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Review article

Counseling in fetal medicine: Congenital cytomegalovirus infection

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ABSTRACT

Although the clinical work-up of CMV in pregnancy has gradually become more accurate, counseling for CMV is still challenging. Despite the potential feasibility of universal prenatal serological screening, its introduction in prenatal diagnosis continues to raise concerns related to its real cost-effectiveness. Contextually, anticipating the confirmation of fetal infection earlier in pregnancy is one of the most pressing issues to reduce the parental psychological burden. Amniocentesis is still the gold standard and recent data have demonstrated that it could be performed before 20 weeks of gestation, provided that at least 8 weeks have elapsed from the presumed date of maternal seroconversion. New approaches, such as chorionic villus sampling (CVS) and virome DNA, even if not yet validated as confirmation of fetal infection, have been studied alternatively to amniocentesis to reduce the time-interval from maternal seroconversion and the amniocentesis results. Risk stratification for sensorineural hearing loss (SNHL) and long-term sequelae should be provided according to the prognostic predictors. Nevertheless, in the era of valacyclovir, maternal high-dose therapy, mainly for first trimester infections, can reduce the risk of vertical transmission and increase the likelihood of asymptomatic newborns, but it is still unclear whether valacyclovir continues to exert a beneficial effect on fetuses with positive amniocentesis. This review provides updated evidence-based key counseling points with GRADE recommendations.

Introduction

Cytomegalovirus (CMV) represents a public health concern, affecting 0.67 % of live births. [1] CMV can present as a primary infection (PI) or non-primary infection (NPI), when either a later reactivation or a reinfection occurs. Although affected newborns are mostly asymptomatic, congenital CMV (cCMV) can cause sensorineural hearing loss (SNHL) and long-term neurological sequelae, occurring both in the symptomatic and asymptomatic infants. [2].

Even if serological screening intercepts maternal seroconversion for CMV, controversies mainly based on the cost-effectiveness of a universal serological screening have led to different policies worldwide. Amniocentesis is currently the gold standard for fetal infection confirmation and even quantitative results related to the amniotic fluid viral load have been demonstrated as potential determinants of post-natal outcomes, but available data are still not generalizable.

A crucial step in parental counseling is to identify fetuses at higher risk of sequelae and prenatal imaging, both ultrasound (US) and magnetic resonance imaging (MRI), plays a pivotal role in the cCMV risk stratification. [3] Finally, recent evidence demonstrates a beneficial role for antiviral therapy to prevent vertical transmission, leading to the potential reconsideration of the current prenatal management of women with suspected CMV infection. [4].

The current body of evidence on the management of cCMV infection is still controversial regarding diagnosis, prognosis, and treatment. The aim of this review is to provide updated answers to a list of common

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clinical questions that may emerge in our daily practice as fetal medicine consultants coping with CMV infection.

Methods

We collected a list of common clinical questions emerging in our daily practice as fetal medicine consultants working at University Hospital referral centers. A systematic search strategy was performed through Medline, Embase, Cinahl and Web of Sciences databases were searched electronically up to January 2023, utilizing combinations of the relevant medical subject heading (MeSH) terms, key words, and word variants for "cytomegalovirus", "CMV", "infection", "pregnancy" and "outcome". Selection criteria were restricted to English language. Reference lists of relevant articles and reviews were hand searched for additional reports. Studies focusing on other infections were excluded as these were beyond the scope of this review.

The quality of the evidence was assessed using the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) guidelines in one of four levels: high (further research is very unlikely to change our confidence in the estimate of effect); moderate (further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate); low (further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate); and very low (any estimate of effect is very uncertain). [5] The results of the quality assessment for each evidence-based answer are shown in Table 1.

Clinical questions

How does CMV affect pregnant women?

CMV belongs to the Herpesviridae family, and the human being is its sole host, colonized via community exposure (mainly occupational contact with young children) or bloodborne transmission. [2] When PI occurs, CMV has a replication phase and later a hematogenous dissemination. Afterwards, the infection reaches a latency phase, until any immunocompromising or interfering conditions trigger its reactivation. Reactivation or even reinfection with a new viral strain are called NPI.

When acquired during pregnancy, PI is asymptomatic in about 90 % of women, while it may cause a mononucleosis-like clinic with mild febrile illness and other nonspecific symptoms (rhinitis, pharyngitis, myalgia, fatigue and dermatological manifestations) in the remaining cases. Abnormal liver function tests, atypical lymphocytosis, hemolytic anaemia are laboratory findings that should require prompt investigation for CMV. Conversely, NPI generally does not result in maternal symptoms, unless immunocompromised.

In contrast to other conditions, such as influenza virus or COVID-19, pregnancy does not appear to worsen the clinical course of maternal infection. [6].

How does CMV affect the fetus?

Similarly to pregnant women, roughly 90 % of children develop an asymptomatic infection. However, cCMV represents a noteworthy concern for the SNHL and long-term neurological sequelae, occurring both in the symptomatic and asymptomatic infants. [1,2] The risk of vertical transmission of CMV in utero increases with advancing gestation, but the risk of fetal/neonatal complications is inversely proportional to the age at infection. Vertical transmission to the fetus occurs more frequently after a maternal PI (MPI) than a non-primary one, with the risk being around 30–40 % and 1–2 %, respectively, but NPI is hard to diagnose and possibly underestimated. [3] In fact, although the latter is associated with a lower risk of transmission, it occurs more frequently, according to the worldwide seroprevalence, which is roughly 80 %, therefore the consequential burden of disease should be considered as relevant. [7] Furthermore, a recent *meta*-analysis concluded that type of

Table 1
Summary of evidence-based key counseling points.

