 5 patients rejected amniocentesis and therapy was stopped after 12 weeks. All patients who did the therapy tested negative for the viral DNA at amniocentesis. (15/15, 100%).

6 Role of second trimester ultrasound in prediction of newborns neurologic damage after maternal Cytomegalovirus infection

Rationale

After this necessary introduction that is a little summa of this PhD Programme and justify the drop in the number of cases useful to be enrolled in the initial project, we focused our attention on another serious challenging problem for Fetal Medicine Specialists that usually cope with women with CMV infection.

In Italy, according the law n. 194/78 it is possible to stop the pregnancy until 22 weeks of gestational age, despite the abnormalities that could be found later at ultrasound. So, in the management of these women, especially for diagnosis of infection performed in the first trimester, the ultrasound anatomic assessment is extremely important, playing a very important role in the decision about carrying on with the pregnancy, when possible. The main objective in this collection of cases was to find a correlation between cerebral symptoms at birth and abnormalities found at referral US assessment performed in the second trimester, in other words we wanted to check the sensitivity of ultrasound in the prediction of severe CMV neonatal disease.

Materials and Methods

This was a retrospective collection of all cases of primary congenital CMV infection reported in our unit over a period of 9 years (2013–2022). Only cases of fetal infection following confirmed maternal primary infection in the first trimester (MPI) and newborns with confirmed CMV infection on blood/saliva or urine were included. This study was conducted in conformity with the principles and regulations of the Helsinki Declaration. The protocol was approved by the Ethics Committee “Area Vasta Emilia Centro—AVEC”, Bologna, Italy (protocol no. 104/2017/O/Oss and 593/2020/Oss/AouBo).

All cases were assessed virologically and reviewed to confirm and date the maternal primary infection. Diagnosis of maternal primary CMV infection was based on:

a. seroconversion identified on two sequential serum samples, with negative CMV-specific immunoglobulin G (IgG) in the first sample and positive CMV-IgG in the second sample in the same laboratory, or
b. positive CMV-specific IgG and CMV-IgM, and low IgG avidity (different commercial kit)

The estimated time since the onset of primary infection was determined based on criteria described previously50. Briefly, positive CMV-IgM/negative CMV-IgG indicated up to 15 days after onset of infection; positive CMV-IgM/positive CMV-IgG with avidity index < 20% indicated 2–4 weeks after onset of infection; positive CMV-IgM/positive CMV-IgG with avidity index of 20–40% indicated 5–7 weeks after onset of infection; and positive CMV-IgM/positive CMV-IgG with avidity index of 40–60% indicated 8–12 weeks after onset of infection. Infection was defined as periconceptional when it occurred within 4 weeks before or 2 weeks after the date of conception, first-trimester when it occurred between 2 and 14 weeks of gestation, second-trimester when it occurred between 14 and 28 weeks' gestation and third-trimester when it occurred after 28 weeks' gestation. Cases with high IgG avidity in the first trimester were considered as non-primary infections and were excluded from the study.

All the included cases were neonates who had a confirmatory postnatal diagnosis of CMV infection by saliva or urine sample with a positive detection of viral DNA from polymerase chain reaction (PCR). Not all the included cases had amniocentesis, as it was usually offered to all women during the second trimester but it not always was accepted. Amniocentesis was usually performed at least 8 weeks following seroconversion and not earlier than 17 weeks of gestation.51 Targeted US examination was performed, using a Voluson Expert E8 or E10 machine (General Electric, Zipf, Austria), at 20-22 weeks, at 28-32 weeks and at 34-36 weeks every fortnight from referral until delivery or termination of pregnancy (TOP). US features suggestive of fetal CMV infection were recorded as part of serial targeted fetal US examination. All US reports performed during the pregnancy were retrospectively reviewed and a synthetic conclusion was notified for each of them according to the features that were reported: normal US, extracerebral features, or cerebral features. The MRIs were offered to every patients but not always performed. No maternal or fetal sedation or contrast agents were used. The women were placed in a supine or lateral decubitus position. The radiologists all had over 15 years of experience in the interpretation of fetal MRI studies, and were aware of the results of the US at the time that the MRI images were interpreted.

