Improvement of pregnancy outcome following primary CMV infection after the introduction of valacyclovir in the clinical practice in Italy, updated data from a large real-life multicenter study

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BACKGROUND: Congenital cytomegalovirus infection (cCMV) is a leading cause of neurodevelopmental impairment and congenital sensorineural hearing loss (SNHL). Since December 2020, valacyclovir (VCV) is reimbursed by the Italian National Health System to reduce maternal—fetal CMV transmission following primary CMV maternal infection.

OBJECTIVE: The aim of this study is to evaluate the effectiveness of VCV in preventing fetal CMV infection, termination of pregnancy, miscarriage, congenital CMV infection at birth and presence of signs/symptoms of cCMV in newborns.

STUDY DESIGN: The current study provides an update on previously published data from a large multicenter observational Italian study including women with primary CMV infection acquired during the periconceptional period and up to 24 gestational weeks between January 2010 and June 2025. Primary outcomes are CMV transmission at amniocentesis, termination of pregnancy, miscarriage, transmission at birth (positive CMV DNA on newborn's urine), symptomatic cCMV, and a composite outcome (occurrence of transmission at amniocentesis or at birth or termination of pregnancy or miscarriage).

RESULTS: Overall, 834 pregnancies were included (826 singletons and 8 sets of twin pregnancies, total 842 fetuses); 588 fetuses received prenatal VCV (576 singletons and 12 twins) and 254 were untreated (250 singletons and 4 twins). CMV-positive amniocentesis occurred in 17.2% (64/373) of VCV-treated fetuses compared with 27.9% (38/136) of untreated one (*P*=.007, aOR 0.53 [95% CI: 0.34, 0.85]). Termination of pregnancy and miscarriage occurred in 4.7% (26/555) and 0.7% (4/555) of treated fetuses vs 10.0% (25/250) and 1.6% (4/250) of untreated one, respectively (*P*=.004, aOR 0.46 [95% CI: 0.25, 0.78] and *P*=.24, aOR 0.45 [95% CI: 0.11, 1.80]). Neonatal CMV DNA in urine was detected in 23.6% (124/525) and 27.1% (60/221) of newborns from treated and untreated pregnancies, respectively (*P*=.31, aOR 0.83 [95% CI: 0.58, 1.19]). The prevalence of symptomatic congenital infection at birth was significantly less frequent in the VCV group (3.6% 19/525 vs 10% 22/221, *P*=.001, aOR 0.34 [95% CI: 0.18, 0.64]). The composite outcome occurred less frequently in treated pregnancies (27.2% vs 35.8%, *P*=.012, aOR 0.67 [95% CI: 0.49, 0.91]). VCV-related adverse events were reported in 6.7% of cases and were mild or moderate.

The diagnosis of cCMV infection at birth following a negative amniocentesis was not significantly different among treated and untreated pregnancies (12.9%, 36/280 vs 7.3%, 7/96; *P*=.139). In the group of treated pregnancies with negative amniocentesis the proportion of newborn with cCMV diagnosed at birth was 23.2% (19 of 82), 10.8% (11 of 102) and 6.3% (6 of 96) among those with seroconversion, positive IgG-IgM and low IgG avidity and positive IgG-IgM and intermediate IgG avidity and positive molecular test respectively (*P*=.003).

CONCLUSIONS: Large scale real-life data confirm that VCV improves the outcome of pregnancy with CMV primary infection with a good safety profile.

Keywords: congenital, cytomegalovirus, pregnant, screening, valaciclovir

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AJOG MFM at a Glance

Why was this study conducted?

No large studies evaluating the effectiveness of valacyclovir in improving pregnancy outcomes following primary CMV infection are currently available

Key findings

Real-life data shows that valacyclovir effectively reduces maternal-fetal CMV transmission by the time of amniocentesis and the risk of symptomatic congenital infection at birth with a good safety profile. The rate of congenital CMV diagnosis at birth after a negative amniocentesis is remarkable in treated women with seroconversion occurring during pregnancy.

What does this study add to what is already known?

The study supports the use of valacyclovir in pregnancy for secondary prevention of congenital CMvs. Further investigations are needed to better understand the clinical significance of congenital CMV diagnosis at birth after valacyclovir treatment and negative amniocentesis.

Introduction

Congenital cytomegalovirus infection (cCMV) is the most common congenital infection, affecting approximately 0.5%−2% of newborns. It represents the leading non-genetic cause of sensorineural hearing loss (SNHL) and long-term neurological morbidity,[1] accounting for up to 10% of cerebral palsy cases[2] and 8%−21% of childhood SNHL cases.[3,4] cCMV is acquired transplacentally, and although the risk of vertical transmission increases with advancing gestational age, the risk of fetal injury is highest when primary maternal infection occurs during the periconceptional period or in the first trimester.[5]

In 2020 for the first time a double blind randomized clinical trial involving 90 pregnant women with primary CMV infection acquired in early pregnancy showed that a drug, valacyclovir (VCV) was able to reduce the risk of CMV vertical transmission diagnosed with amniocentesis by 70% compared to placebo.[6] These results were replicated in 2 additional studies carried out in France involving 65[7] and 59[8] treated women respectively and by our Italian multicenter collaborative study group reporting the data of 205 treated women published in 2023.[9]

In December 2020, the Agenzia Italiana del Farmaco (AIFA) approved the inclusion of VCV among drugs fully reimbursed by the Italian National Health Service for the prevention of fetal CMV infection in pregnant women with primary CMV acquired in the periconceptional period or in pregnancy up to 24 weeks of gestation, as well as for the *in utero* treatment of confirmed mild to moderate fetal CMV infection.[10] Since the introduction of reimbursed VCV into routine clinical practice, the number of treated women has steadily increased, allowing this update of our previously published study.[9] The collaborative study group decided to update the analysis when the number of treated patients had at least doubled compared to the previous publication.