CLINICAL QUESTION	UP-TO-DATE ANSWER	QUALITY OF EVIDENCE
1. How does CMV affect pregnant women?	Maternal CMV infection is commonly asymptomatic, and pregnancy does not increase the susceptibility to have worse clinical course of CMV infection than non-pregnant women.	Moderate
2. How does CMV affect the fetus?	The risk of vertical transmission is higher in primary compared to non-primary infection (30–40 % vs 1–2 %), but there are no differences in terms of long-term sequelae once congenital infection has been established.	Moderate
3. Who is at greatest risk of acquiring CMV during pregnancy?	The risk of maternal CMV seroconversion reflects social, demographic, environmental, and obstetrical circumstances. In low income countries, pregnant women are at higher risk of NPI. In high-income countries, women with toddlers at home are at higher risk of PI during pregnancy.	Moderate
4. Which strategies should be recommended to pregnant women for primary prevention of CMV infection?	Preventive strategies are essential to reduce maternal seroconversion. Pregnant individuals should be educated on the importance of such behavioral measures.	Low
5. How to diagnose maternal CMV infection and whom to test?	Diagnosis of MPI is made using serological tests as clinical symptoms of CMV are rather uncommon and non-specific, while in NPI serology and CMV PCR of maternal urine and blood might be misleading due to the different and variable serologic and molecular patterns.	Moderate
6. Universal population-based vs current serological screening: which screening program is more cost-effective?	Cost-effectiveness of universal screening is an area of current investigation and more data is needed, but it should be considered as country-specific, due to different rates of CMV seroprevalence, and related to high dose valaciclovir prophylaxis.	Low
7. How to diagnose fetal CMV infection?	Detection of CMV by PCR in the amniotic fluid obtained from amniocentesis performed 8 weeks after maternal seroconversion is recommended for the diagnosis of cCMV infection antenatally. A false negative result could occur in up to 8 % of cases, but a negative amniocentesis with normal prenatal imaging is not associated with severe SNHL and/or neurodevelopmental impairment at follow-up. Thus, third trimester evaluation is invariably recommended as good clinical practice.	Moderate
8. Are there new approaches to diagnose of fetal infection?	New approaches for earlier confirmation of vertical transmission have been emerging. CVS and virome DNA have shown promising results, but both procedures require further studies to be established and validated.	Low
9. What is the role of ultrasound in assessing	Ultrasound is the primary imaging modality to evaluate fetuses with	Moderate

(*continued on next page*)

Table 1 (*continued*)

CLINICAL QUESTION	UP-TO-DATE ANSWER	QUALITY OF EVIDENCE
fetal infection?	congenital CMV infection. In view of its higher resolution, transvaginal ultrasound should be the preferred method to scan fetuses with CMV infection in cephalic presentation. However, the PPV of ultrasound is poor in the absence of confirmed fetal infection with amniocentesis.	
10. What is the role of prenatal imaging in predicting postnatal outcomes?	Microcephaly is the ultrasonographic sign mostly associated with adverse neurodevelopmental outcome in fetuses with congenital CMV infection. MRI should be used to detect anomalies not easily identified by ultrasound, mainly those involving the cortical surface of the brain and should be recommended in all pregnant women with positive amniocentesis at 28–32 weeks of gestation. Negative imaging findings do not completely rule out adverse outcome, although the large majority of those with normal imaging confirmed at birth have a benign prognosis, with a small residual risk of mild impairment.	Moderate
11. What is the role of laboratory parameters in assessing postnatal outcomes?	Viral load in the amniotic fluid > 1.300.000 copies/mL has been found to be associated with a higher risk imaging anomalies at US and MRI, but this tool should be used as predictor in selected expert centers only and requires further validation.	Low
12. Is gestational age at maternal infection important in determining a poor prognosis?	Early gestational age at maternal infection is one of the main determinants of post-natal adverse outcome in fetuses with congenital CMV infection.	Moderate
13. How should late CMV infection be managed?	There are controversial data regarding outcomes following the late CMV infections and, although milder long-term effects have been described in association, further data are needed to clarify how to manage second and third trimester cCMV. Until robust evidence, amniocentesis to confirm fetal infection and follow-up through US and MRI should be recommended.	Low
14. What is the clinical presentation of neonates with cCMV infection?	Neonatal symptoms at birth are SGA, hepatomegaly, splenomegaly, petechiae, chorioretinitis, central CNS (also CMV-DNA detection in cerebrospinal fluid) and SNHL, elevated liver enzymes or thrombocytopenia. In the long term, symptomatic infection might occur in about 10 % of fetuses with congenital CMV, but asymptomatic infections do exclude permanent sequelae.	Moderate
15. Are there any medical therapies for CMV infection?	Maternal therapy with high dose valacyclovir (2 g every 6 h, 8 g/day) can reduce the risk of vertical transmission, the symptomatic status and other adverse outcomes associated with congenital CMV infection, while HIG is not recommended.	High

CMV: Cytomegalovirus; cCMV: congenital cytomegalovirus; PCR: polymerase chain reacting; MRI: magnetic resonance imaging; HIG: hyperimmune globulin.

maternal infection is not associated with symptomatic newborns' status, SNHL and neurodevelopmental sequelae, once fetal infection has been established, concluding for a similar prevalence of long-term sequelae, regardless of PI or NPI (Fig. 1). [8].

Who is at greatest risk of acquiring CMV during pregnancy?

Worldwide, the CMV seroprevalence in women of reproductive age has been estimated to be about 86 %, with the highest rates reported in the Eastern Mediterranean region (92 %), Western Pacific region (91 %) and African region (90 %) and the lowest in the European region (70 %). [1,7] Maternal seroconversion reflects socio-demographic and obstetrical conditions. The prevalence of CMV is higher in individuals with lower socio-economic status (SES) or household income. [7] Due to their high CMV seroprevalence, pregnant women in low income countries are more likely to be seropositive prior to pregnancy, being at risk of CMV due to NPI, while, in high income countries, childcare workers or childbearing women with less than 3-year-old children or an interpregnancy-interval less than 2 years are at a significantly higher risk of PI during pregnancy. [9,10].

Which strategies should be recommended to pregnant women for primary prevention?

