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

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KEYWORDS: CMV; cytomegalovirus; hearing loss; infection; MRI; neurosonography; ultrasound

CONTRIBUTION

What are the novel findings of this work?
Fetal magnetic resonance imaging (MRI) can detect additional brain anomalies in about 10% of fetuses with congenital cytomegalovirus (CMV) infection and normal neurosonography. CMV viral load was the only independent predictor of fetal anomalies on MRI later in gestation, thus confirming its prognostic value, mostly when associated with antenatal imaging.

What are the clinical implications of this work?
The findings of this study support the value of detailed imaging follow-up throughout the remainder of the pregnancy after the diagnosis of fetal CMV infection and the use of fetal MRI even in cases of negative neurosonography to better predict the postnatal prognosis of infected newborns.

ABSTRACT

Objective *To investigate the role of fetal brain magnetic resonance imaging (MRI) in detecting associated anomalies in fetuses with congenital cytomegalovirus (CMV) infection and normal neurosonography.*

Methods *This was a multicenter, retrospective cohort study of patients examined between 2012 and 2021 in 11 referral fetal medicine centers in Italy. Inclusion criteria were fetuses with congenital CMV infection diagnosed by polymerase chain reaction analysis of amniotic fluid, pregnancies that underwent detailed multiplanar ultrasound assessment of the fetal brain as recommended by the International Society of Ultrasound in Obstetrics and Gynecology, maternal age ≥ 18 years, normal fetal karyotype and MRI performed within 3 weeks after 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 neurosonography at diagnosis. Additional CNS anomalies were classified into anomalies of the ventricular and the periventricular zone, intracranial calcifications in the basal ganglia or germinal matrix, destructive encephalopathy in the white matter, malformations of cortical development, midline anomalies, posterior fossa anomalies and complex brain anomalies. We evaluated the relationship between the incidence of structural CNS malformations diagnosed exclusively on fetal MRI and a number of maternal and gestational characteristics. Univariate and multivariate logistic regression analyses were used to identify and adjust for potential independent predictors of the MRI diagnosis of fetal anomalies.*

Results *The analysis included 95 fetuses with a prenatal diagnosis of congenital CMV infection and normal neurosonography referred for prenatal MRI. 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 on MRI in 40.0% (4/10) of fetuses, destructive encephalopathy in 20.0% (2/10), intracranial calcifications in the germinal matrix in 10.0% (1/10) and complex CNS anomalies in 30.0% (3/10). On multivariate logistic regression analysis, only CMV viral load in the amniotic fluid, expressed as a continuous variable (odds ratio (OR), 1.16 (95% CI, 1.02–1.21);* $P = 0.02$)

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or categorical variable (> 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 on MRI. Associated anomalies were detected exclusively at birth and missed by both prenatal neurosonography and fetal MRI 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 of this 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 comprises the most frequent congenital viral infection, with a birth prevalence of 0.5–1.3%[1–4]. CMV infection is recognized as the leading cause of non-genetic neurosensorial hearing loss and is one of the most common causes of infection-related congenital malformations and impaired neurological outcome[5].

Considering the significant clinical relevance of congenital CMV infection and that it is commonly associated with no or non-specific symptoms, many researchers advocate universal 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 by polymerase chain reaction (PCR) after amniocentesis at approximately 20 weeks' gestation[7]. Once the presence of congenital CMV infection has been established, the main goal of prenatal diagnosis is to determine the prognosis of the pregnancy, 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 a poor prognosis in infected fetuses[7–9].

In the last few years, several studies have reported a significant contribution of fetal magnetic resonance imaging (MRI) to the detection of associated anomalies in fetuses with isolated central nervous system (CNS) malformations undergoing multiplanar assessment of the fetal brain (i.e. neurosonography)[10–15]. A few studies have also investigated the role of fetal MRI in congenital CMV infection; however, their small sample size, inclusion of cases already presenting with ultrasound anomalies and heterogeneity in the prenatal imaging protocols and management adopted preclude the extrapolation of objective evidence to guide clinical practice and prenatal counseling[16].

The aim of this study was to elucidate the role of fetal brain MRI in detecting anomalies in fetuses with congenital CMV infection and normal neurosonography.

METHODS

Study design and participants

This was a multicenter, retrospective cohort study of patients examined between 2012 and 2021 in 11 referral centers in Italy (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' gestation or after 6–8 weeks after maternal seroconversion, as recommended by international guidelines[7], pregnancies that underwent detailed multiplanar neurosonography using transabdominal and/or transvaginal ultrasound as recommended by the International Society of Ultrasound in Obstetrics and Gynecology (ISUOG) guidelines[17,18], fetuses with normal karyotype (including chromosomal microarray, when available), maternal age ≥ 18 years and MRI performed within 3 weeks after the last ultrasound examination.