Our objective was to define the predictive values of these parameters on the prognosis of fetal infection when performed at the end of the second trimester. Categorical variables were summarized using media, median, standard deviation and interquartile range. Groups were compared by using exact Fisher Test and analysis variance (ANOVA). Statistical analysis was performed using Statistical Package for Social Science (SPSS) software, version 27 (IBM Corp. Released 2019. IBM SPSS Statistics for Windows, IBM Corp: Armonk, NY, USA). The significance level was set at $p < 0.05$.

Results

Between 2014 and 2022, 74 fetuses had an antenatal diagnosis of primary CMV infection, confirmed by our laboratories and followed up in our Hospital with regular US scans during the second and third trimesters and, when accepted by the mothers, amniocentesis and a fetal MRI. 5 cases were excluded from the study as the pregnancy was interrupted after the diagnosis. The infection occurred after MPI in the first, second, and third trimester in 62.3% (43/69), 27.5% (19/69), and 10% (7/69) of cases, respectively. Only cases of neonatal confirmed infection following MPI in the first trimester were analysed (n = 43). Magnetic resonance imaging (MRI) was performed only in 38% (26/69) of cases at a median gestational age of 32 +-2 weeks of gestation. Referral US assessment performed in the second trimester was abnormal in 10/69 (14,5%) fetuses: 5/69 (7%) had an extracerebral STA and 5/69 (7%) had a cerebral STA. Normal anomaly scan was found in 59/69 (86%) fetuses. When looking at all fetuses infected in the first trimester, 35% (15/43) had symptoms at birth. All the cases of pregnancies who underwent termination, the infection was acquired

		Symptomatic	n Symptomat	Tot	Sens.	Spec.	PPV	NPV	Youden's J	P[A]
n		18	51	69						
Trimester infection										
- 1°		15	28	43	83%	45%	35%	88%	0,284	0,047
- 2° o 3°		3	23	26						
Anomaly scan										
- Abnormal		6	4	10	33%	92%	60%	80%	0,255	0,016
- Normal		12	47	59						
Amniocentesis										
- Positive		8	19	27	89%	30%	30%	89%	0,185	0,396
- Negative		1	8	9						
- Not performed		*9*	*24*	*33*						
Magnetic Resonance (MR)										
- Positive		2	4	6	29%	79%	33%	75%	0,075	1,000
- Negative		5	15	20						
- Not performed		11	32	*43*						
Trimester infection+	Anomaly scan									
- 1°	- Abnormal	6	3	9	40%	89%	67%	74%	0,293	0,046
	- Normal	9	25	34						
- 2° o 3°	Anomaly scan									
	- Abnormal	0	1	1	0%	96%	0%	88%	-0,043	1,000
	- Normal	3	22	25						
[A] Fisher's Exact Test										

in the first trimester. A mean follow-up of 22.4 months (range 12–48 months) was available for 68/69 (99%) live born neonates. Fig8

Figure 8

Postnatal outcome of fetuses according the second-trimester assessment

Of the 69 fetuses, 59 had a normal referral US assessment performed in the second trimester (86%), but 12 of these (12/69, 17%) newborns were symptomatic at birth. Of the 43 fetuses infected in the first trimester of pregnancy, 15 were symptomatic at birth. The Youden Test showed the highest correlation between the trimester of infection and the presence of symptoms at birth. Youden's *J* statistic is a single statistic test that captures the performance of a dichotomous diagnostic test. The index is a way of summarising the performance of a diagnostic test, Its value ranges from -1 through 1 (inclusive), and has a zero value when a diagnostic test gives the same proportion of positive results for groups with and without the disease, i.e the test is useless. A value of 1 indicates that there are no false positives or false negatives, i.e. the test is perfect. There is a significative difference in acquiring the infection in the first trimester (p=0045) than in the second or third, in terms of being symptomatic at birth. The only anomaly scan seems to have a predictive negative value of the 80%: 10 out of 69 scans were positive for abnormalities with the identification of the half of the symptomatic newborns. The sensitivity remains low (33%).