Materials and methods

MEGAL-ITALI is a multicenter, observational, retrospective−prospective study analyzing data from pregnant women with primary CMV infection referred to participating centers between January 2010 and June 2025. The study was conducted in 14 Italian tertiary care centers (Figure 1).

The study included pregnant women with primary CMV infection acquired in the periconceptional period or in pregnancy up to 24 weeks of gestation.[10]

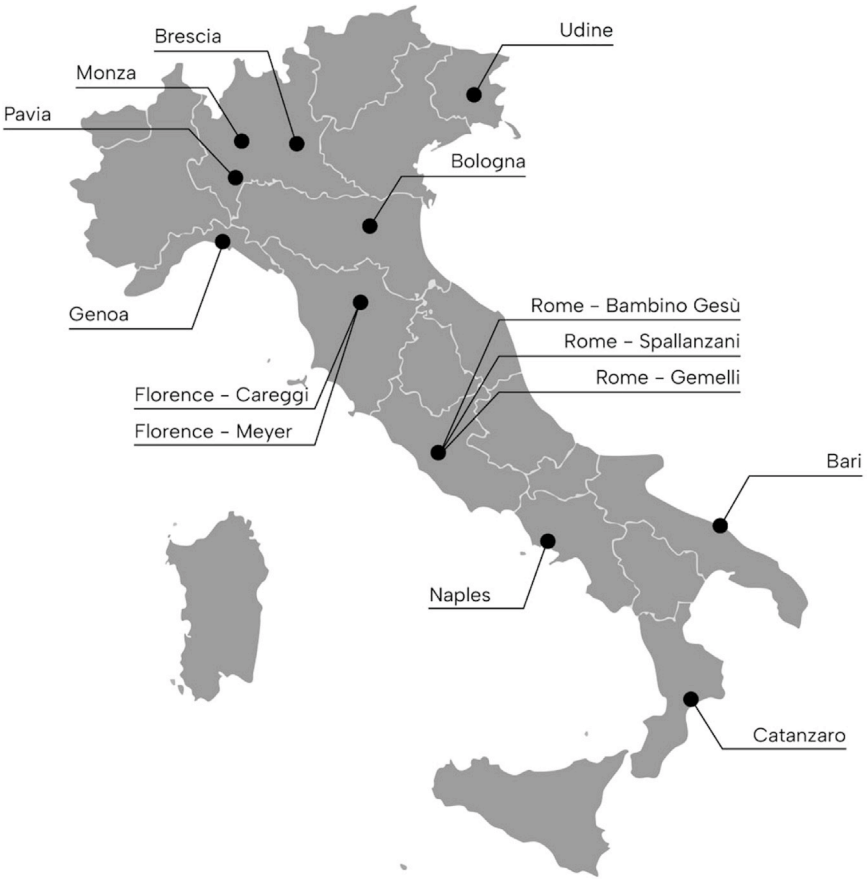
The diagnostic criteria for primary infection were: (1) CMV immunoglobulin G (IgG) seroconversion, defined as a negative IgG test during pregnancy followed by a subsequent positive IgG test; (2) CMV IgG and immunoglobulin M (IgM) positivity with a low IgG avidity index during pregnancy; or (3) CMV IgG and IgM positivity with an intermediate IgG avidity index and detection of CMV DNA in at least one maternal biological sample (blood, saliva, or urine) during pregnancy,[9] according with AIFA criteria.

Exclusion criteria were: diagnosis of CMV infection with CMV-positive amniocentesis at presentation (in this case VCV would be used for tertiary prevention, defined as treatment of confirmed fetal CMV infection aimed to reduce symptomatic neonatal disease, rather than secondary prevention, which refers to maternal treatment to reduce the risk of vertical transmission), maternal infection acquired after 24^{+6} weeks of gestation (according with AIFA criteria for VCV in pregnancy), and absence of outcome data regarding amniocentesis result, termination of pregnancy (TOP), miscarriage, or neonatal CMV DNA in urine.

For each woman, clinical and laboratory data were systematically collected and analyzed. These included the estimated gestational age at maternal CMV infection according to Revello et al.[11] maternal serological and virological CMV results, and details of VCV treatment, such as dosage, duration, and reported adverse events. Data about amniocentesis (gestational age and result), pregnancy outcomes, including miscarriage and TOP were also recorded. Women bearing a twin pregnancy were counted twice. For each newborn, data on the presence of clinical signs or symptoms suggestive of cCMV, CMV DNA detection in urine, and the initiation of antiviral treatment with valganciclovir were collected.

The primary outcomes were transmission at the time of amniocentesis, TOP and miscarriage, CMV DNA detection in neonatal urine within 2 weeks of life and the presence of clinical signs and/or symptoms suggestive of cCMV infection, defined according to ESPID 2017.[12] A composite outcome was also evaluated, defined as the presence of at least one of the following data: CMV-positive amniocentesis, TOP, miscarriage, or positive neonatal CMV PCR in urine.

FIGURE 1
The 14 Italian tertiary care centers involved in the MEGAL-ITALI Study.



