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# Role of fetal magnetic resonance imaging in fetuses with congenital cytomegalovirus infection: a multicenter study

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## CONTRIBUTION

### What are the novel findings of this work?

Fetal MRI can detect additional brain anomalies in about 10% of fetuses with congenital CMV infection and negative neurosonography at first examination. CMV viral load was the only independent predictor of detecting fetal anomalies at MRI at a later gestation, thus confirming its prognostic value, mostly when associated with antenatal imaging.

### What are the clinical implications of this work?

The findings from the study support a detailed follow-up imaging throughout the remainder of the pregnancy after the diagnosis of fetal infection and the use of fetal MRI even in case of negative neurosonography to better predict the postnatal prognosis of infected newborns.

**ABSTRACT**

**Objective:** To investigate the role of fetal brain MRI in detecting anomalies in fetuses with congenital CMV infection undergoing neurosonography.

**Methods:** Multicenter, retrospective, cohort study involving 11 referral fetal medicine centers in Italy from 2012. The inclusion criteria were fetuses with congenital CMV infection diagnosed by PCR analysis of amniotic fluid, detailed multiplanar assessment of the fetal brain as recommended by the International Society of Ultrasound in Obstetrics and Gynecology (ISUOG), normal karyotype and MRI performed within 3 weeks from the last ultrasound examination. The primary outcome was the rate of central nervous system (CNS) anomalies detected exclusively on MRI and confirmed after birth or autopsy in fetuses with a prenatal diagnosis of congenital CMV infection and normal neurosonographic assessment at diagnosis. Additional CNS anomalies were classified into anomalies of the ventricular and the periventricular zone, intra-cranial calcifications in the basal ganglia or geminal matrix, destructive encephalopathy in the white matter, malformations of cortical development, delayed myelinization, midline, posterior and complex brain anomalies. Univariate and multivariate logistic regression analysis were used to identify and adjust for potential confounders.

**Results**: The analysis included 95 fetuses with a prenatal diagnosis of congenital CMV infection and normal neurosonography at first examination. The rate of structural anomalies detected exclusively at fetal MRI was 10.5% (10/95). When considering the type of anomaly, malformations of cortical development were detected at MRI in 40% (4/10) of fetuses, destructive encephalopathy in 20% (2/10), intracranial calcifications in the germinal matrix in 10% (1/10), and complex anomalies in 30% (3/10). At the multivariate logistic regression analysis, only CMV viral load in the amniotic fluid >100,000 copies/ml (OR: 12.0, 95% CI 1.2-124.7, p=0.04) was independently associated with the likelihood of detecting fetal anomalies at MRI, while maternal age (p=0.62), maternal body mass index (BMI) (p=0.73), maternal primary CMV infection (p=0.31), first trimester infection (p=0.685), prenatal therapy (p=0.11), or the interval between ultrasound and MRI (p=0.27) were not. Associated anomalies were detected exclusively at birth and missed at both types of prenatal imaging in 3.8% (3/80) of fetuses with congenital CMV infection.

**Conclusions**: Fetal brain MRI can detect additional anomalies in a significant proportion of fetuses with congenital CMV infection and negative neurosonography. Viral load in the amniotic fluid was an independent predictor of the risk of associated anomalies in these fetuses.

The findings from the study support a longitudinal evaluation using fetal MRI in congenital CMV infection even in cases with negative neurosonography at diagnosis.

## INTRODUCTION

Cytomegalovirus (CMV) is an enveloped, DNA virus of the Herpesviridae family and is the most frequent congenital viral infection, with a birth prevalence ranging from 0.5 to 1.3%.[1-4] CMV infection is recognized as the leading cause of non-genetic neurosensorial hearing loss and one of the most common causes of infection-related congenital malformations and impaired neurological outcome.[5]

Based on the significant clinical relevance and considering that the infection is usually associated with no or nonspecific symptoms, many researchers advocate for maternal serologic screening for CMV in pregnancy, while others defer the antenatal diagnosis to suspected findings at ultrasound.[6]

The gold standard for the diagnosis of congenital CMV infection is the identification of CMV DNA in the amniotic fluid confirmed at polymerase chain reaction (PCR) after amniocentesis at approximately 20 weeks' gestation.[7]