Terminations of pregnancy (TOP)

TOP was performed in 5 cases, all fetuses infected during the first trimester and this was the total number of tops (7% on the totality, 5/74). One fetus had a normal anomaly scan but positive amniotic fluid to CMV DNA and neurofetopathological autopsy reported multi-visceral involvement. Four fetuses had an abnormal cerebral anomaly scan and neurofetopathological autopsy reported CMV encephalitis. As one of the criteria of the study is the presence of symptoms at birth, these cases were excluded from the evaluation of the performance of the ultrasound

Discussion

In this study we demonstrated that the combination of the trimester at diagnosis (first) and the referral US assessment performed in the second trimester are able to predict symptoms at birth in 60% of fetuses diagnosed with CMV infection following a maternal primary infection (p=0.046). Second-trimester assessment was normal in 86% of fetuses, and was abnormal in 15% of fetuses, respectively. When the anomaly scan, performed at a median gestational age of 20 weeks of gestation, was normal, the negative predictive value for the risk of moderate to severe sequelae was 74%. The PPV and negative predictive value (NPV) of MRI for these sequelae were 33 and 75%, respectively; unfortunately, it was not available, because not performed in the 62% of cases. As well the PPV and negative predictive value (NPV) of amniocentisis for these sequelae were 30 and 89%, respectively; unfortunately, it was not available, because it was performed in the 52% of cases.

As the primary prevention strategy has not been found to perform well consistently across studies and, in addition to this, the absence of a CMV vaccine make secondary prevention of congenital infection following MPI a public-health priority52. In line with reports of transplacental transmission rates increasing from around 10% in the periconceptional period to up to 40% at the end of the first trimester53, we observed that 83% (15/18) of the symptomatic neonates had acquired the infection during the first trimester. Even if universal serological screening for CMV in pregnancy is not recommended, recent literature findings might completely change the attitude towards this approach. Cost and the cost-effectiveness of testing and treatment for CMV during pregnancy should be assessed, once more solid evidence on treatment interventions will be available. An economic analysis, conducted in the US, reported that universal maternal serology screening would be cost-effective if a prevention strategy could lead to a reduction in mother to foetus transmission of more than 47% 54. Moreover, several studies have been demonstrating the possible efficacy of Valacyclovir in prevention of vertical transmission of cytomegalovirus after first trimester primary maternal infection. Nissan et al. found that patients with a primary cytomegalovirus infection during the first trimester treated with valaciclovir less likely had a positive amniocentesis for cytomegalovirus (two [11%] of 19 amniocenteses) compared with the placebo group (11 [48%] of 23 amniocenteses; p=0·020. With no clinically significant adverse events reported.55 This new effective therapeutic strategy is going to unavoidably change the diagnostic panorama in the next years.

Strengths

This is, in our knowledge, one of the first study including only cases with documented sequelae at birth and with high risk of developing them as the infection was acquired in the first trimester, indeed, the majority (43/69) of fetuses were infected after maternal primary infection in the first trimester. The evaluation of fetuses, neonates, and children were described in association with a follow-up to over 22 months of age. The evaluation of infected fetuses was based upon US scans, MRI, and amniocentesis. None of the pregnant women included in this cohort received Valaciclovir as an antenatal treatment for fetal infection. In this way, we eliminated a possible confounding factor. This treatment may have reduced the risk of symptoms at birth for those fetuses.

Limitations

The collection of data was, unfortunately, retrospective and low cases underwent MRI and Amniocentesis, as women did not always agree to do these tests in our unit. It could have been very interesting to test the sensitivity and specificity of combination of more than one test (MRI, Amniocentesis, Anomaly scan) as already described in literature56, but, as said, only few cases had all the tests performed.

Conclusion

Second trimester scan represents a miliar stone in the evaluation of prognosis of infected fetuses by CMV and it has a predictive positive value of 67% fetuses infected in the first trimester. Serial assessment by ultrasound is necessary to predict the risk of sequelae occurring in 35% following fetal infection in the first trimester of pregnancy. This combined evaluation by US and trimester of infection should be useful when counselling on the prognosis of cCMV infection, especially for those couples who are in doubt regarding the prosecution of pregnancy as they would be reassured by a negative second trimester scan.

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