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Before late 2020 none of the study centers used VCV for treatment of primary CMV infection acquired in pregnancy. Since late 2020, in accordance with AIFA recommendations,[10] VCV was administered to pregnant women with primary CMV infection acquired from the periconceptional period up to 24 weeks of gestation. The prescribed dosage was either 2 g every 6 hours or 4 g every 12 hours (total 8 g/d). VCV was contraindicated in women with renal impairment (creatinine >1.1 mg/dL or glomerular filtration rate < 90 mL/min).

Treatment was continued until 26 weeks of gestation in women who did not perform amniocentesis. In cases of CMV-positive amniocentesis, therapy was continued for tertiary prevention if ultrasound signs of mild-to-moderate fetal infection were present; otherwise, treatment could be discontinued. Amniocentesis was performed usually after 20 weeks of pregnancy, at least 8 weeks after the estimated date of maternal infection to improve its positive predictive value. In women with negative CMV-DNA on amniotic fluid, the treatment was discontinued after the amniocentesis (from a few days up to some weeks).

During treatment, maternal monitoring included blood tests for renal and hepatic function and CMV DNA quantification in blood at baseline, after 1 week, and subsequently every 14 days.

Data from all participating centers were collected using the REDCap platform.

The study protocol was developed by the coordinating center and approved by the Ethics Committees of all participating centers (code at the coordinating center 17802_bio, Version 3.0, date 21/11/2024).

Statistical analysis

Maternal and neonatal data were analyzed using descriptive statistics. Categorical variables are reported as counts and percentages. Comparisons between treated and untreated groups were performed. Adjusted odds ratios (aOR) with 95% confidence intervals (CIs) were calculated for binary outcomes, using regression models, controlling for gestational age at maternal infection.

The effect of maternal VCV treatment was evaluated on CMV transmission at amniocentesis, TOP, miscarriage, cCMV infection at birth (CMV DNA in neonatal urine), symptomatic cCMV infection, and a composite outcome including any of these events.

Additional focused analyses were carried out to describe neonates born with cCMV after a negative amniocentesis and their mothers and neonates with cCMV born after a positive amniocentesis.

In all tests $P<.05$ was considered statistically significant. Analyses were performed using STATA.

Results

Descriptive analysis

Overall, 834 pregnancies were included (826 singletons and 8 sets of twin pregnancies, total 842 fetuses).

Among the included fetuses, 588 (69.8%), 576 singletons and 12 twins, received prenatal VCV treatment, while 254 (30.2%), 250 singletons and 4 twins, observed before December 2020 were untreated. The untreated group included women enrolled before late 2020, when VCV was not used in the clinical practice for the primary CMV infection acquired in pregnancy.

Maternal characteristics of treated and untreated fetuses did not differ significantly in terms of age, timing of CMV primary infection acquisition and timing of amniocentesis.

Amniocentesis was performed in 509/842 (60.5%) cases, including 63.4%

TABLE 1
Maternal characteristics of treated and untreated fetuses

Variables	*VCV (N 588; 69.8%)*	*NON VCV (N 254; 30.2%)*	*ALL (N 842)*	*P value*
Maternal age at infection, median [IQR], years	32.1 [28.4-35.4]	31.8 [27.6-35.1]	32 [28.2-35.3]	**.46**
Estimated gestational age at maternal infection, n (%), weeks				
Periconceptional	119/588 (20.2%)	60/254 (23.6%)	179/842 (21.3%)	**.50**
1-15	386/588 (65.6%)	157/254 (61.8%)	543/842 (64.5%)	
16-24^{+6}	83/588 (14.1%)	37/254 (14.6%)	120/842 (14.3%)	
Amniocentesis performed, n (%)	373/588 (63.4%)	136/254 (53.5%)	509/842 (60.5%)	**.007**
Gestational age at amniocentesis, median [IQR], weeks	20.4 [20.1-20.8]	20.7 [20.3-21.1]	20.4 [20.1-21]	
Enrollment criteria, n (%)				
Seroconversion	226/588 (38.4%)	82/254 (32.3%)	308/842 (36.6%)	**.001**
Positive IgG and IgM with low IgG avidity	186/588 (31.6%)	114/254 (44.9%)	300/842 (35.6%)	
Positive IgG and IgM with intermediate IgG avidity and *positive PCR*	176/588 (29.9%)	58/254 (22.8%)	234/842 (27.8%)	

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(373/588) of treated and 53.5% (136/254) of the untreated group (*P*=.007).

Among the treated group, IgG seroconversion was the most common inclusion criterion (38.4%, 226/588), whereas among the untreated group, low IgG avidity was more frequently observed (44.9%, 114/254; *P*=.001) (*Table 1*).

Primary outcomes

Maternal–fetal CMV transmission at the time of amniocentesis occurred significantly less frequently among treated fetuses compared with untreated one. CMV-positive amniocentesis was observed in 17.2% (64/373) of treated group and in 27.9% (38/136) of the untreated group (*P*=.007, aOR 0.53 [95% CI: 0.34, 0.85]).

TOP occurred in 4.7% (26/555) of the treated group compared with 10.0% (25/250) of the untreated one (*P*=.004, aOR 0.46 [95% CI: 0.25, 0.78]). Miscarriage was also more frequently observed in the untreated group 1.6% (4/250) than in the treated group 0.7% (4/555), although this difference did not reach statistical significance (*P*=.24, aOR 0.45 [95% CI: 0.11, 1.80]).