Once the diagnosis of congenital CMV infection has been established, the main goal of prenatal diagnosis is to determine the prognosis, i.e. postnatal outcome. In this scenario, gestational age (GA) at infection, the presence of fetal anomalies and the viral load in the amniotic fluid have been described as plausible determinants of poor prognosis in infected fetuses.[7-9]

In the last few years, previous studies have reported a significant contribution of fetal MRI in detecting associated anomalies in fetuses with isolated central nervous system (CNS) malformation undergoing a multiplanar assessment of fetal brain (namely, neurosonography).[10-15] Few studies have also investigated the role of fetal MRI in congenital infections, the small sample size of these studies, the inclusion of cases already presenting with ultrasound anomalies and the heterogeneity in the prenatal imaging protocol and management adopted do not allow us to extrapolate objective evidence to guide clinical practice and prenatal counselling.[16]

Therefore, the main aim of this study was to elucidate the role of fetal brain MRI in detecting anomalies in fetuses with congenital CMV infection undergoing neurosonography.

## METHODS

### Study design and participants

This was a multicenter, retrospective, cohort study involving 11 referral centers in Italy from 2012 to 2021 (Brescia, Chieti, Foggia, Modena, Naples, Padova, Rome - Catholic University of Sacred Heart, Rome - Sapienza University, Rome – Tor Vergata University, Treviso, Trieste). The study included a non-consecutive series of pregnant women with primary or non-primary CMV infection confirmed by PCR analysis of amniotic fluid.

The inclusion criteria were: fetuses with congenital CMV infection diagnosed by PCR analysis of amniotic fluid obtained by amniocentesis performed after 20 weeks of gestation or after 6 to 8 weeks after maternal seroconversion as recommended by international guidelines,[7] detailed multiplanar assessment of the fetal brain at trans-abdominal and/or transvaginal ultrasound as recommended by the International Society of Ultrasound in Obstetrics and Gynecology (ISUOG),[17-18] normal karyotype (including chromosomal microarray when available), maternal age ≥ 18 years and MRI performed within 3 weeks from the last ultrasound examination.

Longitudinal, detailed ultrasound follow-up after diagnosis of fetal infection was performed every 2 to 4 weeks, according to each local protocol, including a comprehensive evaluation of fetal growth, placenta, amniotic fluid volume, fetal Doppler and the assessment of potential ultrasound markers of fetal disease.

Fetal MRI was performed according to the standardized planes for fetal brain examination according to ISUOG guidelines for fetal MRI.[19] After birth, all newborns underwent a detailed ultrasound assessment always associated, in case of pre- or post-natal suspicion of anomaly, by MRI.

Cases with ultrasound anomalies at diagnosis and chromosomal anomalies or genetic syndromes detected either before or after birth were excluded. The clinical records were examined, and data collected in a dedicated merged database. STROBE guidelines were followed.[20]

### Outcomes

The primary outcome of the study was to report the rate of CNS anomalies detected exclusively on MRI and confirmed after birth with post-natal MRI or autopsy in fetuses with a prenatal diagnosis of congenital CMV infection and normal neurosonographic assessment at diagnosis. Furthermore, we aimed to report all factors associated with the likelihood of detecting these anomalies at fetal MRI.

For the purpose of this analysis, additional CNS anomalies were classified into:

- Anomalies of the ventricular and the periventricular zone, including ventriculomegaly, periventricular calcifications, intraventricular adhesions, periventricular cysts
- Intra-cranial calcifications in the basal ganglia or geminal matrix
- Destructive encephalopathy in the white matter including hemorrhage, porencephaly or periventricular leukomalacia
- Malformations of cortical development, including lissencephaly, cortical dysplasia, pachygyria, heterotopia, polymicrogyria or schizencephaly
- Midline anomalies, including complete and partial agenesis (ACC), hypoplasia (HCC) and dysgenesis of the corpus callosum or isolated absence of the cavum septum pellucidum
- Posterior fossa anomalies, including all defects involving the cerebellar vermis and/or hemispheres
- Complex brain anomalies, including all defects characterized by the presence of multiple intra-cranial anomalies.