To date, no vaccine is licensed for primary prevention of CMV infection, but a phase-3 randomized-placebo-controlled trial evaluating the efficacy, the safety and reactogenicity of a mRNA-CMV vaccine on seronegative non-pregnant women is currently ongoing (ClinicalTrials.gov identifier: NCT05085366). An alternative strategy to reduce the risk of infection is behavioral recommendations that minimize direct contacts with biological fluids of young children. Several studies reported that not only women but also healthcare workers have inadequate knowledge of preventive measures for cCMV, therefore education on preventive strategies should be a mandatory part of the antenatal clinical counseling. [11,12].

The main recommendations should focus on hygiene measures, such as handwashing in case of contact with children's saliva or urine or avoiding intimate contact, such as kissing and sharing utensils. Several trial provided evidence that seroconversion rate was significantly reduced using these preventive measures, fairly accepted among pregnant women, who demonstrated the capability to sustain behavioral changes, feeling these recommendations worth suggesting and being not concerned about limiting intimate contact with their children [11,12].

How to diagnose maternal CMV infection and whom to test?

As about 90 % of pregnant women are asymptomatic, PI is rarely identified by clinical findings only, thus making serology tests the main tool for diagnosis (Table 2). Conversely, serological tests in NPI are misleading, in view of the possibility of persisting IgM or the presence of IgM that shows a cross reactivity to CMV serological kits. The serological diagnosis of recurrent infection is commonly based on an increased CMV-IgG titer along with high CMV-IgG avidity index and/or positive CMV-IgM, but different serologic patterns may be observed and serologic testing may fail to help with diagnosis. The detection of CMV-DNA by polymerase chain reaction (PCR) analysis in maternal urine and blood might be useful to confirm a NPI, but worth a mention that a negative PCR does not exclude a NPI. [13,14] Thus, the only definitive test of fetal infection after maternal NPI is amniocentesis CMV-PCR, performed after the incidental finding of fetal US abnormalities.

Many researchers have been advocating for maternal serologic screening as an appropriate tool for detecting CMV infection during pregnancy [15], mostly for a precise timing of infection, which

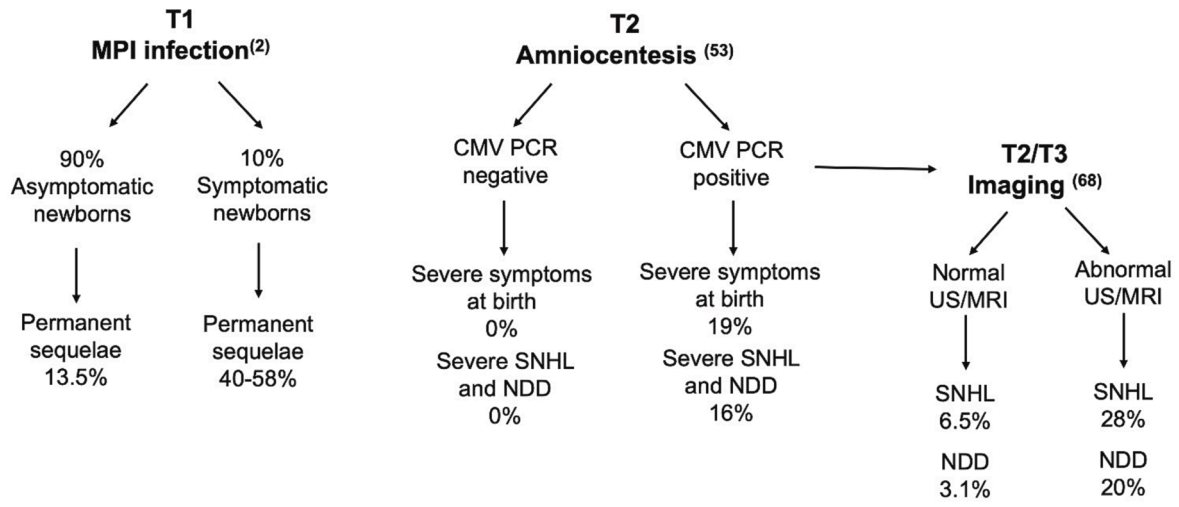

Fig. 1. Flowchart of proposed management of CMV infection during pregnancy T1, first trimester; T2, second trimester; T3, third trimester; PCR, polymerase chain reaction; US, ultrasound; MRI, magnetic resonance imaging; SNHL, sensorineural hearing loss; NDD, neurodevelopmental delay.

Table 2
A guide for the interpretation of serology results.

CMV IgG	CMV IgM	IgG avidity	Recommendations	Interpretation
Negative	Negative	NA	Primary prevention through behavioral measures	**Seronegative uninfected patient**
Negative	Positive	NA	Repeat serology and in case of positive IgG, IgG avidity is requested	**Recent seroconversion should be excluded** with serological confirmation **Exclude a false positive** result due to a cross-reaction with other viral infections
Positive	Negative	NA	Serology might be repeated in cases of suspected fetal infection due to a NPI	**Past infection**
Positive	Positive	High	Serology might be repeated or amniocentesis might be performed to confirm a suspected fetal infection due to a NPI	**Past infection with persisting IgM or NPI**
Positive	Positive	Low	Amniocentesis should be performed to exclude fetal infection	**Recent PI**

NPI: non-primary infection; PI: primary infection; NA: not applicable.

represents one of the most important prognostic predictors. However, universal serological screening is not endorsed by most international societies, unless part of a research context, and therefore maternal serological testing is only triggered by incidental US findings or abnormal maternal blood exams. [16].

Universal population-based vs current serological screening: Which is more cost-effective?