Longitudinal, detailed ultrasound follow-up after diagnosis of fetal CMV infection was performed every 2 to 4 weeks, according to each local protocol, including a comprehensive evaluation of fetal growth, placental structure and function, amniotic fluid volume and fetal Doppler, and assessment of potential ultrasound markers of fetal disease. Fetal MRI was performed using the standardized planes for fetal brain examination according to the ISUOG guidelines for fetal MRI[19]. After birth, all newborns underwent a detailed ultrasound assessment which, in cases of pre- or postnatal suspicion of an anomaly, was followed 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; STrengthening the Reporting of OBservational studies in Epidemiology (STROBE) guidelines were followed[20].

Outcomes

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

For the purposes of this analysis, additional CNS anomalies were classified into: (1) anomalies of the ventricular or periventricular zone, including ventriculomegaly, periventricular calcifications, intraventricular adhesions, periventricular cysts; (2) intracranial

calcifications in the basal ganglia or germinal matrix; (3) destructive encephalopathy in the white matter, including hemorrhage, porencephaly or periventricular leukomalacia and malformations of cortical development, including lissencephaly, cortical dysplasia, pachygyria, heterotopia, polymicrogyria or schizencephaly; (4) midline anomalies, including complete and partial agenesis, hypoplasia and dysgenesis of the corpus callosum or isolated absence of the cavum septi pellucidi; (5) posterior fossa anomalies, including all defects involving the cerebellar vermis and/or hemispheres; (6) complex brain anomalies, including all defects characterized by the presence of multiple intracranial anomalies.

Statistical analysis

We investigated the relationship between the presence of structural CNS malformations diagnosed exclusively on fetal MRI (primary outcome) and a number of maternal and fetal characteristics, including maternal age and body mass index (BMI), GA 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 also planned subgroup analyses according to year at MRI examination (2012–2016 *vs* 2017–2021) and different viral-load cut-offs at quantitative PCR (<100 000, between 100 000 and < 500 000, between 500 000 and 1 000 000 and >1 000 000 copies/mL).

Potential associations between all recorded maternal and fetal parameters and additional CNS malformations detected exclusively on fetal MRI were first evaluated using standard univariate analysis (the χ-square test for categorical variables and the Kruskal–Wallis test for continuous variables). Then, random-effects logistic regression analysis was performed to investigate potential independent predictors of the MRI diagnosis of a fetal anomaly. A stepwise forward process was used for model building, and the following variables were considered for covariate 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 as a categorical variable of more than 100 000 copies/mL, as reported by Guerra *et al.*[21]); (6) prenatal therapy; (7) interval (in weeks) between ultrasound and MRI examinations, included *a priori* as a continuous variable.

Statistical analysis was carried out using Stata version 13.1 (StataCorp., College Station, TX, USA), and statistical significance was defined as two-sided $P < 0.05$ for all analyses.

RESULTS

Maternal characteristics

Of 104 fetuses with congenital CMV infection 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 for prenatal MRI and represent the population of this study, the general characteristics of which are shown in Table 1. The mean maternal age was 31.6 ± 5.8 years and the mean maternal BMI was 25.9 ± 3.9 kg/m^2. When focusing on maternal infection, 87.4% of seroconversions happened in the first trimester and 12.6% in the second. The mean GA at first ultrasound and at MRI was 17.6 ± 4.2 weeks and 26.0 ± 5.1 weeks, respectively. Mean GA at amniocentesis was 20.5 ± 1.4 weeks, while the median viral load was 58 791 copies/mL (interquartile range, 8653–994 750 copies/mL). MRI was performed within 1 week after the last neurosonographic assessment in most (84.2%) cases. Of the included pregnancies, 8.4% underwent therapy with valacyclovir and 16.8% with hyperimmune globulins.