**Statistical analysis**

We investigated the relationship between the presence of structural malformations assessed through fetal MRI (primary outcome) and several maternal and fetal characteristics, including maternal age and body mass index (BMI), gestation at maternal CMV infection, GA at first ultrasound assessment, GA at MRI assessment, interval between last ultrasound examination and MRI, GA at amniocentesis, viral load and prenatal therapy. We planned also sub-group analysis according to year at MRI examination (2012-2016 vs 2017-2021) and different viral loads cut-off at quantitative PCR (<100000/ml, between 100000 and 500000 and >1000000 copies/ml)

The potential association between all recorded maternal and fetal parameters and the two outcomes were first evaluated with standard univariate analyses (chi-squared test for categorical variables; Kruskal-Wallis test for continuous variables).

Then, the potential independent predictors of MRI diagnosis of fetal anomaly were investigated performing a random-effect logistic regression. A stepwise forward process was used for model building, and the following criteria were adopted for covariates selection: (1) maternal age, (2) maternal BMI; (3) maternal primary CMV infection (4) first trimester infection (5) CMV viral load (expressed as a continuous and categorical variable of more than 100000 copies/ml, as

reported by Guerra et al.[21]), (6) prenatal therapy (7) interval, expressed in weeks, between ultrasound and MRI examinations included a priori as a continuous variable.

Statistical significance was defined as a two-sided p-value<0.05 for all analyses, which were carried out using Stata, version 13.1 (Stata Corp., College Station, Texas, USA, 2013).

## RESULTS

### Maternal characteristics

One hundred and four fetuses with congenital CMV infection were included in the study. Brain anomalies were detected at follow-up ultrasound after an earlier normal neurosonogram in 8.7% (9/104) of cases. Ninety-five fetuses with normal neurosonography were referred to prenatal MRI and represent the population of this study. The general characteristics of the study population are shown in Table 1. The mean maternal age was 31.6±5.8 years, while the mean BMI was 25.9±3.9 kg/m$^2$. When focusing on maternal infection, 87.4% of seroconversions happened in the first, while 12.6% in the second trimester. The mean GA at first ultrasound and MRI was 17.6±4.2 and 26.0±5.1 weeks respectively. The mean GA at amniocentesis was 20.5±1.4, while median viral load 58791 (interquartile range, IQR, 8652,5-994750) copies/ml. MRI was performed within one week from the last neurosonographic assessment in most cases (84.2%). Finally, 8.4% of the included women underwent therapy with Valacyclovir, while 16.8% with hyperimmune globulins.

### Synthesis of the results

The rate of structural anomalies detected exclusively at fetal MRI was 10.5% (10/95). When considering the type of anomaly, malformations of cortical development were detected at MRI in 40% (4/10) of fetuses, destructive encephalopathy in 20% (2/10), intracranial calcification in the germinal matrix in 10% (1/10) and complex anomalies in 30% (3/10) (Table 2).

Table 3 shows the results of univariate analysis reporting cases with compared to those without additional anomalies detected at fetal MRI. GA at first ultrasound assessment and GA at MRI were significantly lower (13.8±3.5 vs 18.0±4.1, p< 0.01 and 23.6±4.1 vs 26.3±5.2, p<0.01 respectively) in the group of fetuses with additional anomalies detected at fetal MRI, compared to those without. Likewise, the median viral load at PCR (p=0.003) and the rate of viral load >100,000 copies/ml (p=0.04) were significantly higher in the group of fetuses with additional anomalies detected at fetal MRI, compared to those without. Prenatal therapy with hyperimmune globulins was significantly more common in fetuses with additional anomalies found at MRI, compared to those without (50% vs 12.9%, p=0.01). There was no difference in maternal age, maternal BMI, trimester at maternal seroconversion, interval between ultrasound and MRI and the use of Valacyclovir between the two groups. The rate of associated anomalies detected exclusively at MRI was higher between 2012-2016 compared to 2017-2021: 8/42 vs 2/53, p= 0.020). When assessing the different cut-offs of viral load at CMV, the rate of

additional anomalies was 20% (2/10) below 10000, 10% (1/10) between 10000 and 100000, 10% (1/10) between 100000 and 500000 and 60% (6/10) above 1000000 copies/ml.