The majority of TOP in both groups were decided after a CMV-DNA positive amniocentesis (18 of 26 TOP, 69.2% in the VCV group and 18 of 25 TOP, 72% in the untreated group). Fetal ultrasound or MRI anomalies were detected in 4 and 2 cases in the treated and in the untreated group respectively. In the treated group 2 women opted for TOP after a CMV negative amniocentesis for reasons unrelated to CMV. In the remaining cases (6 cases in the treated group and 7 cases in the untreated group) women declined amniocentesis and performed TOP (no fetal ultrasound or MRI anomalies were detected in these cases).

The diagnosis of cCMV infection, defined as detection of CMV DNA in neonatal urine, was slightly more frequent among newborns from untreated mothers (27.1%, 60/221) compared with those born to treated mothers (23.6%, 124/525); however, this difference was not statistically significant (*P*=.31, aOR 0.83 [95% CI: 0.58, 1.19]).

Conversely, neonatal signs and/or symptoms compatible with cCMV, analyzed according to the 2017 ESPID Expert Consensus Criteria,[12] were significantly less frequent, in the VCV-treated group (3.6%, 19/525) than in the untreated group (10%, 22/221; *P*=.001, aOR 0.34 [95% CI: 0.18, 0.64]).

A composite outcome, defined as the presence of at least one of the following events—CMV-positive amniocentesis, TOP, miscarriage, or positive neonatal CMV PCR in urine—occurred less frequently among women treated with VCV (27.2%, 160/588) compared with untreated women (35.8%, 91/254; *P*=.012, aOR 0.67 [95% CI: 0.49, 0.91]) (Table 2 and Supplementary Figure 1).

A sensitivity analysis of transmission at amniocentesis by diagnostic category showed a consistent effect of VCV across the 3 groups (seroconversion, 17.6% [19/108] vs 37.0% [10/27], aOR 0.37; low IgG avidity, 20.6% [28/136] vs 28.4% [21/74], aOR 0.66; intermediate IgG avidity with positive molecular test, 13.2% [17/129] vs 20.0% [7/35], aOR 0.64), with no significant difference in the effect between the 3 groups (treatment by diagnostic category interaction, *P*=.62).

Additional analysis

The diagnosis of cCMV infection following a negative amniocentesis was evaluated. Overall, 11.4% (43/376) of newborns with negative amniocentesis tested positive for CMV DNA in urine at birth. This finding was more frequent among newborns born to VCV-treated women (12.9%, 36/280) compared with those born to untreated women (7.3%, 7/96), however, the difference was not

TABLE 2
Primary outcomes

Primary outcomes	VCV (N 588; 69.8%)	NON VCV (N 254; 30.2%)	ALL (N 842)	*P* value	aOR	95% CI
Transmission at amniocentesis, N(%)	64/373 (17.2%)	38/136 (27.9%)	102/509 (20%)	.007	0.53	0.34, 0.85
TOP, N(%)	26/555 (4.7%)	25/250 (10.0%)	51/805 (6.3%)	.004	0.46	0.25, 0.78
Miscarriage, N(%)	4/555 (0.7%)	4/250 (1.6%)	8/805 (1.0%)	.24	0.45	0.11, 1.80
cCMV as CMV DNA detected in neonatal urine, N(%)	124/525 (23.6%)	60/221 (27.1%)	184/746 (24.7%)	.31	0.83	0.58, 1.19
Symptomatic cCMV, N(%)	19/525 (3.6%)	22/221 (10%)	41/746 (5.5%)	.001	0.34	0.18, 0.64
Composite outcome	160/588 (27.2%)	91/254 (35.8%)	251/842 (29.8%)	.012	0.67	0.49, 0.91

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statistically significant (*P*=.139). Among the 36 newborns with cCMV diagnosis at birth after negative amniocentesis from VCV-treated mothers, 19 (52.8%) were born to mothers with seroconversion, 11 (30.6%) to mothers with positive CMV IgG-M and low IgG avidity and 6 (16.7%) to mothers with positive CMV IgG-M, intermediate IgG avidity and positivity of one molecular assay. In the group of treated women with negative amniocentesis the proportion of newborns with cCMV diagnosed at birth was 23.2% (19 of 82), 10.8% (11 of 102) and 6.3% (6 of 96) among women with seroconversion, positive IgG-IgM and low IgG avidity and positive IgG-IgM and intermediate IgG avidity and positive molecular test respectively (*P*=.003).

Considering the 7 newborns with cCMV diagnosis at birth after negative amniocentesis from untreated mothers, 2 (28.6%) were born to mothers with seroconversion, 3 (42.9%) to mothers with positive CMV IgG-M and low avidity and 2 (28.6%) from mothers with intermediate avidity and positivity of one molecular assay. In the group of not treated women with negative amniocentesis the proportion of newborns with cCMV diagnosed at birth was 11.8% (2 of 17), 5.8% (3 of 52) and 7.4% (2 of 27) among women with seroconversion, positive IgG-IgM and low IgG avidity and positive IgG-IgM and intermediate IgG avidity and positive molecular test respectively (*P*=.711).

Considering the group of mothers with newborns with cCMV diagnosis after a negative amniocentesis according to the timing of VCV discontinuation, no statistically significant difference was observed between cases in which VCV was discontinued shortly after a negative amniocentesis (≤22 weeks) and those in which treatment was continued up to 26 weeks (21 of 204, 10.3% vs 8 of 46, 17.4%; *P*=.17).

