

PRIORITY TOPICS IN THE DIAGNOSIS AND MANAGEMENT OF CMV IN PREGNANCY

A TARGETED GUIDELINE



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Reported interests:

All experts and stakeholders consulted within this report were selected because of their involvement in the topic of diagnosis and management of cytomegalovirus in pregnancy. Therefore, by definition, each of them might have a certain degree of conflict of interest to the main topic of this report.

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LIST OF ABBREVIATIONS

ABBREVIATION	DEFINITIONS
AGREE II	Appraisal of Guidelines for Research & Evaluation, version II
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
BELPreg	Belgian pregnancy exposure registry
BERA	Brainstem Evoked Response Audiometry
CBC	Complete blood count
cCMV	Congenital cytomegalovirus
CI	Confidence interval
CID	Cytomegalic Inclusion Disease
CMV	Cytomegalovirus
CNS	Central nervous system
COS	Centre for developmental disorders
DNA	Deoxyribonucleic Acid
EtR	Evidence-to-Recommendation
ESPID	European Society for Paediatric Infectious Diseases
FU	Follow-up
GDG	Guideline Development Group
GRADE	Grading of Recommendations Assessment, Development and Evaluation
GP	General practitioner
GW	Gestational weeks
HAS	Haute Autorité de Santé (French National Authority for Health)
IgG	Immunoglobulin G
IgM	Immunoglobulin M
INAMI/RIZIV	National Institute for Health and Disability Insurance (see NIDHI)
IQR	Interquartile range
IUGR	Intrauterine growth restriction
LoE	Level of Evidence
MeSH	Medical Subject Headings
MRI	Magnetic Resonance Imaging
NIHDI	National Institute for Health and Disability Insurance
NPI	Non-primary infection
NPP	Negative predictive value
OR	Odds Ratio



PCR	Polymerase Chain Reaction
PECO	Population, Exposure, Comparator, Outcome
PICO	Population, Intervention, Comparator, Outcome
PIRTS	Participants, Index test, Reference standard, Target condition, Study design
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
QUADAS	Quality Assessment of Diagnostic Accuracy Studies
QUAPAS	Quality Appraisal of Prognostic Accuracy Studies
RCT	Randomised controlled trial
ROB2	Risk of Bias 2
ROBINS I	Risk Of Bias In Non-randomised Studies of Interventions
SAE	Severe adverse event
TOP	Termination of pregnancy
US	Ultrasound
VVK	Vlaamse Vereniging voor Kindergeneeskunde



■ SCIENTIFIC REPORT

1 INTRODUCTION

1.1 Background

Cytomegalovirus (CMV) is a common infection worldwide, and the leading cause of congenital infection in developed countries(1).

During pregnancy, pregnant patients can become infected with CMV either by primary infection (when an individual acquires the virus for the first time and lacks previous immunity)(2), or non-primary infection, when a previously acquired (preconceptional) infection reactivates or when the patient gets reinfected with a new CMV strain(2).

Currently, no vaccine against CMV is available. Therefore, primary prevention in pregnant patients relies on behavioral measures aimed at reducing exposure to bodily fluids from potential carriers, particularly young children(3).

Between 1996 and 2007, 60.8% of pregnant patients in Brussels were found to have preconceptional CMV antibodies, indicating prior exposure(4). Among seronegative pregnant patients, primary CMV infection occurs in approximately 0.15% to 2% of pregnancies(5).

The clinical spectrum of congenital CMV (cCMV) infection ranges from asymptomatic disease to severe multisystem involvement. Hearing loss and neurological impairment are the most common long-term sequelae. Approximately 10–15% of infected infants are symptomatic at birth, and up to 90% of these symptomatic children develop long-term complications. Among infants who are asymptomatic at birth, 5–15% may later develop neurosensory sequelae, most commonly hearing loss(6,7).

While CMV infection is common, fetal CMV syndrome (symptomatic disease that occurs with cCMV vertical transmission) is considered a rare disease according to European standards (estimated frequency 1-5/ 10 000 in the general population^a).

While the probability of vertical transmission increases with advancing gestational age at the time of maternal primary infection, the risk of foetal damage and long-term sequelae decreases when infection occurs later in pregnancy. The estimated risk of sequelae declines from 28.8% following periconceptional infection to 19.3% in the first trimester, 0.9% in the second trimester, and 0.4% in the third trimester(8).

For infants with symptomatic cCMV infection at birth, postnatal treatment with valganciclovir has been shown some potential of reducing the risk of long-term sequelae, particularly hearing loss (9).

In addition, a small randomised controlled trial published in 2020 demonstrated a 70% reduction in maternofetal transmission in cases of primary infection occurring in early pregnancy, when pregnant patients were treated with high-dose valaciclovir(10). The reduction of vertical transmission was later confirmed in a number of observational studies(11). Overall, these results highlight the potential role of antiviral therapy in improving outcomes following primary maternal CMV infection.

^a <https://www.orpha.net/en/disease/detail/294>

1.1.1 KCE 2015 guidelines

KCE produced guidelines on the subject of CMV in pregnancy in 2015, in report 248 “what are the recommended clinical assessment and screening tests during pregnancy?” where one of the research questions concerned whether universal CMV screening for primary CMV infection in pregnancy should be performed in Belgium (12).

The conclusion of the 2015 guideline development group (GDG) on universal screening, was that there was insufficient data to support routine screening in all pregnant patients for CMV (see Figure 1), due to lack of studies comparing CMV universal screening during pregnancy to no screening.

Nevertheless, the GDG also contextualized the fact that, in routine practice in Belgium, a lot of health care practitioners were convinced that CMV screening was essential, at least during the first trimester of pregnancy. Another consideration was that patients would most likely prefer to know.

Consequently, the report issued a weak recommendation to perform a one-off screening, preferably before or at the start of pregnancy, and an additional recommendation was added emphasizing the importance of providing comprehensive information and obtaining patient consent when offering CMV testing.

A strong recommendation was made to discuss primary prevention measures for CMV with pregnant patients.

Figure 1 – Recommendations on CMV screening from the 2015 KCE guidelines

KCE 2015	Recommendation	Strength of Recommendation	Level of Evidence
	- There is insufficient evidence to support routine screening in all pregnant women for cytomegalovirus infection. A single serological test preferably prior to pregnancy can be useful as it may encourage (non-immune) women, to take preventive measures or as it may reassure (at least partially) those who are immune. - In case serological tests for cytomegalovirus infections are offered, pregnant women and their partners should be informed in detail about all the possible consequences and asked for their consent.	Weak	Very low
New KCE 2015	Recommendation	Strength of Recommendation	Level of Evidence
	- Despite a lack of clearly proven benefits, discuss primary prevention measures with pregnant women to reduce the risk of cytomegalovirus infection, such as: - Regularly wash your hands, especially after contact with saliva or urine of small children (e.g. changing diapers) or wear protective gloves when changing diapers or handling children's dirty laundry. - Clean toys, countertops, and other surfaces that come into contact with young children's bodily fluid.	Strong	Very low
New KCE 2015	Policy recommendation	Strength of Recommendation	Level of Evidence
	More data on the diagnostic accuracy of serological tests, value of amniocentesis and imaging, clinical evolution of infected infants and harmful effects for healthy pregnancies need to be collected in the Belgian context, in order to evaluate the benefits and harms, both on the short term and on the long term, of cytomegalovirus screening appropriately.	NA	NA

1.1.2 Current practices in antenatal CMV screening and management in Belgium

Serological testing for CMV primary infection in Belgium is based on a combination of 3 serological markers: IgG, IgM and IgG avidity, where IgM indicates a possible recent immune response to CMV, IgG confirms past exposure or established immunity, and IgG avidity helps distinguish between a recent primary infection (low avidity) and an older infection (high avidity). Generally, primary infection is diagnosed in case of positive IgG and IgM in a patient known to have a negative IgG status before pregnancy(13).