At the multivariate logistic regression analysis, only CMV viral load >100,000 copies/ml (OR: 12.0, 95% CI 1.2-124.7, p=0.04) was independently associated with the likelihood of detecting fetal anomalies at MRI, while maternal age (p=0.62), maternal BMI (p=0.73), primary infection (p=0.31), first trimester infection (p=0.685), prenatal therapy (p=0.11), or the interval between ultrasound and MRI (p=0.27) were not (Table 4).

Associated anomalies were detected exclusively at birth and missed at both types of prenatal imaging in 3.8% (3/80) fetuses with congenital CMV infection, all consisting in malformations of cortical development.

## DISCUSSION

### Summary of the main findings

The findings of this study show that in fetuses with prenatal diagnosis of congenital CMV infection and normal neurosonography at first examination, the rate of additional structural anomalies detected exclusively by fetal brain MRI was 10.5%, highlighting the important role of MRI for the antenatal imaging of fetal CMV infection. Cortical, destructive and complex anomalies were among the most common malformations identified at MRI. CMV viral load was the only independent predictor of detecting fetal anomalies at MRI at a later gestation, thus confirming the prognostic value of laboratory findings, mostly when associated with antenatal imaging. Finally, the rate of anomalies detected exclusively at birth and missed at prenatal imaging was 3.8%.

### Strengths and limitations

Our study is one of the largest studies exploring the role of fetal brain MRI in fetuses with congenital CMV infection undergoing neurosonography. The relatively homogenous sample size, the inclusion of cases examined using a multiplanar approach as proposed by ISUOG guidelines[17-18] and the short time interval between US and MRI represent the main strengths of this study. The retrospective non-randomized design represents the main limitation of the study and led to challenges in obtaining all the details on the imaging for all the fetuses in the participating centers. Moreover, pharmacological management of congenital infection and therefore the use of certain therapies, such as Valacyclovir or hyperimmune globulins, were deferred to the discretion of each local clinician, without a shared consensus among the participant centers.

### Implications for clinical practice and research

CMV infection is the most common congenital infection and has been described as an "unmet public health issue", due to the high burden of short- and long-term sequelae, mostly affecting CNS and neurodevelopmental outcome.[6]

An accurate prenatal prediction of postnatal prognosis of fetuses with CMV infection is still challenging, and therefore parental counselling is often based on observational studies that date back to many years ago and do not adequately take into account the role of prenatal imaging in predicting perinatal and neurodevelopmental outcomes.

Children with congenital CMV infection and normal fetal brain findings using ultrasound examination have intuitively better outcomes in terms of cognitive, language, motor,

emotional-behavioral, and executive functioning.[22] However, defining prenatally a fetal CMV infection as asymptomatic prenatally is still challenging.

CMV has a peculiar tropism for the neurons in the periventricular zone, thus potentially affecting neuronal migration and proliferation.[23] Therefore, current guidelines suggest that parents should be counselled that CMV-related anomalies can be evident even 12 weeks or more after maternal seroconversion.[7] In the present study, additional anomalies were detected at follow-up ultrasound in about 9% of cases, thus confirming that fetuses with CMV infection should undergo intensive follow-up using neurosonography, as well as fetal MRI, in order to recognize associated anomalies which can be identified only later in pregnancy.

The role of MRI in congenital infection represents another peculiar issue. In the last two decades, the use of fetal MRI as a complementary imaging tool to improve the knowledge and the detection rate of fetal anomalies has significantly increased, thus leading to a more accurate prediction of short- and long-term prognosis.[10-15]

In the present study, we reported that MRI was able to identify about 10% of anomalies not detected even by neurosonography, and in particular those involving the cortical surface of the brain, thus confirming the excellent diagnostic accuracy of MRI for brain migration disorders. In this scenario, fetal MRI should be always recommended in cases of congenital CMV infection even with negative neurosonography in order to identify a subset of fetuses at higher risk of adverse neurodevelopmental outcome.