Both maternal and neonatal universal screening program are not routinely offered and recommended worldwide. However, reasons for which universal screening should be advocated are multifold, starting from the promising data of a roughly 70 % reduction in vertical transmission with valacyclovir in-*utero* treatment. [4,17] Early serology allows a more precise timing of maternal seroconversion which is necessary for the counseling and the understanding of fetal prognosis. Moreover, the implementation of a universal maternal screening might increase the otherwise missed subtle fetal findings, given that the targeted US of known infected fetuses has a 91 % of sensitivity and 96 % of negative predictive value. [18] Nevertheless, controversial results found in cost-effectiveness analysis are inevitable, since population with different seroprevalence, healthcare costs and prenatal therapies, hyperimmune globulins (HIG) or valacyclovir, have been considered. The first issue to be addressed is that in countries with low seroprevalence, MPI is mainly responsible for cCMV, but this may not apply for countries with a maternal seroprevalence over 90 %, where NPI is the main source for cCMV. [7] The second relevant issue is that, so far, these analysis have considered different treatments and the current body of evidence suggest that only valacyclovir, due to its promising data, could be considered for population-based screening cost-effectiveness. A recent study stated that valacyclovir prophylaxis to be cost-effective must achieve at least 75.9 % efficacy in reducing the risk of vertical transmission of primary CMV, [19] but a recent *meta*-analysis found that the risk reduction is just below 70 % with high dosage. [4] Well-designed large scale RCT are urgently needed to confirm this data.

How to diagnose fetal CMV infection?

Definitive diagnosis is only possible by amniotic fluid PCR analysis of CMV-DNA. [3] A time-interval between infection and amniocentesis less than 8 weeks and a gestational age at amniocentesis less than 18 weeks have usually been considered among the most important risk factors for false negative results, although recent data showed no significant difference in terms of sensitivity between an invasive procedure performed after 17 weeks or after 20 weeks of gestation when the interval between maternal seroconversion and amniocentesis was more than 8 weeks. [20] Faure-Bardon et al., in a 2021 prospective study on 214 women, confirmed that amniocentesis between 17 and 20 weeks, as long as 8 weeks had elapsed from MPI, provided a sensitivity for detecting cCMV of 95.8 %, specificity of 100 %, positive predictive value (PPV) of 100 % and negative predictive value (NPV) of 97.7 % [21].

Reported cases of cCMV infection with a negative amniocentesis are worth mentioning due to a delayed placental transmission, with later fetal infection and a viral load at amniocentesis that is too low to be detectable. [22] A recent *meta*-analysis has reported an 8 % rate of false negative results, defined as newborns shedding CMV in the urine at birth with negative amniocentesis. [22] However, this scenario was not

associated with fetal insult and long-term sequelae, even if transmission had occurred. [22] Nevertheless, the included studies in this *meta*-analysis did not clearly report abnormalities at the prenatal imaging after negative amniocentesis, thus counseling should carefully underline that a negative amniocentesis is not associated with neonatal consequence in case of a normal imaging. Consequently, although undetected CMV-DNA in the amniotic fluid might be reassuring, pregnant women should be evaluated once more during the third trimester as a good clinical practice, especially in countries where third trimester scanning is not routinely performed.

Are there new approaches to diagnose fetal infection?

Emerging approaches have been focusing on earlier diagnosis to reduce the anxiety of vertical transmission and the potential choice of the termination of pregnancy (TOP). A recent study of 37 CMV-PCR obtained from chorionic villus sampling (CVS) demonstrated a sensitivity of 50 %, a specificity of 100 %, a NPV of 100 % and a PPV of 91 %, thus suggesting that a negative CMV-PCR in the trophoblast after 12 weeks could be useful to exclude CMV-related embryopathy leading to significant sequelae. [23].

Likewise, the potential feasibility of genome-wide cell-free DNA (cf-DNA) to investigate the prevalence of viral DNA in pregnant women have been underlined, without being altered due to various aneuploidies. This screening testing is usually performed during the first trimester and, as CVS, could be implemented for earlier diagnosis of fetal CMV infection. In fact, a low fetal fraction has been associated with the absence of virome DNA, that may suggest a placental or fetal origin of viral DNA, but further studies are needed to fully understand and confirm if the presence of viral cfDNA comes from either the placenta or the fetus, thus potentially suggesting an ongoing CMV-induced placentitis or an occurred fetal infection. [24].

What is the role of ultrasound in assessing fetal infection?

In the absence of a universal serological screening, US is the mainstay for triggering the diagnosis of fetal infection. The ultrasonographic findings are grouped in placental, extracranial and cranial features, following the natural history of CMV infection (Table 3, Figs. 2-4); [3] these signs may be detected from about 12 weeks after MPI and the presence of any of these findings cannot be considered diagnostic but merely suggestive of cCMV. [3].

Maternal viremia is associated with placental invasion, which may result in placentomegaly, defined as a placental thickness greater than 40 mm, although other authors reported an increasing placental thickening by week of gestation from 16 to 36 weeks [3].

Table 3
Ultrasound findings in fetal infection with CMV (adapted from Leruez-Ville M.) [2].

Placental findings	
Placentomegaly and placental calcifications	**2 %**
Extracerebral findings	
Small for gestational age (SGA) or fetal growth restriction (FGR)	**9 %**
Hyperechogenic bowel	**13 %**
Pericardial effusion, pleural effusion, hydrops, skin edema	**~ 1 %**
Ascites	**4.2 %**
Hepatomegaly and/or splenomegaly	**3.8 %**
Liver calcifications	**1.2 %**
Polyhydramnios	**< 1 %**
Oligohydramnios	**3.4 %**
Cerebral findings	
Microcephaly	**6 %**
Hydrocephalus	**3.6 %**
Ventriculomegaly	**6.1 %**
Cerebral calcifications	**6.3 %**
Hyperechogenic periventricular halo	**3 %**
Subependymal cysts	**1.7 %**
Abnormal gyration (heterotopias and polymicrogyria)	**<1%**

After placenta invasion, the most frequent extracerebral findings are the hyperechogenic bowel (13 % of cases) and fetal growth restriction (FGR) (9 % of cases), and the most frequent cerebral abnormalities are ventriculomegaly (Fig. 2a), microcephaly (Fig. 2b) and cerebral calcifications, [2] that are usually associated with a first trimester PI and are considered not only suggestive for cCMV, but also prognostic of symptomatic newborns. [25] Other US signs are more subtle, as the periventricular echogenic halo (Fig. 2c). A suspected cCMV is one of the main indications for a targeted neurosonography (NSG), recommended at each follow-up examination in a referral center, performed by experienced operators, as this exam has a much higher diagnostic potential compared with the screening evaluation. Moreover, the transvaginal US gives a better resolution of the fetal brain, allowing detailed study of the periventricular parenchyma and should be the preferred approach when assessing infected fetuses in cephalic presentation, also to assess cortical formation (Fig. 2d).