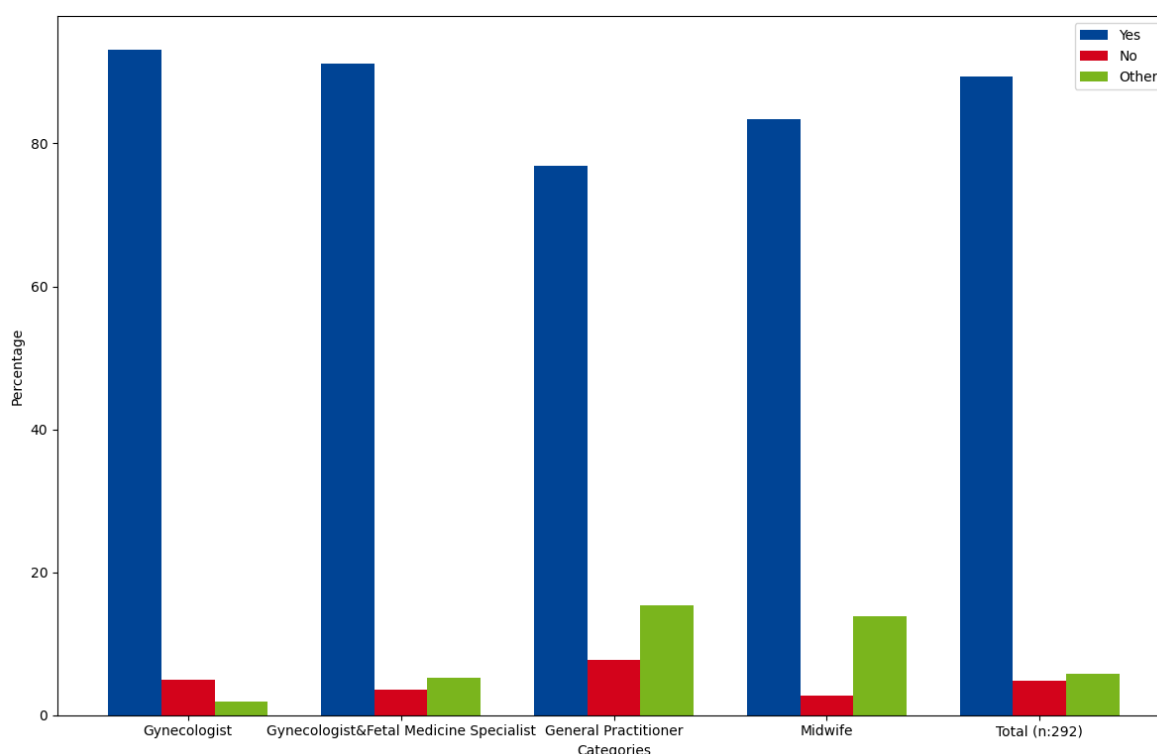
In case of unclear serological results, the National Reference Center for Congenital Infections^b (*Nationaal Referentiecentrum voor Congenitale Infecties / Centre national de référence des infections congénitales*), performs a panel of serological tests aimed at confirming, ruling out, or dating a primary CMV infection in pregnant patients. Test turnaround time is 1 to 5 working days, and the samples are stored for a minimum of three years.

Routine CMV serological testing during pregnancy is not reimbursed in Belgium. The reimbursement, previously available, was withdrawn in June 2017, although it remains in case of symptoms compatible with infection^c.

To have an updated picture of the current practices in CMV testing and management, we designed an online survey directed to midwives, general practitioners (GPs), and gynaecologists who were involved in pregnancy follow-up and/or were specialists in foetal medicine. We report a summary of the findings, the interested reader can find the complete survey report in [Appendix 1](#).

We disseminated our survey via social media and professional associations' websites and analysed 298 complete survey responses. Of these, 6 were foetal medicine specialists not involved in the medical follow-up of pregnant patients, 57 were foetal medicine specialists who were also involved in medical follow-up, and 160 gynaecologists, 39 general practitioners, and 36 midwives who all provided medical follow-up for pregnant patients.

Figure 2 – Proportion of healthcare practitioners who proposed CMV screening at the first trimester of pregnancy



A large majority of practitioners reported offering CMV serology in the first trimester (see Figure 2).

^b <https://www.sciensano.be/en/nrc-nrl/national-reference-center-nrc-congenital-infections-toxoplasma-rubella-cytomegalovirus-and>

^c https://www.inami.fgov.be/SiteCollectionDocuments/nomenclatureart24_20170601_20170731_01_portee.pdf

The most commonly cited reason for CMV testing was to enable targeted imaging follow-up in the event of seroconversion. Preventing vertical transmission through valaciclovir was less frequently cited overall but was the primary reason among foetal medicine specialists. In contrast, GPs were the least likely to cite this rationale, suggesting either limited awareness of treatment options or hesitancy to propose treatments not supported by formal guidelines.

Foetal medicine specialists were specifically asked about their approach to managing CMV infections acquired during pregnancy. Just over half reported systematically proposing valaciclovir treatment, while others were more selective. Decisions around treatment were influenced by factors such as the patient's socioeconomic status, since valaciclovir is currently not reimbursed in Belgium for antenatal CMV infection, and the patients carry the whole financial burden of treatment, with public prices ranging from 25.57€ to 26.62€ for a 42-tablet box of 500 mg, depending on the pharmaceutical brand (approximately 10 euros each day of treatment)^d.

Practices regarding indications for treatment, duration, and follow-up imaging varied. While most offer treatment only in first-trimester infections, some extend treatment to other scenarios. Treatment often continues until amniocentesis, but some practitioners continue until delivery. Few described their approach in cases where amniocentesis is refused. Follow-up imaging approaches also varied in the context of a negative amniocentesis.

In addition, although the majority of consensus guidelines(14) say that the only indication for medical termination of pregnancy (TOP) is the presence of severe foetal anomalies, our survey highlighted that, according to the perception of practitioners, couples may still choose TOP at any point during the clinical pathway. That is either after the notion of seroconversion, after the results of a positive amniocentesis, after the discovery of minor foetal anomalies and, as recommended, after the discovery of severe foetal anomalies.

The results of our survey highlighted how there is a significant proportion of practitioners that propose CMV testing during pregnancy (90% among our respondents, although this proportion may be an overestimation since our sample may be affected by selection bias), but also heterogeneity in the follow-up of patients who seroconvert.

1.1.3 *Diagnosis and treatment of congenital CMV in the newborn*

Since April 2024 valganciclovir qualifies in Belgium for category B reimbursement when used to treat newborns and infants under 13 months who have symptomatic congenital CMV infection and/or CMV-related illness^e.

Newborns are recommended to be tested at birth whenever there are signs suggestive of a congenital CMV infection, regardless of maternal serological status.

The CMV working group of the Flemish association for Pediatrics (Vlaamse Vereniging voor Kindergeneeskunde – VVK) provided in June 2022 recommendations for diagnosis, treatment and follow-up in newborns(9). A summary of the recommendations is reported in Table 1 (modified from the original publication).

A recent publication carried out in 50 hospitals in Flanders demonstrated that only 1.65% of newborns were tested for CMV, when the frequency of microcephaly alone is estimated at 3%, highlighting how some newborns may miss the opportunity for treatment(9).

^d https://www.cbip.be/fr/chapters/12?frag=10793&view=pvt&vmp_group=31674

^e <https://www.cbip.be/fr/chapters/12?frag=8900243>



Table 1 – Guideline for the Diagnosis and Treatment of Congenital CMV by VVK 2022

Guideline for Diagnosis and Treatment of Congenital CMV		
Step 1: Screening and work-up		
Indications	Screening	Work-up
Microcephaly or proportional IUGR Clinical suspicion of congenital CMV ¹ Proven maternal seroconversion Abnormal BERA or hear-screening Neurologic abnormalities ² Central nervous involvement ³ Ophthalmological findings ⁴	PCR CMV on urine (d1 – d21), or PCR CMV on saliva (at least 2h after breastfeeding) with confirmation on PCR urine *	• Cerebral ultrasound • MRI of the brain (including cerebellopontine angle in case of hearing loss) • Lab test (CBB, ALT/AST, bilirubin) • Fundoscopy of the eye • BERA (Brainstem Evoked Response Audiometry)
Step 2: Management and follow-up		
Classification	Treatment	Follow up
Asymptomatic:	No indication for treatment	• Audiological: at 3 months, annually at 1-2-3-4 years • Vestibular: at 6 months • Ophthalmological: at 12 months • COS: at 12 months
Mild Up to 2 non-significant or transient findings (e.g., IUGR, petechiae, mild liver/spleen enlargement, mild lab abnormalities)	No indication for treatment	• Audiological: at 3 and 6 months, biannually until 3 years, and at 4 years • Vestibular: at 6 months
Moderate More than 2 mild symptoms or persistent abnormalities (>2 weeks), or mild brain imaging findings	Consider treatment in consultation with expert	• Ophthalmological: at 12 months • COS: at 4 months (in case of CNS-involvement)
Severe CNS involvement (e.g., seizures, chorioretinitis, microcephaly, significant brain abnormalities), severe single or multi-organ involvement, life-threatening disease, or isolated sensorineural hearing loss.	Start oral valganciclovir 16 mg/kg/12h during 6 months**	

1 Symptoms: petechiae or purpura, thrombocytopenia or anaemia, hepatosplenomegaly or elevated transaminases, conjugated hyperbilirubinemia, eye abnormalities. 2 Neurological signs: lethargy, hypotonia, convulsions, elevated protein in cerebrospinal fluid. 3 Central nervous system involvement: calcifications (specifically lenticulostriate vasculopathy), periventricular cysts, ventricular dilation, subependymal pseudocysts, germinolytic cysts, white matter abnormalities, cortical atrophy, migration disorders, cerebellar hypoplasia. 4 Ophthalmological findings: chorioretinitis, retinal haemorrhages. *If the screening result is negative despite strong clinical suspicion, it should be repeated. ** Exception: bilateral severe hearing loss. COS: centre for developmental disorders. In the updated version of 2024 small changes are made in the VVK guidelines, whereas ophthalmologic findings are no longer mentioned as indications for screening, and prenatal findings of IUGR are specified as extra indication for screening.