Among newborns with cCMV diagnosis after negative amniocentesis, 3 cases presented clinical signs and/or symptoms compatible with cCMV without signs of brain disruption, and all were born to mothers treated with VCV (3 of 36, 8.3% newborns from VCV-treated mothers vs 0 of 7 newborns from untreated mothers). In this subgroup, the estimated timing of maternal infection was periconceptional in one case and between 11 and 15 weeks of gestation in 2 cases. The duration of maternal VCV treatment was 39, 63, and 88 days; 2 women received VCV 2 g 4 times daily, and one received 4 g twice daily. VCV treatment was discontinued after a negative amniocentesis in all cases, at 21 weeks in 2 cases and at 26 weeks in one case. Reported neonatal cCMV manifestations included bilateral temporopolar white matter abnormalities with cyst-like appearance in one case, small for gestational age (SGA) in another case, and thrombocytopenia with petechiae in the third case. The newborn with SGA was not treated with valganciclovir after birth, while the remaining 2 were treated with valganciclovir, in particular the newborn with CNS involvement was treated for 180 days, while the duration of treatment of the other one is unknown. Regarding follow-up, the newborn with thrombocytopenia and petechiae was reported to be healthy by the parents with no clinical abnormalities. As far as the newborns with CNS involvement and SGA is concerned, audiological follow-up was consistently normal up to 22 months and up to 24 months of age respectively (Table 3).

Among neonates born after CMV-positive amniocentesis, signs and/or symptoms of cCMV were observed less frequently in the VCV-treated group (30.8%, 12/39) than in the untreated group (35.3%, 6/17), however, this difference does not have a statistical significance (*P*=.739).

Among the VCV-treated women with positive amniocentesis, 18 of 64 (28.1%) opted for TOP, 45 of 64 (70.3%) continued the pregnancy, and one had a miscarriage. Among the 18 women opting for TOP, 4 presented with ultrasound features suggestive of fetal disease (3 mild to moderate and 1 severe), while in the remaining cases no ultrasound abnormalities were reported.

Among women with positive amniocentesis who continued pregnancy, 6 were lost to follow-up. Among the remaining 39 women with available follow-up data 10/39 (25.6%) presented at least one fetal ultrasound or laboratory finding suggestive of mild to moderate

TABLE 3
Features of symptomatic cCMV after negative amniocentesis

Newborns with symptomatic cCMV after negative amniocentesis	Prenatal treatment with VCV, days	Dosage	Timing of maternal infection	Signs/symptoms of cCMV	Post natal treatment with valgancyclovir	Follow-up
1	88	2 g q6h	Periconceptional	CNS involvement: white matter abnormalities, cyst	Yes (180 days)	Audiological and psychomotor: normal up to 22 months
2	39	4 g q12h	11-15 GW	SGA	No	Audiological and psychomotor: normal up to 24 months
3	63	2 g q6h	11-15 GW	Severe thrombocytopenia and petechiae	Yes (unknown duration)	Unknown (reported to be healthy by the parents)

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fetal disease according to Leruez-Ville,[13] while 29 (74.4%) did not. Of these 10 mothers, 9 continued VCV until delivery or at least until 37 weeks of pregnancy. Among this group, 5 out of 9 newborns (55.6%) were asymptomatic at birth. The newborn whose mother did not receive VCV despite the presence of signs of fetal disease was asymptomatic at birth.

Within the group of 29 women with positive amniocentesis without ultrasound or laboratory sign of mild to moderate fetal diseases, 15 (51.7%) discontinued VCV shortly after amniocentesis (median week of discontinuation of 22.7 (IQR 20.9−26.8), while 14 women (48.3%) continued VCV until delivery or at least until 37 weeks. Prevalence of asymptomatic cCMV was 66.7% (10 out of 15) among those whose mother discontinued VCV shortly after amniocentesis and 78.6% (11 out of 14) among those whose mother continued VCV until delivery or at least until 37 weeks (P=.68).

Characteristics of treatment

In the cohort of 588 VCV-treated fetuses, the median treatment duration was 8.28 weeks (IQR 5.7−11.9). A total of 14.8% of women received treatment for 1−29 days, while 85.2% were treated for ≥30 days. The median gestational age at treatment initiation was 15.14 weeks (IQR 12.14−18.7). The treatment delay, defined as the difference between the estimated date of infection and treatment initiation had a median of 6.7 weeks (IQR 4.2−10.4).

The most commonly prescribed VCV dosage was 2 g every 6 hours (585 out of 588, 99.5%), while only 3 out of 588 women (0.5%) received 4 g every 12 hours.

Regarding the tolerability profile of VCV, adverse events were reported in 39 out of 578 women (6.7%), with missing data for 10 patients. Most events were classified as mild (31 cases) or moderate (8 cases), with no severe or life-threatening adverse effects observed.

No statistically significant differences were observed across treatment duration groups (P=.23) (Table 4).

Discussion

Principal findings

In this large multicenter Italian cohort, maternal treatment with VCV following primary CMV infection during the periconceptional period or early pregnancy was associated with improved maternal and neonatal outcomes. In particular, VCV-treated women showed significantly lower rates of maternal−fetal transmission at amniocentesis (−47%), TOP (−54%), symptomatic cCMV at birth (−66%), and composite adverse outcomes compared with untreated women (−33%).