Prediction of outcome in fetuses with CMV infection is challenging. Although the presence of associated anomalies at ultrasound represents the strongest risk factor for impaired neurocognitive outcome,[7] prenatal counselling when no anomaly is detected at prenatal imaging is more challenging. We have previously reported that in fetuses with congenital CMV and no anomalies at prenatal imaging confirmed at birth, symptomatic infection was found in 1.5% of infants while the overall rate of a neurodevelopmental anomaly was 3.1%.[16] Compared with fetuses infected in the second or third trimester, those infected in the first trimester had a relatively higher risk of having an additional anomaly detected on follow-up ultrasound or MRI, abnormal neurodevelopmental outcome and hearing problems. Therefore, parents whose pregnancy is complicated by CMV infection and no anomaly is detected at prenatal imaging, can be reassured about the low risk of symptomatic infection and adverse outcome. However, prenatal imaging cannot completely rule out adverse events related to the infection, especially sensorineural hearing loss, which cannot be anticipated prenatally.

Viral load in the amniotic fluid represents another peculiar issue. Only few studies have addressed the diagnostic performance of CMV load in predicting the outcome of fetuses with

CMV. Guerra et al reported a viral load of >/=$10^3$ at quantitative PCR predicts mother-child infection with 100% probability, while that >/=$10^5$ the development of a symptomatic infection.[21] In the present study, viral load in the amniotic fluid was independently associated with associated anomalies at fetal MRI. Furthermore, 60% of the associated anomalies detected at MRI were more common when amniotic fluid viral load was higher than $10^6$. In this scenario, future studies should be directed at elucidating the optimal cut-off of CMV load in the amniotic fluid able to predict an adverse outcome, especially in fetuses not presenting anomalies at ultrasound and MRI.

**Conclusions**

Fetal MRI can detect additional brain anomalies in about 10% of fetuses with congenital CMV infection and negative neurosonography at first examination. Viral load in the amniotic fluid significantly affects the risk of associated anomalies in these fetuses.

The findings from the study support a detailed follow-up imaging throughout the remainder of the pregnancy after the diagnosis of fetal infection and the use of fetal MRI even in case of negative neurosonography to better predict the postnatal prognosis of infected newborns.

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**Table 1:** Selected gestational and fetal characteristics of 95 singleton pregnancies with congenital CMV infection and normal ultrasound undergoing prenatal magnetic resonance imaging.

| Characteristic | Value |
|---|---|
| Maternal age in years (mean±SD) | 31.6±5.8 |
| Maternal BMI in $Kg/m^2$ (mean±SD) | 25.9±3.9 |
| Primary CMV infection, n (%) | 92 (96.8%) |
| Non-primary CMV infection, n (%) | 3 (3.2%) |
| Trimester at seroconversion, n (%)<br>• Frist Trimester<br>• Second Trimester | <br>83 (87.4%)<br>12 (12.6%) |
| GA at first ultrasound assessment in weeks, (mean±SD) | 17.6±4.2 |
| GA at MRI in weeks, (mean±SD)<br>• <24 weeks<br>• >24 weeks | 26.0±5.1<br>43 (45.3%)<br>52 (54.7%) |
| Interval between ultrasound and MRI in weeks, (mean±SD)<br>• 1 week<br>• >2 weeks | 0.8±1.3<br>80 (84.2%)<br>15 (15.8%) |
| Gestational age at amniocentesis in weeks, (mean±SD) | 20.5±1.4 |
| Viral load at qPCR (n/ml), median (IQR) | 58791 (8652.5-994750) |
| Viral load >100000 copies/ml, n (%) | 43 (45.3%) |
| Prenatal therapy with Valacyclovir, n (%) | 8 (8.4%) |
| Prenatal therapy with hyperimmune globulins, n (%) | 16 (16.8%) |

*SD, standard deviation; BMI, body mass index; CMV, cytomegalovirus; MRI, magnetic resonance imaging; PCR, polymerase chain reaction, IQR, interquartile range*

**Table 2:** Additional central nervous system (CNS) anomalies diagnosed on prenatal magnetic resonance imaging (MRI) and after delivery or autopsy in fetuses with a confirmed congenital CMV infection, according to the type of anomaly.