1.2 Objective

Considering recent evidence and evolving practices in the testing and treatment of CMV during pregnancy, a new Belgian guideline is developed to support healthcare professionals involved in its management. The guideline aims to harmonize clinical practices and improve outcomes for both mothers and infants. It offers evidence-based recommendations focused on priority areas identified by experts and stakeholders for the diagnosis and management of CMV in pregnancy.

1.3 Target users

This guideline is primarily intended for healthcare professionals involved in both primary and secondary care. Specifically, it targets obstetricians, midwives, foetal medicine specialists, general practitioners and other clinicians managing pregnant patients at risk of or affected by CMV infection. It aims to support clinical decision-making and promote consistent care in the diagnosis, and management of CMV during pregnancy.

1.4 Scope

This is a **targeted guideline covering priority topics in antenatal CMV management, to harmonise the pathway of care of pregnant patients that made the informed decision of having a CMV test during pregnancy.**

The chosen point of view is that of the individual patient.

This guideline does not cover the topic of universal CMV screening in pregnancy.

Conditions for updating guidelines on universal CMV screening in pregnancy

The establishment of a clear pathway of care is necessary and propaedeutic to studying the opportunity a universal screening policy in the future (see [Appendix 2](#)).

Conditions that would prompt the submission by KCE of a proposal to update guidelines on universal CMV screening in pregnancy are:

- High quality comparative evidence of the effectiveness of screening
- Reimbursement of valaciclovir for pregnant patients with CMV in pregnancy



2 METHODOLOGY

This guideline was developed based on scientific evidence in accordance with the KCE guideline development methodology(15).

2.1 Defining the scope of the guideline

The first step in a targeted guideline is to define which aspects will be covered, to this end a search for recent guidelines on diagnosis, treatment and/or follow-up of CMV in pregnancy was performed on Medline and Embase. The retrieved guidelines for this scoping phase were appraised with AGREE II(16) by one reviewer and discussed with the KCE research team (see [Appendix 3](#)). A longlist of topics for research questions was compiled based on these guidelines, within the current scope.

We established a Guideline Development Group (GDG) and stakeholder group (for details about GDG and stakeholder group composition see [Appendix 4](#)) including representatives from all relevant professional fields (gynecologists, pediatricians, virologists, otolaryngologists, midwives, general practitioners, radiologists, policymakers) as well as patient representatives.

A longlist of research questions was presented as an online survey (see [Appendix 5](#)) and was distributed to retrieve input on the priority of topics for research questions from experts and stakeholders. Proposals for new topics were encouraged.

2.2 Development of the research questions

Based on the results of the survey, questions that were consistently rated as high priority and considered feasible within the scope of the guideline were retained.

The prioritisation was presented and discussed with the GDG and Stakeholder group. Final research questions were formulated:

On diagnosis

- 1-Should practitioners use serologic tests to diagnose non-primary CMV infection (NPI) in pregnant patients?

On treatment

- 2-Should high dose Valaciclovir be used in pregnant patients with a primary CMV infection?

On management

- 3-In pregnant patients with confirmed primary CMV infection, and confirmed cCMV at birth, what is the frequency of CMV-related sequelae in the foetus or child in those who had a previous positive amniocentesis compared to those who had a previous negative amniocentesis?
- 4-In pregnant patients with a confirmed primary CMV infection and a negative amniocentesis result, should management include enhanced imaging (regular ultrasound, MRI) compared routine imaging only?
- 5-In pregnant patients with a confirmed primary CMV infection, should amniocentesis be performed at 17 weeks of gestation compared to a later gestational age?

All clinical questions were structured using appropriate evidence frameworks tailored to the type of question (interventional, diagnostic, or prognostic), such as PICO (Population, Intervention, Comparison, Outcome), PECO (Population, Exposure, Comparison, Outcome), and PIRTS (Population, Index test, Reference standard, Target condition, Setting), and were shared, discussed and finalised in collaboration with the GDG.

According to KCE methodology, recommendations are made based on data available on outcomes that are considered *critically important* for patients. Outcomes that are considered *important* but not critical by patients have a secondary role(15). To prioritise critical and important outcomes, we consulted patients' representatives in the GDG. The results of the consultation were presented and discussed with the GDG (see [Appendix 6](#) for methods and definitions) and presented at a stakeholder group meeting.

The following outcomes were prioritised as critical for decision making:

In the foetus or child:

- **severely symptomatic foetus**
- **termination of pregnancy**
- **severely symptomatic newborn**
- **cerebral palsy**
- **cognitive developmental delay**
- **severe adverse events (SAE) following treatment in the pregnant patient**
- **SAE following treatment in the newborn**

In diagnostic questions, if no direct evidence was retrieved for critical outcomes, we reported outcomes related to accuracy measurements alone.

According to KCE methodology(15), and after discussion with the GDG, minimal important difference thresholds for critical outcomes were defined as follows:

12 events per 1000 individuals distinguishing trivial from small effects

34 events per 1000 individuals distinguishing small from moderate effects

67 events per 1000 individuals distinguishing moderate from large effects

2.3 Evidence review

All questions were addressed using a de novo methodology. The GRADE-ADOLOPMENT(17) approach could not be applied, as all existing guidelines assessed using the AGREE instrument were consensus-based and did not meet the methodological requirements for adolopment.

All reviews used the same three databases: MEDLINE (via Ovid or PubMed), Embase, and the Cochrane Library, from inception to the day of the search.

The search strategies were adapted across questions to match the relevant PICO/PIRTS/PECO frameworks (see Table 1). One reviewer screened titles, abstracts, and full texts according to predefined eligibility criteria and study designs relevant to each question. Reference lists of included studies, and of excluded systematic and non-systematic reviews were checked to identify additional publications. The GDG reviewed the selected papers and had the opportunity to add references that would have been missed by the research team.

Included studies focused on (diagnostic) randomised controlled trials (RCTs), observational (comparative) studies and/or prospective/retrospective diagnostic accuracy studies (in which the index test(s) and the reference standard test are applied to the same patients in a cross-sectional design). The design included was defined by the type of review (e.g. intervention vs diagnostic/ prognostic). The following study types were excluded: narrative reviews, case reports, studies presented as conference abstract only or if no full text was available, and studies in which it was not possible to extract the required data.

Quality appraisal was done using ROB 2 (Risk of Bias 2)(18) for RCTs, ROBINS I (Risk of bias in non-randomised studies of interventions)(19) for observational studies, QUADAS2 (Quality Assessment of



Diagnostic Accuracy Studies 2)(20) for diagnostic accuracy studies and QUAPAS (Quality Assessment of Prognostic Accuracy Studies)(21) for prognostic studies.

For quantitative synthesis, we performed pairwise or diagnostic/prognostic meta-analysis, depending on the research question.

We applied random-effects pairwise meta-analysis models to account for clinical and methodological heterogeneity across studies. For both interventional and non-interventional analyses evaluating the association between a prognostic factor and the outcome, pooled odds ratios (ORs) were calculated. When only two studies were available, we still generated a pooled estimate to provide a single summary measure. Imprecision arising from estimates of between-study variance was addressed by downgrading the certainty of evidence accordingly (see below). For trials or observational studies with zero events in a study arm, a continuity correction of 0.5 was applied. All statistical analyses were conducted using Stata.

In cases where diagnostic/prognostic accuracy was assessed, we performed dedicated diagnostic test accuracy (DTA) meta-analyses using Meta-DiSc 2.0(22), a web-based application developed using the R Shiny framework. Due to the low number of studies, a univariate model with random effects was used.

The certainty of the evidence was evaluated according to GRADE (Grading of Recommendations, Assessment, Development and Evaluation), following KCE methods(15). The GRADIng was done with the aid of the Grade Pro software^f. Certainty of the evidence can be downgraded for five key reasons: risk of bias, inconsistency, indirectness, imprecision, and publication bias. Conversely, it can be upgraded when there is a large effect, a dose–response relationship, or when all plausible confounding would reduce an observed effect. After considering these factors, the overall certainty of evidence is classified into four levels: high, moderate, low, or very low.