Results in the context of what is known

The observed reduction in CMV-positive amniocentesis among treated women is consistent with previous prospective and retrospective studies demonstrating a protective effect of high-dose VCV when administered after maternal primary CMV infection. Previous studies were smaller and performed in single centers where early diagnosis of CMV primary maternal infection and referral to treatment could have been easier compared to a real-life scenario as reported by the present study.[14]

This study provides, in a large scale, multi-center real-life setting, additional evidence supporting the effectiveness of VCV as a secondary prevention strategy of cCMV infection following maternal primary infection in early pregnancy. Compared with our previous report,[9] which included 205 valacyclovir-treated women, the present study nearly tripled the treated population (588 treated fetuses) and extended the observation period by approximately 5 years; the current cohort incorporates the previously reported patients together with newly enrolled women and, in addition to confirming the effect on vertical transmission and symptomatic congenital infection with greater statistical power, provides novel analyses on congenital CMV diagnosed at birth after a negative amniocentesis, stratified by

TABLE 4
Characteristics of valacyclovir treatment

Weeks of treatment, median	8.28 [5.7-11.9]
Days of treatment, median	58 [40-83.5]
Days of treatment	
1-29	86/580 (14.8%)
30-max	494/580 (85.2%)
Median gestational age at treatment initiation, weeks	15.14 [12.1-18.7]
Median gestational age at treatment initiation, days	106 [85-131]
Treatment delay in weeks, median	6.7 [4.2-10.4]
Treatment delay in days, median	47 [30-73]
VCV dosage	
2 g q6h	585/588 (99.5%)
4 g q12h	3/588 (0.5%)
Adverse reaction	39/578 (6.7%)
Classification of adverse reaction	
Headache	16/578 (2.8%)
Asthenia	7/578 (1.2%)
Dizziness	4/578 (0.7%)
Renal toxicity	4/578 (0.7%)
Other	20/578 (3.5%)
Classification of adverse reaction according to severity	
Mild	31/39 (79.5%)
Moderate	8/39 (20.5%)
Severe	0 (0%)
Adverse events according to treatment duration *(Denominator includes women who received VCV in that specific time-interval group)*	
1-14	3/27 (11.1%)
15-29	7/64 (10.9%)
30-44	10/111 (9%)
45-59	3/97 (3.1%)
60-89	9/169 (5.3%)
90-max	7/102 (6.9%)
P value	**.23**

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maternal diagnostic category, and on the use of valacyclovir for confirmed fetal infection.

The study also provides reassuring data on safety of high dose VCV during early pregnancy. The known most serious adverse event related to high dose use during pregnancy is renal toxicity reported in up to 4% in case of use of the 4 g twice daily dosing regimen. In this study in which the majority of patients were treated with the 2 g qid regimen, a reversible creatinine increase was detected only in 0.7% of treated women.

Our results are somewhat in line with a recent published paper reporting the protective effect of VCV even in infection acquired up to 8 to 5 weeks before conception and in patients diagnosed with primary infection following intermediate avidity.[15] Also, in our cohort about one third of treated women were diagnosed with primary infection with an intermediate IgG avidity (plus detection of CMV DNA in one body fluid sample).

Interesting data come from the analysis focused on newborns with cCMV diagnosis at birth after a negative amniocentesis and their mothers. This event appears to be slightly more frequent in the group of mothers treated with VCV compared to those of untreated mothers but not significantly (12.9% vs 7.3%) and within the group of treated mothers appear to be significantly more frequent among women enrolled for seroconversion (23.2%) compared to those enrolled for positive serology with low avidity (10.8%) or positive serology, intermediate avidity and positive molecular test (6.3%).

The prognosis of these newborns is reported to be good according to the available literature,[16] however data come from studies carried out before VCV entered in the clinical practice. VCV may have a modulatory effect on maternal immunity and whether this immunomodulation could affect the severity of fetal infection and disease is unknown.[17,18] Of note, 3 out of 36 newborns with cCMV after negative amniocentesis in the VCV group presented signs or symptoms at birth without signs of brain disruption characteristic of an early infection. More data are needed to understand the relevance of these mild findings at birth and their relevance in terms of possible long term sequelae.

The study also provides some information on VCV use during pregnancy for confirmed fetal infection or disease (so called tertiary prevention). In our cohort, the prevalence of asymptomatic cCMV among newborns with prenatal diagnosis of infection (positive amniocentesis) but no ultrasound or laboratory signs of fetal disease was slightly

higher in those cases who continued VCV until the end of pregnancy compared to those who discontinued the treatment shortly after amniocentesis (78.6% vs 66.7%). Also, a potentially less protective effect of VCV compared to that previously reported by Leruez-Ville et al.[13] was observed among the group of newborns with prenatal ultrasound or laboratory signs of fetal mild to moderate disease and whose mother received VCV until the end of pregnancy. In this subgroup the prevalence of asymptomatic cCMV at birth was 55.6% in our study and 82% in Leruez-Ville et al.'s study.[13]

However it should be considered that our definition of symptomatic cCMV at birth was the one provided by ESPID 2017, while Leruez-Ville et al. in their publication used a different classification.[13] By applying this different classification 6 of 12 symptomatic newborns were re-classified as asymptomatic. Using this classification the proportion of asymptomatic newborns was 66.7% and 85.7% in the group of newborns whose mothers continued VCV until birth with and without prenatal signs of disease respectively and 93.3% in the group of newborns whose mothers discontinued VCV shortly after amniocentesis.