Synthesis of results

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

The results of univariate analysis comparing cases with *vs* those without additional anomalies detected on fetal MRI are shown in Table 3. GA at first ultrasound assessment (13.8 ± 3.5 *vs* 18.0 ± 4.1 weeks; $P < 0.001$) was significantly lower, while the number of patients undergoing MRI at ≤ 24 weeks (70.0% *vs* 42.4%; $P < 0.001$)

Table 1 Maternal and gestational characteristics of 95 singleton pregnancies with congenital cytomegalovirus (CMV) infection and normal neurosonography that underwent prenatal magnetic resonance imaging (MRI)

Parameter	*Value*
Maternal age (years)	31.6 ± 5.8
Maternal BMI (kg/m^2)	25.9 ± 3.9
Primary CMV infection	92 (96.8)
Non-primary CMV infection	3 (3.2)
Trimester at seroconversion	
First	83 (87.4)
Second	12 (12.6)
GA at first US assessment (weeks)	17.6 ± 4.2
GA at MRI diagnosis (weeks)	26.0 ± 5.1
≤ 24 weeks	43 (45.3)
> 24 weeks	52 (54.7)
Interval between US and MRI (weeks)	0.8 ± 1.3
≤ 1 weeks	80 (84.2)
> 2 weeks	15 (15.8)
GA at amniocentesis (weeks)	20.5 ± 1.4
Viral load at qPCR (copies/mL)	58 791 (8653–994 750)
Viral load >100 000 copies/mL	43 (45.3)
Prenatal therapy with valacyclovir	8 (8.4)
Prenatal therapy with hyperimmune globulins	16 (16.8)

Data are given as mean ± SD, *n* (%) or median (interquartile range). BMI, body mass index; GA, gestational age; qPCR, quantitative polymerase chain reaction; US, ultrasound.

was significantly higher in the group of fetuses with additional anomalies detected on prenatal 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 on fetal MRI. Prenatal therapy with hyperimmune globulins was significantly more common in fetuses with additional anomalies found on MRI than in those without (50% *vs* 12.9%; $P = 0.01$). There was no difference in maternal age, maternal BMI, primary CMV infection, 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 on MRI was higher in the period 2012–2016 than in 2017–2021: 8/42 (19.0%) *vs* 2/53 (3.8%) ($P = 0.020$). When assessing the different cut-offs of CMV viral load, the rate of additional anomalies was 20% (2/10) < 100 000; 10% (1/10) between 100 000 and < 500 000; 10% (1/10) between 500 000 and 1 000 000; and 60% (6/10) > 1 000 000 copies/mL.

On multivariate logistic regression analysis, only CMV viral load in the amniotic fluid, expressed as a continuous variable (odds ratio (OR), 1.16 (95% CI, 1.02–1.21); $P = 0.02$) or categorical variable (>100 000 copies/mL) (OR, 12.0 (95% CI, 1.2–124.7); $P = 0.04$), was associated independently with the likelihood of detecting fetal anomalies on MRI, while maternal age ($P = 0.62$), maternal BMI ($P = 0.73$), primary CMV infection ($P = 0.31$), first-trimester infection ($P = 0.69$), prenatal therapy ($P = 0.11$) and the interval between ultrasound and MRI ($P = 0.27$) were not (Table 4).

Table 2 Additional central nervous system (CNS) anomalies diagnosed exclusively on prenatal magnetic resonance imaging (MRI) and after delivery or autopsy in fetuses with confirmed congenital cytomegalovirus infection with normal neurosonography

Outcome	*Fetuses (n = 95)*
Additional anomaly on prenatal MRI	10 (10.5)
Anomalies of ventricular or periventricular zone	0/10 (0)
Intracranial calcifications in basal ganglia or germinal matrix	1/10 (10.0)
Destructive encephalopathy in white matter	2/10 (20.0)
Malformations of cortical development	4/10 (40.0)
Posterior fossa anomalies	0/10 (0)
Midline anomalies	0/10 (0)
Complex CNS anomalies	3/10 (30.0)
Additional anomaly on postnatal MRI	3/80 (3.8)

Data are given as *n* (%) or *n*/*N* (%).

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 cytomegalovirus (CMV) infection in fetuses with normal neurosonography

Variable	*Odds ratio (95% CI)*	P
Maternal age (in years)	0.97 (0.89–1.16)	0.620
Maternal BMI (in kg/m^2)	0.97 (0.78–1.17)	0.726
Maternal primary CMV infection	1.89 (0.30–9.10)	0.312
First-trimester infection	0.61 (0.56–6.71)	0.685
CMV viral load (in copies/mL)	1.16 (1.02–1.21)	0.018
CMV viral load > 100 000 copies/mL	12.04 (1.19–124.73)	0.037
Prenatal therapy	3.77 (0.50–18.20)	0.107
Interval between US and MRI (in weeks)	1.36 (0.67–2.15)	0.273

BMI, body mass index; US, ultrasound.