Full detailed search strategies, quality appraisal, and evidence tables for all selected studies, as well as complete GRADE tables, are provided in Appendices 7–10.

^f <https://www.gradepro.org/>

Table 2 – Overview of review methodology across the five research questions

Question	Type of review	Inclusion criteria	Exclusion criteria	Date of search	Quality appraisal	Notes
1-Use of serologic tests for NPI	Diagnostic	Good quality systematic reviews, diagnostic randomised controlled trials, prospective and retrospective cohort studies, in which the index test and the reference standard are applied to the same patients in a cross-sectional design, case-control studies.	Narrative reviews; Case reports; Studies presented as conference abstract only or if no full text was available	October 28th 2025	QUADAS	Since there is no gold standard for the diagnosis of NPI, the reference standard was presence of cCMV at birth
2-High dose valaciclovir for primary infections	Intervention	Good quality systematic reviews, randomised controlled trials, prospective and retrospective cohort studies, case-control studies.	Narrative reviews; Case reports; Studies presented as conference abstract only or if no full text was available	April 1st 2025	ROB2, ROBINS I	Analysis by trimester of seroconversion, if available
3-Negative amniocentesis when cCMV is detected at birth	Prognostic	Good quality systematic reviews, single arms of randomised controlled trials, prospective and retrospective cohort studies, case-control studies.	Narrative reviews. Case reports. Case series. Studies presented as conference abstract only or if no full text was available. Studies in which it is not possible to classify infected fetuses or children at birth according to their	October 21st 2025	QUAPAS	Analysis by treatment status, analysis by trimester of seroconversion if available.



			amniocentesis result. Studies where index and/or reference standard testing not include CMV DNA PCR.			
4- Follow up of negative amniocentesis	Intervention	Good quality systematic reviews, randomised controlled trials, prospective and retrospective cohort studies, case-control studies.	Narrative reviews; Case reports; Studies presented as conference abstract only or if no full text was available	October 21st 2025	ROB2 , ROBINS I	Analysis by treatment status, analysis by trimester of seroconversion if available.
5 -Amniocentesis at 17 weeks of gestation	Diagnostic	Good quality systematic reviews, diagnostic randomised controlled trials, prospective and retrospective cohort studies, in which the index test and the reference standard are applied to the same patients. Case-control studies.	Narrative reviews. Case reports. Studies presented as conference abstract only or if no full text was available. Studies in which it is not possible to calculate diagnostic characteristics of the tests according to timing of amniocentesis. Studies where index test and reference standard testing does not include CMV DNA PCR.	October 21st 2025	QUADAS	Analysis by treatment status, analysis by trimester of seroconversion if available.

Study selection criteria included studies, and the quality appraisal of each included study were discussed with the GDG. Results from the literature review were presented to the GDG and discussed to ensure a shared understanding of the evidence base and its implications for the recommendations.

2.4 From evidence to recommendation

The evidence base for each research question, including the underlying evidence, evidence summaries, and evidence-to-recommendation (EtR) considerations, is presented in the individual chapters of this report and the related appendices.

Recommendations were formulated as strong or conditional, in accordance with the certainty of the supporting evidence and the balance between desirable and undesirable consequences of the alternative management options (see Table 3 and Table 4). In line with GRADE principles, additional considerations included patients' values and preferences, quality of the evidence, and estimated costs or resource use (see Table 5).

Table 3 – Strength of recommendation according to the GRADE system

GRADE	Definition
Strong	The desirable effects of an intervention clearly outweigh the undesirable effects (<i>the intervention is to be put into practice</i>), or the undesirable effects of an intervention clearly outweigh the desirable effects (<i>the intervention is not to be put into practice</i>)
Conditional	The desirable effects of an intervention probably outweigh the undesirable effects (<i>the intervention probably is to be put into practice</i>), or the undesirable effects of an intervention probably outweigh the desirable effects (<i>the intervention probably is not to be put into practice</i>)

Table 4 – Interpretation of strong and conditional recommendations

Implications	Strong recommendation	Conditional recommendation
For patients	Most individuals in this situation would want the recommended course of action, and only a small proportion would not.	The majority of individuals in this situation would want the suggested course of action, but many would not.
For clinicians	Most individuals should receive the intervention. Adherence to this recommendation according to the guideline could be used as a quality criterion or performance indicator. Formal decision aids are not likely to be needed to help individuals make decisions consistent with their values and preferences	Recognize that different choices will be appropriate for individual patients and that you must help each patient arrive at a management decision consistent with his or her values and preferences. Decision aids may be useful helping individuals making decisions consistent with their values and preferences.
For policy makers	The recommendation can be adopted as policy in most situations.	Policy-making will require substantial debate and involvement of various stakeholders.

Source: (Andrews 2013) (23)

**Table 5 – Factors that influence the strength of a recommendation**

Factor	Comment
Balance between clinical benefit and harms	The larger the difference between the desirable and undesirable effects, the higher the likelihood that a strong recommendation is warranted. The narrower the gradient, the higher the likelihood that a conditional recommendation is warranted
Quality of evidence	The higher the quality of evidence, the higher the likelihood that a strong recommendation is warranted
Values and preferences	The more values and preferences vary, or the greater the uncertainty in values and preferences, the higher the likelihood that a conditional recommendation is warranted
Costs (resource allocation)	The higher the costs of an intervention, i.e. the greater the resources consumed, the lower the likelihood that a strong recommendation is warranted

Sources: (Andrews 2013, Andrews 2013)(23)

When the evidence collected did not justify routine use of an intervention because evidence was insufficient or highly uncertain, a research recommendation could be formulated (23)

In addition, **clinical practice guidance** was formulated to provide practical implementation advice based on expert opinion where such guidance was considered necessary.

The KCE research team drafted the initial recommendations based on the evidence review. These draft recommendations were presented, discussed and adapted, first with a restricted working group within the GDG and then with the whole GDG, following a detailed presentation of the evidence. During the meeting, that was held on 20th Jan 2025, agreement was sought by consensus on the direction of effect, strength, and wording of each recommendation.

After the meeting, GDG members were invited to provide written feedback and suggestions for clarification. Based on this feedback, the recommendations and accompanying guidance were further refined.

Following this process, the report underwent **external validation**. **After external validation the draft recommendations and accompanying guidance were circulated to the stakeholder group to assess understanding, clarity, and readability.**

2.5 Dissemination and implementation

A range of different methods to raise awareness of the guideline exist. These include standard approaches which are applied by KCE, such as:

- issuing a press release at the moment of publication on the KCE website;
- addressing questions from the press;
- notifying experts, stakeholders and patient organisations of the publication;
- publicising the guideline through the KCE's alerts;
- using social media channels;
- and publicising the guideline's main points of interest in a journal.

For the applicability of the guideline, we refer to the section on evidence to recommendation which discusses facilitators and barriers, such as resources, cost or patient's opinions on the topic under study.

3 SHOULD PRACTITIONERS USE SEROLOGIC TESTS TO DIAGNOSE NON-PRIMARY CMV INFECTION IN PREGNANT PATIENTS?

3.1 Recommendations

	Strength of recommendation	Level of evidence
<i>Practitioners should not use serology (new IgM appearance or significant IgG rise) to diagnose non-primary CMV infection in pregnant patients with known preconceptional CMV IgG seropositivity</i>	Strong	Very low

3.2 Introduction

Non-primary maternal CMV infection (NPI) can occur when maternal immunity, established by positive CMV IgG, exists prior to pregnancy. It can be due either to reinfection by a new viral strain or by a latent CMV reactivation(24).

In immunocompromised patients, CMV NPI is diagnosed by CMV shedding in bodily fluids. However, as pregnant patients are usually immunocompetent, CMV shedding may be intermittent or may not manifest in NPI(25,26).

If IgM is detected at the beginning of a pregnancy, the presence of a high IgG avidity can also exclude a primary CMV infection, as this indicates that the infection occurred more than three months previously(27). The appearance of IgM in a patient previously IgM-negative, or a significant increase in IgG, have been hypothesised as markers of NPI in pregnant patients(28).

Since there is no gold standard for the diagnosis of non-primary maternal CMV infection in pregnant patients, we focused on the use of serology as a marker of cCMV in children of pregnant patients with a known preconceptional IgG positivity.

3.3 Research questions and search

“Is IgG kinetics and/or IgM detection a useful marker of the diagnosis of cCMV in children of pregnant patients with positive IgG and negative IgM prior to pregnancy?”

The PIRTS developed for the search, and all details related to the analysis of the evidence can be found in [Appendix 7](#).

3.4 Evidence

3.4.1 Included studies and methodological quality

Four observational studies were identified for answering this research question, two retrospective and one prospective cohort study, and one nested case-control study.