However, the accuracy of US alone is poor in fetuses whose infectious status is unknown, revealing cCMV only in one-third of cases, with a PPV of 35 %, whereas it increases to up to 95 % in case of serologically proven MPI and positive amniocentesis. [25,26] The increased accuracy of US in known-infected fetuses is also due to the longitudinal imaging assessment, recommended every 2–4 weeks in case of positive amniotic fluid to identify structural anomalies potentially impacting the short- and long-term outcomes of the newborn. [27].

What is the role of prenatal imaging in predicting postnatal outcomes?

US represents the mainstay of surveillance and prognosis of postnatal course in cCMV infection. Signs of CMV infection can occur in about 4–9 % of cases, thus highlighting the need for follow-up targeted US during pregnancy. [27–29].

Detection of CNS anomalies is among the main determinants of adverse neurodevelopmental impairment in cCMV. [27] The commonest cranial abnormalities are ventriculomegaly (Fig. 2a), followed by periventricular abnormalities and temporal cysts and other parenchymal lesions. However, only microcephaly (Fig. 2b) has been clearly associated with more than 95 % of probability of adverse neurodevelopmental outcome. [30] Periventricular hyperechogenicity, or the halo sign (Fig. 2c), which is a subtle sonographic marker, not visible on MRI, is worth mentioning as it is one of the most frequent findings in cCMV. If isolated, the halo sign was mostly associated with mild ventriculitis, microglial nodules and CMV-infected cells with no evidence of white-matter necrosis, whereas non-isolated halo sign revealed white-matter and cortical necrosis. Although these results come from a small sample, they introduce a new classification in isolated and non-isolated halo sign variants, with potential different prognostic significance that need to be further assessed. [31].

When exploring the outcomes of fetuses with positive amniocentesis, the overall rate of an associated CNS anomaly detected on a follow-up US is 4.4 %, while the rates of those detected exclusively on prenatal MRI or on postnatal imaging is 5.8 % and 3.2 %, respectively. However, normal sonographic imaging in fetuses with positive amniocentesis does not completely rule out the development of SNHL and minor neurodevelopmental abnormalities, although the risk of severe neurodevelopmental outcome is less common. [28,29,32] In cases without an associated anomaly detected pre- or postnatally, symptomatic status, neurodevelopmental anomaly and SNHL is found in 1.5 %, 3.1 % and 6.5 % of children, respectively. These findings highlight the role of a thorough US follow-up during pregnancy, but fetal MRI should be considered as a complementary exam, in order to detect anomalies, especially those involving the cortical surface, which can be missed at US. Its advantages include visualization of the entire brain parenchyma and detection of white matter anomalies, of which white matter hyperintense signal on T2-weighted images is subjective with a prognostic value still difficult to interpret. [28,29,33,34] In conclusion, MRI