These data underline the importance of additional studies on alternative strategies for treatment of confirmed fetal infection. In this view the results of the ongoing clinical trial comparing letermovir to VCV for prenatal treatment of confirmed CMV fetal infection are eagerly awaited.[19]

Clinical implications

The results of the study provide support to clinicians for the management of pregnant women with primary CMV infection. The results indicate that VCV can be used successfully and safely in patients satisfying simple diagnostic criteria of CMV primary infection within 24 weeks of pregnancy by combining serological and molecular tests. Moreover, the results may help the managing physician in more adequately counselling pregnant women with primary CMV infection by providing expected rates of cCMV diagnosis at amniocentesis and at birth (including diagnosis at birth after a negative amniocentesis) with or without treatment and the rate of symptomatic cCMV.

Strengths and limitations

One of the limitations of this study is its multicenter nature which implies some degree of heterogeneity in clinical management, diagnostic algorithms, and laboratory assays across participating centers, even though all centers employed VCV as recommended by the AIFA. An additional potential limitation could be the use of intermediate-avidity test plus a positive molecular test as a criterion for primary infection since detection in maternal biological samples could potentially reflect viral reactivation, reinfection, or prolonged shedding rather than recent primary infection. However it should be considered that the ECCI consensus[20] considers intermediate avidity test (confirmed by a second test on the same sample) as indication for secondary prevention.

Despite these limitations, this study represents the largest real-world cohort evaluating pregnancy outcome after the implementation of VCV in the clinical practice at country level. The consistency of the observed protective effects across multiple clinically relevant outcomes, combined with the favorable safety profile, supports the integration of VCV into standardized management pathways for primary CMV infection in pregnancy. Future prospective studies with harmonized diagnostic protocols and long-term follow-up are warranted to further refine treatment strategies.

Conclusions

In this large multicenter real-world cohort, maternal treatment with high-dose VCV following primary CMV infection during the periconceptional period or early pregnancy was associated with a significant reduction in maternal–fetal transmission at amniocentesis, TOP and symptomatic cCMV infection.

VCV showed a favorable safety and tolerability profile, with no severe adverse events observed, supporting its prolonged use during pregnancy. These findings provide strong real-life evidence supporting the use of VCV as an effective secondary preventive strategy in the management of primary CMV infection in pregnancy.

Overall, this study reinforces the clinical value of early diagnosis and treatment and supports inclusion of VCV into standardized care protocols for pregnant women with primary CMV infection. ■

CRediT authorship contribution statement

Lorenzo Zammarchi: Writing – review & editing, Writing – original draft, Validation, Supervision, Project administration, Methodology, Investigation, Data curation, Conceptualization. **Anna Beltrami:** Writing – original draft, Data curation. **Giuseppina Liuzzi:** Investigation, Data curation. **Federica Ciccone:** Data curation. **Filippo Lagi:** Methodology, Formal analysis. **Beatrice Borchi:** Investigation, Conceptualization. **Lucia Pasquini:** Investigation. **Elisabetta Venturini:** Writing – review & editing, Formal analysis. **Luisa Galli:** Project administration. **Nigritella Brianese:** Investigation. **Lina Rachele Tomasoni:** Investigation, Data curation. **Sofia Lovatti:** Investigation. **Vania Spinoni:** Investigation. **Serena Messali:** Investigation. **Antonella Vimercati:** Investigation. **Francesco Di Gennaro:** Investigation. **Sara Dalena:** Data curation. **Maria Teresa Tassone:** Investigation. **Daniele Lilleri:** Writing – review & editing, Investigation. **Milena Furione:** Writing – review & editing, Investigation. **Piera d'Angelo:** Investigation. **Alessia Arossa:** Investigation. **Giuseppe Maria Maruotti:** Investigation. **Laura Letizia Mazzarelli:** Investigation. **Serena Salomè:** Investigation. **Francesco Raimondi:** Supervision. **Sara Ornaghi:** Writing – review & editing, Investigation. **Francesca Arienti:** Investigation. **Federica Fernicola:** Investigation. **Mariateresa Sinelli:** Investigation. **Carolina Saffioti:** Investigation. **Erica Ricci:** Investigation. **Alessio Mesini:**

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Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.ajogmf.2026.102156.

References

1. Leruez-Ville M, Foulon I, Pass R, Ville Y. Cytomegalovirus infection during pregnancy: state of the science. Am J Obstet Gynecol 2020;223:330–49. https://doi.org/10.1016/j.ajog.2020.02.018.

2. Smithers-Sheedy H, Raynes-Greenow C, Badawi N, et al. Congenital cytomegalovirus among children with cerebral palsy. J Pediatr 2017;181. https://doi.org/10.1016/j.jpeds.2016.10.024. 267-271.e1.

3. Korver AM, de Vries JJ, Konings S, et al. DECIBEL study: congenital cytomegalovirus infection in young children with permanent bilateral hearing impairment in the Netherlands. J Clin Virol 2009;46(Suppl 4):S27–31. https://doi.org/10.1016/j.jcv.2009.09.007.

4. Avettand-Fenoël V, Marlin S, Vauloup-Fellous C, et al. Congenital cytomegalovirus is the second most frequent cause of bilateral hearing loss in young French children. J Pediatr 2013;162:593–9. https://doi.org/10.1016/j.jpeds.2012.08.009.

5. Chatzakis C, Ville Y, Makrydimas G, Dinas K, Zavlanos A, Sotiriadis A. Timing of primary maternal cytomegalovirus infection and rates of vertical transmission and fetal consequences. Am J Obstet Gynecol 2020;223. https://doi.org/10.1016/j.ajog.2020.05.038. 870-883.e11.