Tang 2025(29) is a prospective cohort study from China including 695 pregnant patients with positive CMV IgG and negative CMV IgM during first or second trimester who delivered by caesarian section. In these pregnant patients, peripheral blood samples were collected on the same day as the caesarian section and tested for CMV IgG and IgM by enzyme-linked immune-absorbent assay, while amniotic fluid was collected during the caesarian section and tested for CMV DNA to diagnose cCMV. Results were reported on the proportion of patients with positive IgM in patients with amniotic fluid positive and negative for CMV DNA. The limitation of this study is the potential inclusion of primary CMV infection



and the exclusion of patients not undergoing caesarian section, which may lead to a selection bias. CMV DNA was also not tested in the urine of newborns which is the gold standard for cCMV detection

Huang 2024(30) is a nested case-control study from China with data on 48 cases with cCMV and 144 controls without cCMV taken from 6602 enrolled pregnant patients with seropositive results (IgG) early in pregnancy (before 23 weeks gestational age). Neonatal cCMV was screened by CMV DNA in saliva and/or urine samples within 13 days of birth and confirmed by saliva and urine samples within 21 days of birth. Maternal blood and urine samples were collected during middle and late pregnancy and retrospectively tested for IgM and IgG avidity (in maternal serum) and CMV DNA (in maternal blood and urine). Results were available on the proportion of patients with positive IgM and with 4-fold increase in IgG for both newborns with and without cCMV. Limitations include the case-control design with retrospective testing of serology and the potential inclusion of primary CMV infections.

Hadar 2017(31) is a retrospective cohort study performed in Israel reporting on 107 neonates with symptomatic cCMV, 12 of those following a non-primary maternal CMV infection with pregestational immunity i.e. positive CMV IgG. The diagnosis of cCMV in the neonates was confirmed by a positive urinary shell vial culture or PCR for CMV taken shortly after birth and up to two weeks following delivery. Laboratory data including full maternal serologic data (IgM, IgG and IgG avidity) were extracted from medical charts and computerized databases. This study reported only the proportion of positive IgM during pregnancy in primary and non-primary maternal CMV infections. A limitation of this study is the exclusion of neonates with asymptomatic cCMV and no cCMV as well as patients with insufficient data. Other limitations include the retrospective design of the study and the lack of reported threshold for IgM positivity.

Périllaud-Dubois 2022(32) is a retrospective cohort study from France with 53 pregnant patients with NPI during pregnancy, confirmed by documented maternal CMV immunity before pregnancy (positive IgG and high IgG avidity index before 10 weeks of gestation), and confirmed cCMV in the foetus or neonate (positive CMV PCR in urine samples). Retrospective analyses were performed on serum samples collected during pregnancy and included kinetics of serological and molecular CMV markers (IgG, IgM, IgG avidity and CMV PCR). Doubling of the antibody titer was used to define a significant rise in IgG. Results were reported on the proportion of patients with a rising IgG titer, positive IgM and/or positive PCR. However, results on the latter two (IgM and PCR) could not be distinguished because the criterion used was 'at least one marker of infection'. Other limitations of this study are the retrospective design and the exclusion of children without cCMV.

3.4.2 Results

Table 6 – Summary GRADE tables of serology for NPI

Marker	Studies & participants	Patients with cCMV	Patients without cCMV	Effect estimate	Certainty of the evidence
IgM positivity	Hadar 2017 (n=12); Huang 2024 (n=48); Tang 2025 (n=5)	Hadar: 3/12 (25%;)	/		⊕○○○ Very low
		Huang: 2.1% (1/48)	Huang: 4.2% (6/142)	OR 0.49 (95%CI 0.05 to 4.19)	
		Tang: 0% (0/5)	Tang 1.6% 11/690	OR 5.45 (95%CI 0.28 to 104.62) The OR direction is driven by the continuity correction	

Significant IgG rise among cCMV cases	Périllaud-Dubois 2022 (n=53); Huang 2024 (n=48)	Périllaud-Dubois: 5.7% (3/53)	/		⊕○○○ Very low
		Huang: 8.5% (4/48)	Huang : 1.4% (2/142)	OR 5.91 (95%CI 1.05 to 33.32)	

The results from the above-mentioned primary studies were not suitable for meta-analysis because of heterogeneity and the lack of consistent diseased versus non-diseased comparators.

None of the studies reported diagnostic accuracy measurements, and the computation post-hoc of accuracy measurements is hindered by the methodological limitations of the body of evidence, therefore we report only proportions of serologic testing positivity in pregnancies resulting in cCMV and not resulting in cCMV (when available).

IgM positivity has been described in only 0 to 2.1% of patients with a non-primary infection, compared to 1.6 to 4% of patients without infection (29,30). No significant difference in IgM positivity was identified between groups. A higher proportion of IgM positivity of 25% was described by a third, but non-comparative study(31).

A significant IgG rise has been described in 8.5% of patients with non-primary cCMV, compared to 1.4% of patients without infection(30). Non-comparative data from a second study showed a proportion of IgG rise in 5.7% of patients with cCMV(32).

Conclusions

In pregnant patients with a known preconceptional IgG seropositivity, the appearance of IgM in serum was observed in a very small proportion of patients who had a cCMV positive newborn. This proportion may not differ from that in patients whose newborns were not cCMV positive. The evidence remains very uncertain (very low level of evidence).

In pregnant patients with a known preconceptional IgG seropositivity, a significant increase in IgG was observed in a very small proportion of patients who had a cCMV positive newborn. This proportion may be slightly higher than in patients whose newborns were not cCMV positive. The evidence remains very uncertain (very low level of evidence).

3.5 Evidence to recommendations

3.5.1 Balance between clinical benefits and harms

From the literature, we conclude that serological markers are unreliable to detect a non-primary CMV infection that causes cCMV at birth. Performing a serological test for this aim can lead to misclassification and either false reassurance due to a missed NPI/cCMV or unnecessary alarm in case of a false positive test. Moreover, there are no available treatment options for NPI during pregnancy.

We conclude that the overall balance between clinical benefits and harms is negative. The expected clinical benefits are minimal to none, while there is potential harm from misclassification and unnecessary alarm.

3.5.2 Quality of evidence

The quality of evidence is very low overall. The evidence comes only from observational, mostly retrospective cohort or case-control study designs without blinding, with inappropriate exclusions and substantial missing or selective data contributing to a serious risk of bias.



Included studies are very heterogeneous with different reference standards, variable or undefined thresholds and timings of serological detection as well as differing laboratory platforms. Included studies have overall small sample sizes and small number of events, leading to serious imprecision with wide or non-estimable confidence intervals.

3.5.3 Patient preferences

Patients are unlikely to prefer undergoing an unreliable serological test to diagnose a non-primary infection.

3.5.4 Cost and resource allocation

Resources should not be allocated to performing an unreliable serologic test to diagnose non-primary infection.

4 SHOULD HIGH DOSE VALACICLOVIR BE USED IN PREGNANT PATIENTS WITH A PRIMARY CMV INFECTION?

4.1 Recommendations

	Strength of recommendation	Level of evidence
<i>For pregnant patients with a known CMV primary infection, before establishment of foetal infection</i>		
<i>Practitioners may offer high dose valaciclovir in pregnant patients with primary CMV infection in the periconceptional period and first trimester</i>	Conditional	Very low
<i>For pregnant patients with a known CMV primary infection and an established foetal infection</i>		
<i>In the context of research, evidence should be gathered on the use of high dose valaciclovir in pregnant patients with a CMV infected foetus.</i>	Research recommendation	

CLINICAL PRACTICE GUIDANCE

Outside the domain of research, the Guideline Development Group suggests:

- To treat pregnant patients with confirmed periconceptional or first trimester primary CMV infection with a dose of 4 times 2 grams per day of valaciclovir, administered until 21 weeks of gestation, and at least 7 weeks after the estimated timing of the maternal infection.
- To test pregnant patients before starting treatment, to check the number of platelets, the creatinine level and the level of AST or ALT to exclude thrombocytopenia, liver cytolysis or kidney dysfunction.
- To monitor the number of platelets, the creatinine level and the level of AST or ALT every 2-3 weeks to detect thrombocytopenia, kidney- or liver dysfunction. (If detected, stopping the medication should be considered).
- To encourage all pregnant patients with a primary CMV infection, both those undergoing treatment with high dose valaciclovir and those not receiving any pharmacological treatment, to register in the nationwide BELpREG pregnancy registry in our country (<https://belpreg.be> or <https://belpreg.be/fr/>) to support safety monitoring and evidence generation.

POLICY RECOMMENDATION



We recommend temporary reimbursement of valaciclovir for the indications specified in this guideline.