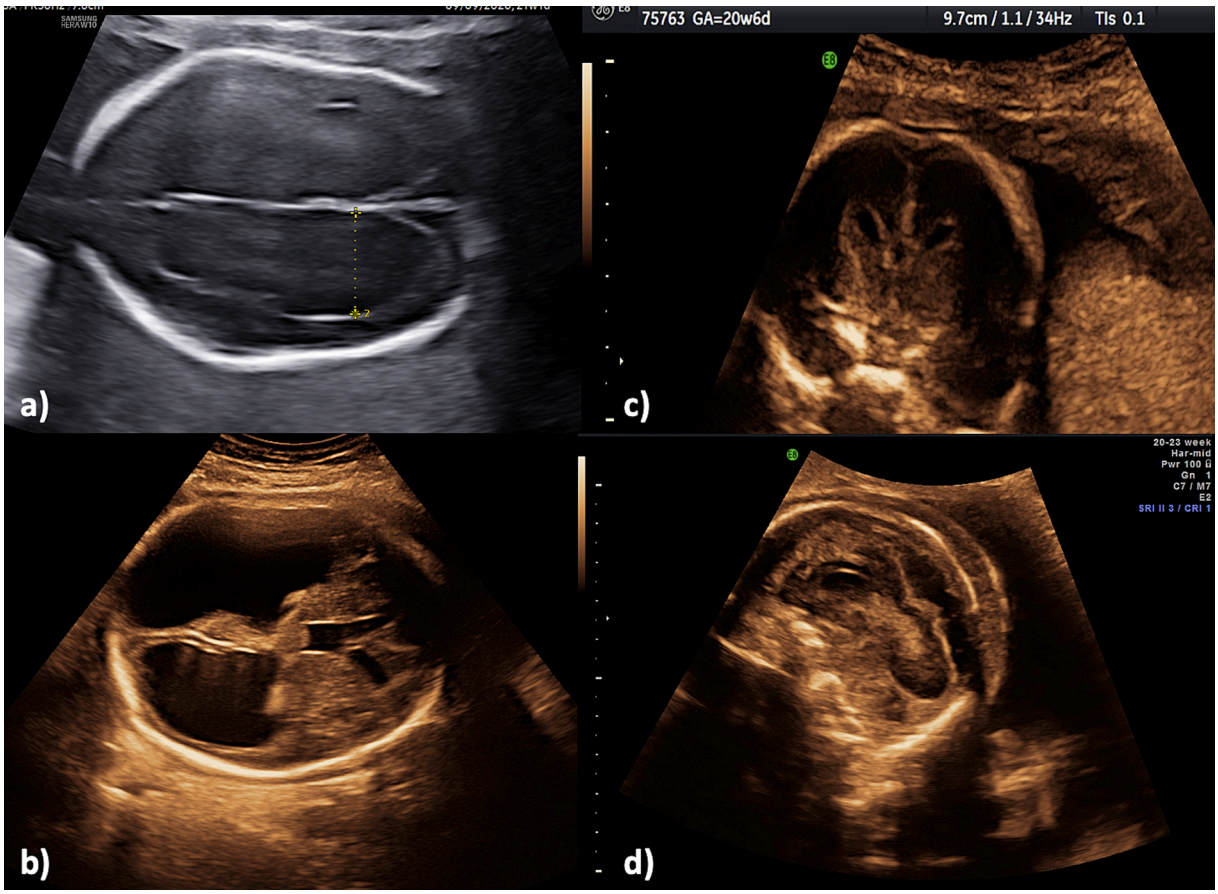

Fig. 2. Sonographic features of congenital CMV infection (a) severe ventriculomegaly; (b) ventriculomegaly and microcephaly; (b) periventricular echogenic halo; (d) abnormal cortical formation.

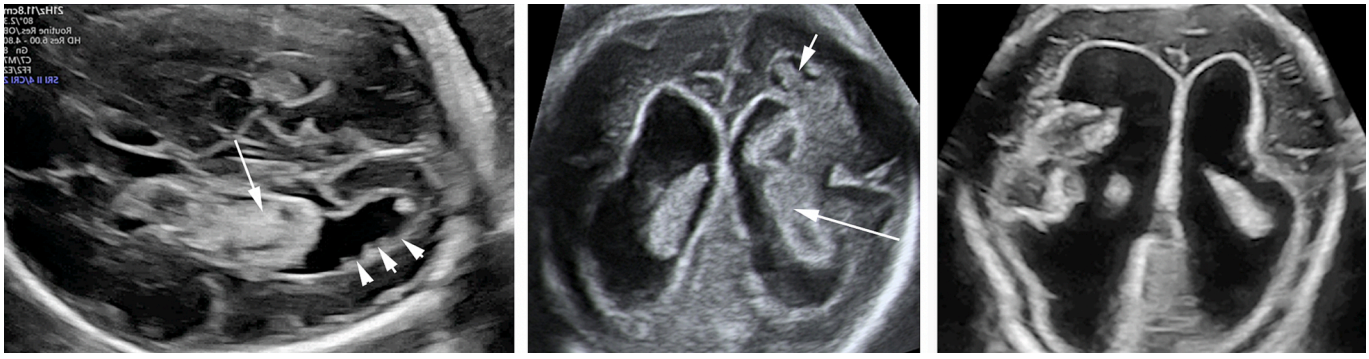

Fig. 3. Clastic pattern of a ventriculomegaly (irregular borders of the lateral ventricles and echogenic deposits within the ventricular cavity and the periventricular zone).

should be recommended in case of positive amniocentesis at 28–32 weeks of gestation. [35].

What is the role of laboratory parameters in assessing postnatal outcomes?

The amniotic viral load is the most examined laboratory parameter for the assessment of the postnatal outcomes. Worth a mention that it is related to the weeks of gestation at which amniocentesis is performed and to the time-interval from maternal seroconversion. The prognostic value of viral load comes from different sources and a recent multicenter study showed high CMV viral load as an accurate predictor for detection of anomalies at follow-up imaging – both at US and MRI – even in fetuses with normal imaging at the initial diagnosis. [36] CMV viral load revealed an optimal cut-off point at $>$ 1,310,520 copies/mL with a sensitivity of 66.7 %, a specificity of 84.3 % [36] However, further independent validation studies of viral load as a predictor of outcomes are needed and the specific threshold of prognostic CMV copies should be confirmed. Therefore, this parameter should be used only in selected and research centers.

Fetal thrombocytopenia at cordocentesis has also been evaluated as a poor prognostic feature, but controversial in its use because it is associated with a higher fetal loss risk, thus not routinely recommended. In fact, US and viral load by amniotic fluid alone and with fetal blood samples showed respectively a NPV of 95 % and 100 %, respectively. Some argue that a 5 % increase in NPV is not worth the increased risk of fetal loss. [2].

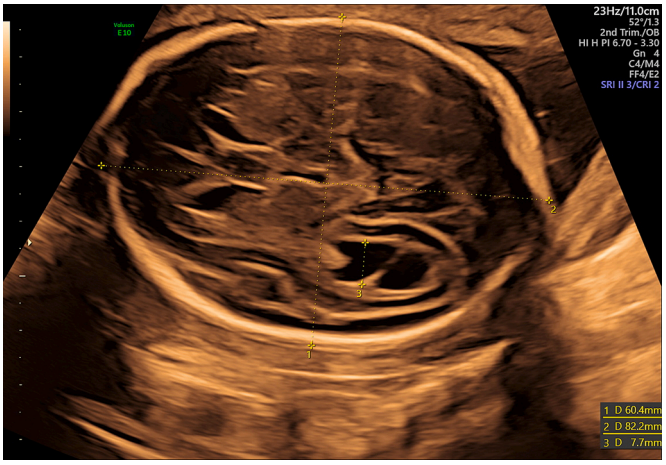

Fig. 4. Intraventricular synechiae.

Is gestational age at maternal infection important in determining a poor prognosis?

As for other viral infections, gestational age at maternal infection represents one of the most important prognostic factors, with the risk of vertical transmission increasing as gestational age increases. The risk of vertical transmission ranges from 5.5 % at the preconception period, to 21–36.8 % during the periconceptional period and first trimester, up to 40.3 % and 66.2 % during the second and third trimester, respectively. [37] However, it has been also reported that the higher is the gestational age at maternal infection, the lower is the risk of a symptomatic newborn with adverse sequelae. [4,37] When focusing on SNHL, a recent retrospective study reported it in 28 % of cases after PI occurred before 14 weeks of gestation, whereas none of the infected fetuses during the second and the third trimester had hearing sequelae. [38] Furthermore, newborns with first trimester infection also have a higher risk for late-onset-SNHL, thus requiring thorough assessments and investigations during the first years of life.[78].