6. Shahar-Nissan K, Pardo J, Peled O, et al. Valaciclovir to prevent vertical transmission of cytomegalovirus after maternal primary infection during pregnancy: a randomised, double-blind, placebo-controlled trial. Lancet 2020;396(10253):779–85. https://doi.org/10.1016/S0140-6736(20)31868-7.

7. Faure-Bardon V, Fourgeaud J, Stirnemann J, Leruez-Ville M, Ville Y. Secondary prevention of congenital cytomegalovirus infection with valacyclovir following maternal primary infection in early pregnancy. Ultrasound Obstet Gynecol 2021;58:576–81. https://doi.org/10.1002/uog.23685.

8. Egloff C, Sibiude J, Vauloup-Fellous C, Benachi A, Bouthry E, Biquard F, Hawkins-Villarreal A, Houhou-Fidouh N, Mandelbrot L, Vivanti AJ, Picone O. New data on efficacy of valacyclovir in secondary prevention of maternal-fetal transmission of cytomegalovirus. Ultrasound Obstet Gynecol. 2023;61(1):59–66. doi:10.1002/uog.26039. Erratum in: Ultrasound Obstet Gynecol. 2023;61(4):541. doi:10.1002/uog.26195.

9. Zammarchi L, Tomasoni LR, Liuzzi G, et al. Treatment with valacyclovir during pregnancy for prevention of congenital cytomegalovirus infection: a real-life multicenter Italian observational study. Am J Obstet Gynecol MFM 2023;5:101101. https://doi.org/10.1016/j.ajogmf.2023.101101.

10. Farmaco Agenzia Italiana del. Inserimento del medicinale valaciclovir (originatore o biosimilare) nell'elenco dei medicinali erogabili a totale carico del Servizio sanitario nazionale. Gazzetta Ufficiale della Repubblica Italiana; 2020. p. 322.

11. Revello MG, Lazzarotto T, Guerra B, Spinillo A, Ferrazzi E, Kustermann A, Guaschino S, Vergani P, Todros T, Frusca T, Arossa A, Furione M, Rognoni V, Rizzo N, Gabrielli L, Klersy C, Gerna G. CHIP Study Group. A randomized trial of hyperimmune globulin to prevent congenital cytomegalovirus. N Engl J Med 2014;370(14):1316–26. https://doi.org/10.1056/NEJMoa1310214.

12. Luck SE, Wieringa JW, Blázquez-Gamero D, et al. Congenital cytomegalovirus: a European expert consensus statement on diagnosis and management. Pediatr Infect Dis J 2017;36:1205–13. https://doi.org/10.1097/INF.0000000000001763.

13. Leruez-Ville M, Ghout I, Bussières L, et al. In utero treatment of congenital cytomegalovirus infection with valacyclovir in a multicenter, open-label, phase II study. Am J Obstet Gynecol 2016;215(4):462.e1–462.e10. https://doi.org/10.1016/j.ajog.2016.04.003.

14. Chatzakis C, Shahar-Nissan K, Faure-Bardon V, et al. The effect of valacyclovir on secondary prevention of congenital cytomegalovirus infection following primary maternal infection acquired periconceptionally or in the first trimester of pregnancy: an individual patient data meta-analysis. Am J Obstet Gynecol 2024;230. https://doi.org/10.1016/j.ajog.2023.07.022. 109-117.e2.

15. Fourgeaud J, Barry M, Bourgon N, Veyrenche N, Guilleminot T, Ville Y, et al. Cytomegalovirus vertical transmission rate according to IgG avidity value and valacyclovir treatment of maternal primary infection in the first trimester of pregnancy. Clin Infect Dis 2025;81:e417–23. https://doi.org/10.1093/cid/ciaf437.

16. Chatzakis C, Sotiriadis A, Dinas K, Ville Y. Neonatal and long-term outcomes of infants with congenital cytomegalovirus infection and negative amniocentesis: systematic review and meta-analysis. Ultrasound Obstet Gynecol 2023;61(2):158–67. https://doi.org/10.1002/uog.26128.

17. Arcieri F, Vasta A, Sorrenti S, et al. Antenatal imaging and neonatal outcome in infants with congenital Cytomegalovirus infection: the effect of Valaciclovir. J Clin Med 2026;15(2):809. https://doi.org/10.3390/jcm15020809.

18. Brosh-Nissimov T, Benshalom-Tirosh N, Bucris E, Morad H, Zuckerman NS, Tepperberg Oikawa M. Recurrent congenital cytomegalovirus infection in a sequential pregnancy with severe sequelae, and a possible association with prophylactic valacyclovir treatment: a case report. Int J Infect Dis 2022;125:93–5. https://doi.org/10.1016/j.ijid.2022.09.038. Epub 2022 Oct 10. PMID: 36229004.

19. ClinicalTrials.gov. Prenatal treatment of congenital cytomegalovirus infection with letermovir versus valaciclovir (CYMEVAL3-step2). ClinicalTrials.gov identifier: NCT05446571. Available at: https://clinicaltrials.gov/study/NCT05446571

20. Leruez-Ville M, Chatzakis C, Lilleri D, et al. Consensus recommendation for prenatal, neonatal and postnatal management of congenital cytomegalovirus infection from the European congenital infection initiative (ECCI). Lancet Reg Health Eur 2024;40:100892. https://doi.org/10.1016/j.lanepe.2024.100892. Erratum in: Lancet Reg Health Eur. 2024 Jun 24;42:100974. 10.1016/j.lanepe.2024.100974..

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