This reimbursement should be accompanied by transparent patient information on expected benefits and remaining uncertainties, and a systematic and structured national data collection system embedded in routine clinical practice. Such data collection is necessary to monitor safety and nationwide patterns or trajectories of use, and to generate evidence that can inform future reassessment of clinical and economic value in the Belgian context. A formal re-evaluation of this recommendation should be planned after 3-5 years, based on the accumulated clinical data and economic evaluation.

4.2 Introduction

Antiviral drugs like aciclovir and valaciclovir have been used in high doses to prevent CMV disease in transplant recipients (4 x 2g per day for 90 days) (33). They have also been used to treat herpes simplex infections during pregnancy (2 x 500 mg per day for 3-10 days)(34).

In the context of treatment of antenatal CMV, valaciclovir was initially investigated in fetuses with symptomatic congenital CMV infection as a form of tertiary prevention aimed at reducing long-term sequelae. Its good placental transfer, achieving therapeutic concentrations in maternal blood, fetal blood and amniotic fluid(35), together with its apparently good tolerability in pregnant patients(36), provided the rationale for subsequent studies assessing whether valaciclovir could prevent vertical CMV transmission in pregnant patients with a primary infection(10).

Although we dispose only of limited data, the safety profile of valaciclovir in pregnancy seems good⁹. Practitioners in Belgium are already offering valaciclovir to their patients, as we observed in our survey on current practices on CMV testing and management and as observed in the real-world data collection in the BELpREG pregnancy registry in our country (see [Appendix 1](#)).

4.3 Research question and search

A search was made on Medline, Embase and Cochrane databases for this research question:

“In pregnant patients with primary CMV infection, should valaciclovir (8 g/day) be used compared to placebo or no treatment, to reduce the risk of CMV-related sequelae in the foetus or child?”

The PICO developed for the search, and all details related to the analysis of the evidence can be found in [Appendix 8](#).

4.4 Evidence

4.4.1 Included studies and methodological quality

One RCT and four retrospective cohort studies were identified on the topic of secondary prevention (use in pregnant patients with a known primary infection, before establishment of foetal infection with amniocentesis).

Two retrospective cohort studies were found on the topic of tertiary prevention (use in pregnant patients with a known primary infection and an established foetal infection after the results of amniocentesis).

⁹ <https://www.lecrat.fr/8595/>



4.4.1.1 Secondary prevention

Shahar-Nissan 2020 is the only included RCT(10). It is a double blind monocentre study from Israel with a sample size of 100 pregnant patients with a primary CMV infection during the periconceptual period or first trimester up to 16 weeks of gestation. Participants were randomised 1:1 between 2 x 4g per day of valaciclovir and placebo until amniocentesis, which was planned at ≥ 21 weeks of gestation and ≥ 7 weeks after estimated maternal infection. Limitations of this study included a per protocol analysis excluding patients because of non-adherence, TOP before amniocentesis and non-viable pregnancies after randomisation. There were also missing data on amniocentesis from seven fetuses (four treated and three untreated) with the results being imputed depending on the PCR result at birth (thereby assuming 100% accuracy of amniocentesis in this group). Although follow-up was accurately specified in the publication, part of the information was collected by phone-call and there is no information on missing data.

Three of the four included retrospective cohorts(37–39) compared a group of pregnant patients with primary CMV infection treated with valaciclovir and their offspring with a control group consisting of pregnant patients that either declined treatment or that did not receive treatment because they presented before valaciclovir was used. As in every observational study, patients in the control group may differ from those treated with valaciclovir and therefore introduce selection bias.

Egloff 2023 is a multicentre study from France(37) with a sample size of 143 that included pregnant patients regardless of the timing of their primary CMV infection (periconceptual, first, second or third trimester). The aim of the study was to assess frequency of vertical transmission at amniocentesis. Valaciclovir 8g was mostly given until amniocentesis, which was performed at ≥ 21 weeks of gestation and ≥ 6 weeks after estimated time of infection. Treatment could be continued until birth, if the result of amniocentesis was positive (positive DNA CMV PCR) after discussion with the parents. No amniocentesis was performed in 8% of valaciclovir and 42% of the control group, which could affect the primary outcome. Propensity score matching was used for timing of infection, which may be an overadjustment. No critical outcomes were reported in a comparative way. There is non-comparative data on adverse events in the mother.

Faure Bardon 2021 is a monocentre cohort from France(38) including 130 pregnant patients with a periconceptual or first trimester primary CMV infection. Treatment with 2 x 4g per day of valaciclovir was given until amniocentesis (≥ 8 weeks after maternal infection and ≥ 17 weeks of gestation) and could be continued until birth after a positive amniocentesis. The primary outcome was CMV PCR at amniocentesis and no comparative results were available on critical outcomes. Propensity score matching was used for timing of infection and gestational age, but there is a risk for over-adjustment because of the low number of events. Adverse events in the mothers were only described in the treatment group.

Zammarchi 2023 is a multicentre study from Italy(39) that included a total of 447 patients with a periconceptual, first or second trimester infection up to 24 weeks of gestation. Treatment with valaciclovir 4 x 2g was given until amniocentesis (≥ 8 weeks after estimated timing of primary infection and ≥ 20 1/7 weeks of gestation) and could be continued in case of a positive amniocentesis with abnormal findings on ultrasound. If amniocentesis was refused (n=180), treatment was continued until 26 weeks of gestation. The primary outcome reported was CMV PCR at amniocentesis. Limitations of this study were the retrieval of clinical data from electronic or paper medical records according to the availability in the different participating centres, which may be incomplete and the risk of over-adjustment by inverse probability weighting due to a low number of events.

Amir 2023 is a monocentre study from Israel(40) with a retrospective cohort (N=195) and an historical control group (N=47). The protocol for treatment was slightly different including dosing of either 2 x 4g or 4 x 2g valaciclovir per day and starting treatment a maximum 9 weeks after a periconceptual infection and 8 weeks after a first trimester infection until amniocentesis between 20-22 weeks of gestation. However, a major limitation of this study was that it relied on the placebo-treated participants from the only available RCT(10). Therefore, this study could not be included in meta-analyses. Another

problem was the exclusion of terminations of pregnancy from the analysis. Adverse events including nausea and acute renal failure were only reported in treated patients.

Among the selected studies only two reported results on critical outcomes(10,39).

4.4.1.2 Tertiary prevention

The studies on tertiary prevention (prevention of sequelae in a CMV infected foetus) included one cohort study and a phase II study with one arm compared to a historical control group.

Jaquemard 2007(35) is a monocentre cohort study from France where 20 pregnant patients with primary CMV infection and confirmed foetal infection (positive CMV PCR on amniocentesis) with biological and/or ultrasound markers of infection were treated with valaciclovir 4 x 2g per day from amniocentesis (median 28 weeks, range 22-34 weeks) until delivery or TOP. This treatment group was compared to a control group consisting of 24 pregnant patients that presented at 19-38 weeks of gestation with a symptomatic intrauterine infection but either declined treatment or presented 12 months before treatment was offered. The primary outcome was viral load and drug concentrations. Outcomes of symptomatic treated and untreated infants were described case by case, but not directly compared, except for 'poor outcome', a composite outcome composed of TOP, severe Cytomegalic Inclusion Disease (CID)(41) and miscarriage. No matching or adjustments were made to reduce confounding..

Leurez-Ville 2016 is a phase II study performed in 6 centres in France(36). Valaciclovir 4 x 2g was offered to 41 pregnant patients with a CMV infected foetus and one extracerebral ultrasound feature compatible with CMV infection and/or one isolated cerebral abnormality and/or low platelets or high CMV DNA viral load in foetal blood. Foetuses with severe ultrasound brain abnormalities were excluded. Treatment started after positive amniocentesis (> 21 weeks) and continued until delivery, TOP, or 26 weeks of treatment. The treated patients were compared to a historical control: 47 cases with matching inclusion criteria for the ultrasound abnormalities reported in three suitable published studies(35,42,43). The primary outcome was frequency of asymptomatic neonates. The outcomes TOP and adverse events were only described in treated patients and not compared.