The data on the rate of neurodevelopmental disability, including cerebral palsy, seizures or chorioretinitis have been controversial, mostly for the second and the third trimester cases. Among the most recent evidence, although some studies have associated milder sequelae to later infections, [39] others showed no neurological impairment in case of second and third trimester infections. [40,41].

How should late CMV infection be managed?

Clinical work-up for late infections, despite their higher vertical transmission rates, is challenging and still unclear, mostly because there is a lack of robust data that conclusively exclude SNHL and long-term neurological sequelae following second and third trimester infections. So far, few studies reported no neonatal consequences related to MPI after the first trimester. [38] Moreover, other reassuring results could be extracted considering that newborns with urinary CMV shedding at birth, albeit negative amniocentesis, have no clinical consequences. This is most likely due to a late transplacental CMV transmission, that, with normal prenatal imaging, has not been linked with severe SNHL and post-natal sequelae. [22] Therefore, on one hand, it could be speculated that an intra-amniotic viral colonization occurred later than the second trimester does not increase the clinical burden of cCMV, posing justifiable questions about the reasonability of performing late amniocentesis to confirm fetal infection. On the other hand, conflicting results come from the latest study with the largest sample of women with late CMV infections, [39] which registered a proportion of composite outcomes (SNHL or neurodevelopmental sequelae) following second trimester cCMV of 7 %, with 3 % of partial and unilateral SNHL and 5 % of minor neurological anomalies, such as mild verbal and motor delay, whereas only one case of very slight motor delay was reported in third trimester PI. Another controversial result is that the incidence proportion of abnormal prenatal findings on US or MRI was not significantly correlated to hearing loss or neurodevelopmental abnormalities. [39] In conclusion, given the lack of robust evidence, more accurate data need to be collected to understand the outcomes of late infections and whether US and MRI could represent a prognostic factor as for MPI of first trimester. As good clinical practice, amniocentesis could be considered to confirm fetal infection and US and MRI should be performed in order to further clarify their accuracy in the prediction of postnatal outcomes following late infections.

What is the clinical presentation of neonates with cCMV infection?

Several clinical features have been described among newborns with cCMV infection, from a completely asymptomatic to a symptomatic status. The definition of symptomatic status currently includes neonatal symptoms, like small for gestational age (SGA), hepatomegaly, splenomegaly, petechiae, chorioretinitis, central nervous system (CNS) impairment (also considering CMV DNA detection in cerebrospinal fluid) and SNHL with associated biochemical anomalies, such as elevated liver enzymes or thrombocytopenia. [2,35] The symptomatic status is further distinguished in mild, moderate and severe status, based on the grade of association of symptoms, [35] although the most severe consequences of cCMV infection – SNHL and long-term neurological sequelae – might occur both in symptomatic and asymptomatic newborns. [2] This postnatal classification has a good correlation with the prenatal one, defining infected fetuses according to the presence and the severity of symptoms: asymptomatic fetuses are those with no US, MRI and fetal blood anomalies. Mild or moderate symptomatic fetuses are those with isolated biological abnormalities without brain lesions or with isolated anomalies, such as hyperechogenic bowel, mild ventriculomegaly or isolated calcifications; severe symptomatic fetuses are those with severe US anomalies, such as ventriculomegaly, intracerebral hemorrhage, associated with thrombocytopenia. [35] This classification can be used to better counsel pregnant women and to discuss TOP.

Given the 36.8% chance of fetal infection after MPI acquired during the first trimester, the impairment may not be present at birth, but in the long term, around 90 % of children are asymptomatic and 10 % are symptomatic with permanent sequelae that might be registered in 13.5 % and 40–58 %, respectively [2,37].

Are there any medical therapies for cCMV infection?

The absence of a CMV vaccine and a limited public knowledge on primary prevention advocate for secondary prevention of cCMV infection following maternal primary infection. Until 2020, there was no approved treatment option for CMV infection during pregnancy. Growing evidence has demonstrated beneficial effects of high doses of oral valacyclovir (2 g every 6 h, 8 g/day) which is considered as a safe drug with a very low rate of adverse maternal effects, mostly due to acute renal failure, promptly resolved after discontinuation of the drug. [4] High-dosage oral valacyclovir, administered from the diagnosis of MPI before 14 weeks of gestation until amniocentesis, showed a significantly lower risk of vertical transmission and a trend toward reduction of CMV-related fetal and neonatal morbidity in case of first trimester infections, while no significant difference was found for MPI in the periconceptional period, in the second or third trimester of pregnancy. [4] Furthermore, when considering symptoms at birth, women treated with valacyclovir faced an increased likelihood to have asymptomatic infants, and even a significant reduction in the rate of symptomatic disease postnatally among fetuses with mild symptomatic disease, from 82 % to 43 %. [42].

The most comprehensive evidence on this topic has been recently provided by an individual patient data *meta*-analysis including 527

patients that showed a 65 % reduction of the vertical transmission rate of CMV for both the periconceptional period and the first trimester and a 70 % decrease of the rate of neonatal infection for both these periods. Furthermore, valacyclovir was associated with 80 % reduction of the rate of TOP because of CMV-associated severe fetal findings. Finally, the overall rate of severe side effects was 2.1 %. [43].

Although these promising data have been demonstrated for MPI, the use of valacyclovir as a treatment for mildly infected fetuses and NPI is not standard care as only one quality paper and a few case reports deal with these issues. [42].