| **Outcome** | **Fetuses (n= 95)** |
|---|---|
| Additional anomaly detected on prenatal MRI | 10 (10.5) |
| Type of prenatally diagnosed anomaly | |
| • Anomalies of the ventricular or periventricular zone | 0/10 (0) |
| • Intra-cranial calcifications in the basal ganglia or germinal matrix | 1/10 (0) |
| • Destructive encephalopathy | 2/10 (20) |
| • Malformations of cortical development | 4/10 (40) |
| • Posterior fossa anomaly | 0/10 (0) |
| • Midline anomalies | 0/10 (0) |
| • Complex anomaly | 3/10 (40) |
| Additional CNS anomaly on postnatal MRI | 3/80 (3.8) |

**Data are shown as n/N (%). MRI, magnetic resonance imaging*

**Table 3:** Selected gestational and fetal characteristics of fetuses with congenital CMV infection with vs without additional central nervous system anomaly found on prenatal magnetic resonance imaging (MRI)

| **Characteristic** | **Additional anomaly (n= 10)** | **No additional anomaly (n=85)** | **p-value** |
|---|---|---|---|
| Maternal age in years, (mean±SD) | 31.4±7.5 | 31.6±5.7 | 0.86 |
| Maternal BMI in Kg/m2, (mean±SD) | 26.1±4.0 | 25.9±4.0 | 0.68 |
| Primary CMV infection, n (%) | 9 (90) | 83 (97.6) | 0.29 |
| Trimester at seroconversion<br>• First trimester<br>• Second trimester | <br>9 (90)<br>1 (10) | <br>74 (87.1)<br>11 (12.9) | <br>1.000<br>1.000 |
| GA at first ultrasound assessment in weeks, (mean±SD) | 13.8±3.5 | 18.0±4.1 | <0.001 |
| GA at MRI (weeks), n (%)<br>• <24 weeks<br>• >24 weeks | 23.6±4.1<br>7 (70)<br>3 (30) | 26.3±5.2<br>36 (42.4)<br>49 (57.6) | <br><0.001<br>0.18 |
| Interval between ultrasound and MRI (weeks), n (%)<br>• 1 week<br>• >2 weeks | 0.95±0.8<br>8 (80)<br>2 (20) | 0.8±1.3<br>72 (84.7)<br>13 (15.3) | 0.48<br>0.66 |
| GA at amniocentesis in weeks, (mean±SD) | 20.2±0.8 | 20.5±1.4 | 0.08 |
| Viral load at qPCR (n/ml), (Median and IQR) | 1672500 (509312-4344327) | 40500 (5228-52000) | 0.003 |
| Viral load >100000 copies/ml, n (%) | 8 (80) | 35 (41.1) | 0.04 |
| Prenatal therapy with Valacyclovir, n (%) | 0 (0) | 8 (9.4) | 0.59 |
| Prenatal therapy with hyperimmune globulins, n (%) | 5 (50) | 11(12.9) | 0.01 |

*SD, standard deviation; BMI, body mass index; CMV, cytomegalovirus; GA, gestational age; IQR: interquartile range; MRI, magnetic resonance imaging; PCR, polymerase chain reaction.*

**Table 4:** Logistic regression models evaluating potential independent predictors of prenatal magnetic resonance imaging (MRI) diagnosis of additional central nervous system anomalies associated with congenital CMV infection

| Variables | Odd ratio (95% CI) | p-value |
|---|---|---|
| Maternal age in years | 0.97 (0.8-1.1) | 0.620 |
| Maternal BMI in $kg/m^2$ | 0.97 (0.8-1.2) | 0.726 |
| Maternal primary CMV infection | 1.89 (0.3-9.1) | 0.312 |
| First trimester infection | 0.61 (0.6-6.7) | 0.685 |
| CMV load >100000 copies/ml | 12.04 (1.2-124.7) | 0.037 |
| Prenatal therapy | 3.77 (0.8-18.9) | 0.107 |
| Interval between US and MRI | 1.36 (0.8-2.3) | 0.273 |

*BMI, body mass index; CMV, cytomegalovirus; US, ultrasound; MRI, magnetic resonance imaging.*