4.4.2 Results

4.4.2.1 Results on secondary prevention

We report below the results of the comparison of critical outcomes in maternal primary CMV infection treated with high dose valaciclovir versus no treatment or placebo. severely symptomatic newborn

Table 7 – Summary GRADE table for use of valaciclovir for secondary prevention

Outcomes	N° of participants (studies) Follow-up	Certainty of the evidence (GRADE)	Relative effect (95% CI)	Anticipated absolute effects	
				Risk with Placebo or no treatment	Risk difference with Valaciclovir 8g/day
<i>severely symptomatic foetus - not reported</i>	-	-	-	-	-
<i>All-cause termination of pregnancy</i>	549 (1 non-randomised study and 1 RCT)	⊕○○○ Very low	OR 0.41 (0.19 to 0.92)	112 per 1,000	63 fewer per 1,000 (from 89 fewer to 8 fewer)



Termination of pregnancy in patients with positive amniocentesis	72 (1 non-randomised study and 1 RCT)	⊕○○○ Very low	OR 0.36 (0.12 to 1.07)	479 per 1,000	230 fewer per 1,000 (from 380 fewer to 17 more)
Severely symptomatic newborn	482 (1 non-randomised study and 1 RCT)	⊕○○○ Very low	OR 0.17 (0.06 to 0.51)	91 per 1,000	74 fewer per 1,000 (from 85 fewer to 42 fewer)
motor developmental delay at follow-up	not reported	-	-	-	-
cognitive developmental delay at follow-up	not reported	-	-	-	-
cerebral palsy	not reported	-	-	-	-
epilepsy	not reported	-	-	-	-
Severe adverse events	90 (1 RCT)	⊕○○○ Very low	Not estimable	0 events	0 events

Despite the identification of five studies on secondary prevention, only two of them, one RCT(10) and one retrospective cohort study(39) reported on critical outcomes. The RCT included only periconceptional and first trimester infections that were treated until amniocentesis, whereas the cohort study also included second trimester infections. In the cohort study treatment could be continued after a positive amniocentesis in case of abnormal ultrasound and was done until 26 weeks if amniocentesis was not performed. In the RCT treatment was stopped at amniocentesis.

The pooled results of the two studies on termination of pregnancy for 549 fetuses (255 treated and 294 untreated) showed a significantly lower number of (all cause) TOPs with treatment compared to no treatment or placebo. This reduction was also statistically significant in case of a positive amniocentesis, but not statistically significant in case no amniocentesis was performed. As expected, the frequency of TOPs appeared not to be affected by treatment with valaciclovir in case of negative amniocentesis. Certainty of the available evidence on this outcome, however, is very low, mainly because of the serious risk of bias and serious imprecision due to small sample sizes and/or low number of events. Since the RCT did not distinguish critical outcomes for periconceptional infections and first trimester infections, we could not stratify the meta-analysis per period of infection for this outcome. The data of the observational study suggest that the frequency of TOPs may be statistically significantly reduced in first trimester infections only.

In the RCT a distinction was made between positive amniocenteses with and without symptoms. In case of a positive amniocentesis, 60% of pregnancies terminated in untreated patients were asymptomatic, whereas all pregnancies terminated in treated patients were symptomatic. The hypothesis is that treatment offers the mothers some kind of reassurance on the low risk for sequelae in the child. However, this observation was based on only on 15 positive amniocenteses (4 in the treated group and 11 in the placebo group). This distinction is not available for the observational study.

The meta-analysis of the same two studies on a total of 482 children (228 treated and 254 untreated) showed an impact of treatment on severely symptomatic newborns (1.8% in treated versus 9.1% in not treated patients). Since the RCT did not distinguish results of periconceptional infections and first trimester infections, we could not stratify the meta-analysis per period of infection for this outcome.

Based on the data from the observational study, the protective effect of treatment was not statistically significant in the different subgroups (periconceptional, first and second trimester infections) but followed the same protective trend.

In our prioritisation of outcomes (see [Appendix 6](#)), severely symptomatic newborns were defined according to the classification published by the VVK(9), and corresponds to indications to postnatal treatment with valganciclovir. This definition includes isolated hearing loss as a severe symptom. In the studies severe symptoms were mainly hearing loss in the RCT and the combination of hearing loss and abnormal CNS involvement based on brain imaging in the observational study.

Adverse events in the treated mothers were described in all five studies on secondary prevention, but only the RCT reported comparative data between treated and untreated patients. A meta-analysis of data was not possible. The adverse events reported were mostly aspecific and mild, and no severe adverse events were described(39). Acute renal failure, a well-known adverse event linked to high dose valaciclovir, was reported in the 4 observational studies and occurred in 0.5 to 2% of the treated patients. However, none of the acute renal failures was considered an SAE, as they always resolved after suspending or stopping the treatment with valaciclovir. The absence of reported severe adverse events in these studies so far does not exclude them entirely. Data on more pregnant patients treated with valaciclovir are necessary to draw stronger conclusions on the safety of the medication, especially for rare adverse events.

CONCLUSIONS ON SECONDARY PREVENTION

The number of TOPs may be lower in pregnant patients with primary CMV infection treated with valaciclovir compared to pregnant patients with primary CMV not treated with valaciclovir, but the evidence remains very uncertain (very low certainty of evidence).

Severely symptomatic newborns may be lower in pregnant patients with primary CMV infection treated with valaciclovir compared to pregnant patients with primary CMV infection not treated with valaciclovir, but the evidence remains very uncertain (very low certainty of evidence).

No critical outcomes were observed in second or third trimester infection, but the evidence remains very uncertain (very low certainty of evidence).

The number of SAEs may not be increased in pregnant patients with primary CMV infections treated with valaciclovir compared to pregnant patients with primary CMV infection not treated with valaciclovir but the evidence remains very uncertain (very low level of evidence).

There is no reported evidence for the other critical outcomes: severely infected fetuses, motor developmental delay, cognitive developmental delay and cerebral palsy.

4.4.2.2 Results on tertiary prevention

We report below the results of the comparison of critical outcomes in maternal primary CMV infection and confirmed symptomatic foetal infection, treated with high dose valaciclovir versus no treatment or placebo.

The two identified primary studies on tertiary prevention were observational and provided extremely low-certainty evidence due to serious risk of bias, indirectness because of inclusion of only symptomatic foetal infections, and imprecision because of small sample sizes and small number of events. In Leruez-Ville 2024 (36), the historical control group used as the comparator included a selection of patients from the study by Jacquemard 2007(35). Reported outcomes differed between studies, preventing meta-analysis for critical outcomes. In Jacquemard 2007(35), comparative data were available for termination of pregnancy (TOP) and hearing loss, as well as for a composite outcome of TOP, miscarriage, and severe CID (Cytomegalic Inclusion Disease). In Leruez-Ville 2024(36), comparative data were available only for asymptomatic neonates, while individual symptoms were reported exclusively in the treatment group.



Based on outcomes from only one study(35), the frequency of TOP may be reduced with treatment compared to no treatment. The certainty of evidence is extremely low, because of high risk of bias, serious indirectness and very serious imprecision. Conversely, severely symptomatic newborns may be more frequent with treatment than without. This likely reflects the timing of treatment (initiated only after symptomatic foetal infection was detected, when irreversible damage had already occurred) and the greater likelihood of termination in untreated pregnancies showing worsening or persistent cerebral abnormalities. The certainty of evidence for this outcome is also extremely low, for the same reasons as above, with additional extremely serious imprecision. No adverse events were reported.

Table 8 – Summary GRADE table for use of valaciclovir for tertiary prevention

Outcomes	No of participants (studies) Follow-up	Certainty of the evidence (GRADE)	Relative effect (95% CI)	Anticipated absolute effects	
				Risk with Placebo or no treatment	Risk difference with Valaciclovir 8g/day
<i>severely symptomatic foetus</i>	not reported	-	-	-	-
<i>motor developmental delay at follow-up</i>	not reported	-	-	-	-
<i>cognitive developmental delay at follow-up</i>	not reported	-	-	-	-
<i>cerebral palsy</i>	not reported	-	-	-	-
<i>epilepsy</i>	not reported	-	-	-	-
<i>adverse events</i>	not reported	-	-	-	-
<i>termination of pregnancy (tertiary prevention)</i>	45 (1 non-randomised study)	⊕○○○ Very low	OR 0.50 (0.15 to 1.68)	500 per 1,000	167 fewer per 1,000 (from 370 fewer to 127 more)
<i>severe symptoms (hearing loss and severe CID)</i>	45 (1 non-randomised study)	⊕○○○ Very low	OR 3.83 (0.37 to 40.02)	42 per 1,000	101 more per 1,000 (from 26 fewer to 593 more)

CONCLUSIONS ON TERTIARY PREVENTION

- The number of TOPs may be lower in pregnant patients with primary CMV infection and a symptomatic infected foetus who are treated with valaciclovir, compared with those not treated. The evidence remains extremely uncertain (very low level of evidence).
- Severely symptomatic newborns may be more frequent in pregnant patients with primary CMV infection and a symptomatic infected foetus treated with valaciclovir than in those not treated. The evidence remains extremely uncertain (very low level of evidence).
- There is no evidence for the following critical outcomes: severe adverse events, severely symptomatic fetuses, motor developmental delay, cognitive developmental delay, and cerebral palsy.
- There is no evidence for critical outcomes in pregnant patients with CMV infected fetuses that are asymptomatic.