On the other hand, studies on CMV HIG failed to prove any benefit in two RCTs in which the therapy was started in the second trimester: HIG use did not result in a lower incidence of a composite of congenital CMV infection or perinatal death compared with placebo, [44] and also did not significantly modify the course of primary CMV infection during pregnancy. [45] Also when considering long term outcomes, CMV HIG

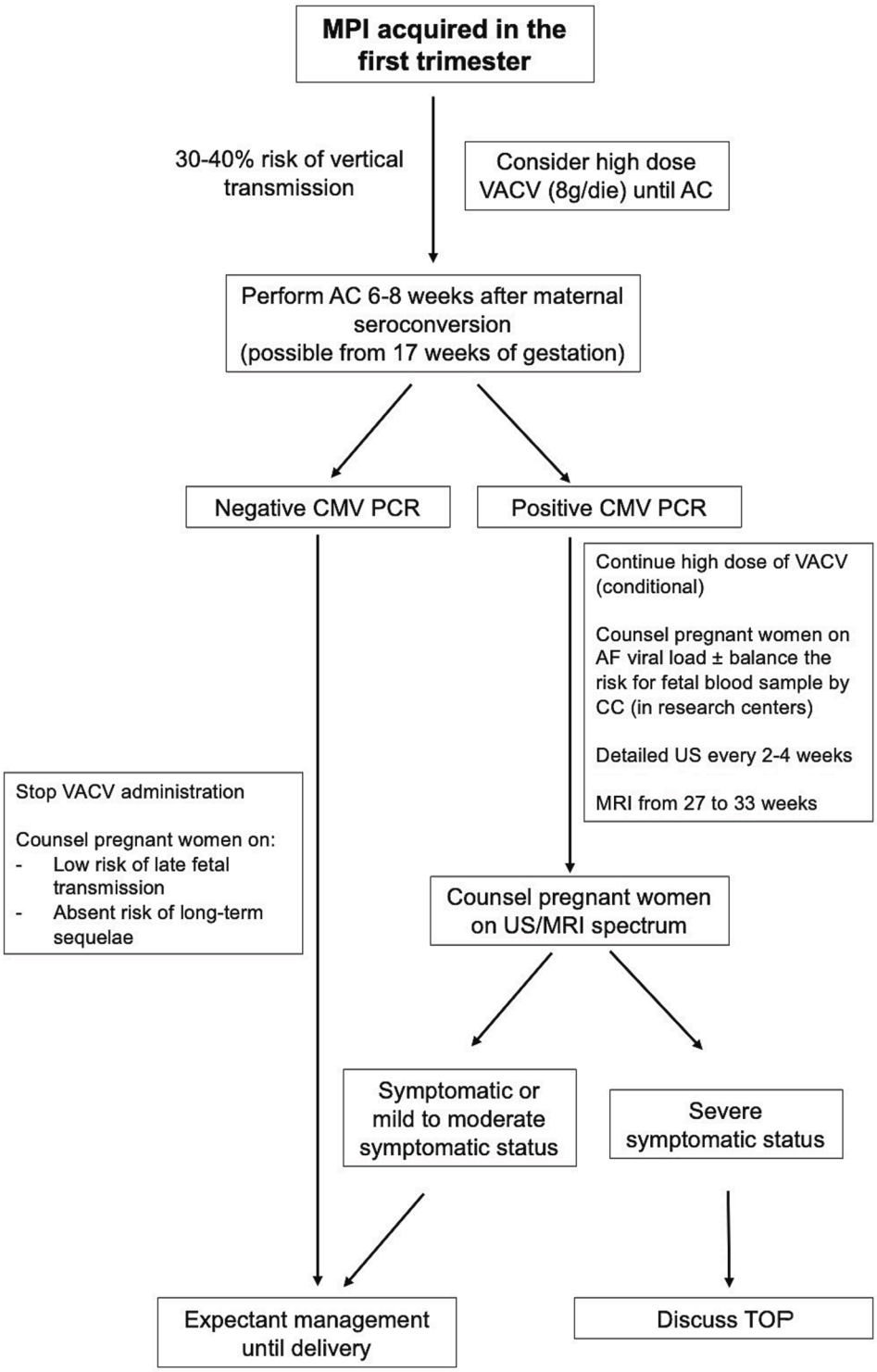

Fig. 5. Potential approach for diagnosis and management when primary infection is acquired in the first trimester. MPI, maternal primary infection; VACV, Valacyclovir; AC, amniocentesis; PCR, polymerase chain reaction; US, ultrasound; AF, amniotic fluid; CC, cordocentesis; MRI, magnetic resonance imaging; TOP, termination of pregnancy;

did not improve two-year hearing or developmental outcomes. [46].

Furthermore, there are other aspects that make HIGs a less tolerable prenatal treatment for cCMV: the drug administration routes, since HIG regimen requires hospital admission for infusions, and the strict criteria based on the antibodies threshold that must be satisfied to start this therapy, while valacyclovir therapeutic criteria consider solely the maternal first trimester MPI. For these reasons, CMV-specific HIG therapy is not recommended.

Conclusion

This review provided evidence-based answers to the most common clinical questions when dealing with congenital CMV infection, according to the most recent literature, contributing with a potential approach for diagnosis and management when MPI is acquired in the first trimester (Fig. 5).

Further studies are needed to validate the cost-effectiveness of the universal screening for CMV infection in the first trimester of pregnancy and to evaluate the role of new approaches for earlier confirmation of vertical transmission. Regarding antenatal therapy, large RCTs will establish whether prenatal valacyclovir could decrease the risk of fetal structural anomalies, symptomatic infection and neurocognitive impairment in fetuses with confirmed congenital CMV infection, and also whether this therapy could be applied also for NPI. Once the vertical transmission has been established, evidence is still lacking on the actual role of viral load in the amniotic fluid as a predictor of adverse outcomes. MFM attendings should always consider maternal psychological stress and endorse a dedicated parental support.

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CRediT authorship contribution statement

Elena D'alberti: Writing – original draft. **Giuseppe Rizzo:** Supervision. **Asma Khalil:** Supervision, Conceptualization. **Ilenia Mappa:** Writing – review & editing. **Maria Elena Pietrolucci:** Methodology. **Giulia Capannolo:** Methodology. **Sara Alameddine:** Methodology. **Sara Sorrenti:** Writing – original draft. **Fabrizio Zullo:** Writing – original draft. **Antonella Giancotti:** Supervision. **Daniele Di Mascio:** Supervision, Conceptualization. **Francesco D'antonio:** Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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