Table 3 Selected maternal and gestational characteristics of pregnancies with congenital cytomegalovirus (CMV) infection and normal neurosonography, with *vs* without additional central nervous system anomaly found on prenatal magnetic resonance imaging (MRI)

Parameter	*Additional anomaly (n = 10)*	*No additional anomaly (n = 85)*	P
Maternal age (years)	31.4 ± 7.5	31.6 ± 5.7	0.86
Maternal BMI (kg/m^2)	26.1 ± 4.0	25.9 ± 4.0	0.68
Primary CMV infection	9 (90.0)	83 (97.6)	0.29
Trimester at seroconversion			
First	9 (90.0)	74 (87.1)	1.00
Second	1 (10.0)	11 (12.9)	1.00
GA at first US assessment (weeks)	13.8 ± 3.5	18.0 ± 4.1	< 0.001
GA at MRI (weeks)	23.6 ± 4.1	26.3 ± 5.2	0.11
≤ 24 weeks	7 (70.0)	36 (42.4)	< 0.001
> 24 weeks	3 (30.0)	49 (57.6)	0.18
Interval between US and MRI (weeks)	0.95 ± 0.8	0.8 ± 1.3	0.72
≤ 1 weeks	8 (80.0)	72 (84.7)	0.48
> 2 weeks	2 (20.0)	13 (15.3)	0.66
GA at amniocentesis (weeks)	20.2 ± 0.8	20.5 ± 1.4	0.08
Viral load at qPCR (copies/mL)	1 672 500 (509 312–4 344 327)	40 500 (5228–52 000)	0.003
Viral load > 100 000 copies/mL	8 (80.0)	35 (41.2)	0.04
Prenatal therapy with valacyclovir	0 (0)	8 (9.4)	0.59
Prenatal therapy with hyperimmune globulins	5 (50.0)	11 (12.9)	0.01

Data are given as mean ± SD, *n* (%) or median (interquartile range). BMI, body mass index; GA, gestational age; qPCR, quantitative polymerase chain reaction; US, ultrasound.

Associated anomalies – all of which comprised malformations of cortical development – were detected exclusively at birth and missed on both prenatal neurosonography and fetal MRI in 3.8% (3/80) fetuses with congenital CMV infection.

DISCUSSION

Summary of main findings

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

Strengths and limitations

This is one of the largest studies exploring the role of fetal brain MRI in fetuses with congenital CMV infection with normal neurosonography. The relatively homogeneous sample, the inclusion of cases examined using a multiplanar approach as proposed by the ISUOG guidelines[17,18] and the short time interval between ultrasound and MRI represent the main strengths of the study. Its main limitation is its retrospective non-randomized design. Moreover, pharmacological management of congenital infection and, therefore, the use of certain therapies, such as valacyclovir and hyperimmune globulins, was left to the discretion of each local clinician, without a shared consensus among the participating 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', owing to the high burden of short- and long-term sequelae, mostly affecting the CNS and neurodevelopmental outcomes[6]. Accurate prenatal prediction of the postnatal prognosis of fetuses with CMV infection is still challenging, and parental counseling is often based on the results of observational studies that date back many years 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 on ultrasound examination have better outcomes in terms of cognitive, language, motor, emotional–behavioral and executive functioning[22]. However, defining prenatally a case of fetal CMV infection as asymptomatic 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 counseled 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 that can be identified only later in pregnancy.

The role of MRI in congenital infection remains unclear. In the last two decades, the use of fetal MRI as a complementary tool to ultrasound for improving the knowledge and detection rates of fetal anomalies has significantly increased, thus leading to a more accurate prediction of short- and long-term prognoses[10–15]. In the present study, MRI was able to identify about 10% of anomalies not detected 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 always be 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.

The prediction of outcome in fetuses with CMV infection is challenging. The presence of associated anomalies on ultrasound represents the strongest risk factor for impaired neurocognitive outcome[7], but prenatal counseling when no anomaly is detected at prenatal imaging is more difficult. We previously reported that, in fetuses with congenital CMV infection and no anomalies on prenatal imaging and 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 in which no anomaly is detected by 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 unusual issue. Only a few studies have addressed the diagnostic performance of CMV load in predicting the outcome of fetuses with CMV. Guerra *et al.*[21] reported that a viral load of $\geq 10^3$ copies/mL at quantitative PCR predicts mother–child infection with 100% probability, and a viral load of $\geq 10^5$ copies/mL the development of a symptomatic infection. 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 by MRI were more common when the 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 on ultrasound and MRI.

Conclusions

Fetal MRI can detect additional brain anomalies in about 10% of fetuses with congenital CMV infection and negative neurosonography. Viral load in the amniotic fluid significantly affects the risk of associated anomalies in these fetuses. The findings of this study support the importance of detailed follow-up imaging throughout the remainder of the pregnancy after the diagnosis of fetal CMV infection, and the use of fetal MRI even in cases of negative neurosonography to better predict the postnatal prognosis of infected newborns.

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