4.5 Evidence to recommendations

4.5.1 Balance between clinical benefits and harms

Secondary prevention

Reported benefits of valaciclovir use include a lower rate of sequelae with treatment such as hearing loss and brain imaging abnormalities at birth.

A lower rate of positive amniocenteses after treatment may be the driver behind the reduced frequency of TOPs due to positive amniocentesis alone. The administration of preventive medication may be reassuring for parents, leading to less unnecessary terminations of pregnancy.

Reported harms of the treatment with valaciclovir include mild and aspecific adverse events in the mother, but no severe adverse events, although information on more treated patients will be necessary to draw conclusion on the safety of the medication.

Acute renal failure was reported in up to 2% of treated patients(39), but was reversible with suspension of treatment.

Other disadvantages of the treatment include the need to take a high number of pills per day (16 x 500 mg).

Tertiary prevention

Regarding tertiary prevention no clear benefit of treatment has been reported, because the results on critical outcomes including termination of pregnancy and severely symptomatic newborns were not significantly different between treatment and no treatment groups. Moreover, there was a trend towards more severely symptomatic newborns after treatment compared to no treatment, but that may be explained by the decreased number of TOPs in the treated group.

4.5.2 Quality of evidence

Secondary prevention

The quality of evidence for the use of valaciclovir in secondary prevention was evaluated to be very low.

The included primary studies were all small with a low number of events. Most of them were retrospective observational studies. The only RCT was at high risk of bias mainly due to deviations from the intended intervention (intention to treat analysis) and missing outcome data (imputation results of amniocentesis).

Among the studies there were differences in definitions of patients' categories (notably periconceptual infection), in their choice of population (timing of infection for treatment, definition of primary maternal infection, ...), of outcomes definition and collection, and duration of treatment regimen.

None of the studies had a primary outcome that was considered a critical outcome from our patients, so studies were not powered to detect significant differences in critical outcomes.

Tertiary prevention

The quality of evidence for the use of valaciclovir in tertiary prevention was evaluated to be extremely low.

This was due to the inclusion of data from only one very small, partially retrospective cohort study with a serious risk of bias mainly due to patient selection, and a low number of events and non-significant results, leading to very/extremely serious imprecision and inconsistency that actually could not be evaluated.



Since only symptomatic infected fetuses were included in the studies, we lack information on the efficacy of valaciclovir on asymptomatic infected fetuses.

The quality of the studies is probably insufficient to draw any conclusions.

4.5.3 *Patient preferences*

The preference of patients may vary. Some patients would want to be treated to do all things possible to prevent sequelae for their child, while others will refuse any treatment out of fear to harm their child with the medication.

However, patients prefer to have an option rather than no option at all once they know they have a seroconversion. Since the option is now available, patients value shared decision making. Postnatal treatment with valganciclovir for severely affected neonates (and for moderately symptomatic children based on expert opinion) is evaluated by the patients to be burdensome, since it lasts 6 months and requires multiple hospital visits and testing during follow-up. Antenatal treatment with valaciclovir may potentially lower that burden, by lowering the number of children requiring treatment with valganciclovir.

Additionally, regarding tertiary prevention, patients might be more inclined to choose treatment. After a positive amniocentesis, patients know they are in a higher risk group and want to do all that is possible to prevent any further damage, because they have “nothing to lose”. On the other hand, patients are concerned that this approach would allow more severely affected fetuses to survive. Hence, more evidence on the efficacy of tertiary prevention would be welcomed by patients.

4.5.4 *Cost and resource allocation*

In Belgium, valaciclovir is currently already prescribed by some clinicians to pregnant patients with primary CMV infection on an off-label basis, without reimbursement. This results in substantial out-of-pocket costs and leads to unequal access to treatment, whereby receipt of care depends on patients' financial capacity rather than clinical eligibility.

Given a recommendation of valaciclovir for pregnant patients with primary CMV infection, it would be ethically, socially, and from a health-policy perspective indefensible to maintain a situation in which access to the recommended treatment is not financially covered. Introducing such a recommendation without ensuring that the treatment is affordable and accessible to all eligible patients would risk reinforcing existing inequalities and undermining the principle of equal access that underpins the Belgian healthcare system.

On the other hand, since treatment with valaciclovir potentially decreases the number of children needing valganciclovir and possibly life-long care, the resources (medication and follow-up) allocated to valganciclovir and other interventions could be reduced.

5 NEGATIVE AMNIOCENTESIS: WHAT IS THE FREQUENCY OF CMV-ASSOCIATED SEQUELAE IN CHILDREN WITH CCMV AT BIRTH? SHOULD MANAGEMENT INCLUDE ENHANCED IMAGING (REGULAR ULTRASOUND, MRI) COMPARED ROUTINE IMAGING ONLY?

5.1 Recommendations

	Strength of recommendation	Level of evidence
Recommendations for pregnant patients with a primary CMV infection not treated with valaciclovir		
Practitioners may not routinely offer enhanced imaging surveillance in patients with a negative amniocentesis	Conditional	Very low
Recommendations for pregnant patients with a primary CMV infection treated with valaciclovir		
There is insufficient evidence on the use of enhanced imaging to predict the risk of CMV related sequelae in pregnant patients with a negative amniocentesis.		Very low
In the context of research, evidence should be gathered to determine the prognostic accuracy of amniocentesis for CMV-related sequelae in children, and their potential detectability via antenatal imaging, to inform the choice between enhanced imaging surveillance (serial targeted prenatal ultrasound and/or MRI) and routine imaging among pregnant patients with a negative amniocentesis. To completely assess prognostic accuracy, data should be collected also in children born from patients with a known primary infection who have a negative cCMV at birth.	Research recommendation	

CLINICAL PRACTICE GUIDANCE

Outside the context of research, the guideline development group suggests:

For patients treated with valaciclovir

- To not routinely perform enhanced imaging (serial targeted ultrasound and/or foetal MRI) in pregnant patients with a negative amniocentesis for CMV, with the exception of targeted prenatal neurosonography in the third trimester.
- To discuss the potential benefits and harms of enhanced imaging with the pregnant patient, and proceed through shared decision-making, when this is requested.
- To perform ultrasound examinations in a referral centre (a centre where foetal MRI can be performed)

For patients who decline amniocentesis but opt for imaging surveillance

- To include in the surveillance serial ultrasound and foetal MRI, performed in a referral centre



5.2 Introduction

In women with primary CMV seroconversion, mid-pregnancy amniocentesis is performed to assess whether vertical transmission has occurred(44). If CMV virus is detected in the amniotic fluid, the child is at higher risk for sequelae(45).

Follow-up of a positive amniocentesis includes targeted prenatal ultrasound every 2–4 weeks and a foetal MRI at around 32 weeks, unless warning signs prompt earlier assessment(14).

TOP may be considered by the parents in cases of severely affected fetuses, but it is sometimes chosen by parents in the setting of moderate foetal involvement or to avoid any risk based on a positive amniocentesis alone (see [Appendix 1.2.3](#)).

In case of negative amniocentesis, parents are mostly reassured that no vertical transmission has happened, however, it is possible for CMV to be diagnosed at birth despite a negative amniocentesis(45).

Hence, it is important to establish whether the risk of sequelae in babies that have a positive CMV at birth despite a negative amniocentesis is lower compared to that of babies that had a positive amniocentesis, and sufficiently low to avoid unnecessary antenatal imaging follow-up, given the fact that the positive predictive value described for both antenatal ultrasound and antenatal MRI is relatively low, and correlation between some imaging findings and long term functional sequelae is uncertain(46–48).

5.3 Research questions and search

5.3.1 *Amniocentesis to predict the risk of CMV-related sequelae when cCMV is detected at birth*

A search was made for the following research question:

“In pregnant patients with confirmed primary CMV infection, and confirmed cCMV at birth, what is the frequency of CMV-related sequelae in the foetus or child in those who had a previous positive amniocentesis compared to those who had a previous negative amniocentesis?”

We included only studies in which a clear distinction between patients with a previous negative amniocenteses and patients with a previous positive amniocenteses was established at follow-up, and in which at least one of our critical outcomes was reported. We excluded studies in which CMV DNA PCR was not used for either amniocentesis testing and/or testing at birth. We evaluated both treated and untreated patients.

The PECO developed for the search and all details related to the analysis of the evidence can be found in [Appendix 9](#).

5.3.2 *Follow up of negative amniocentesis*

A search was made for the following research question: “In pregnant patients with confirmed primary CMV infection and a negative amniocentesis, does enhanced imaging (e.g., serial ultrasound, foetal MRI) compared with routine imaging improve detection of CMV-related sequelae and lead to better clinical outcomes for the foetus or child?”

The PICO developed for the search and all details related to the analysis of the evidence can be found in [Appendix 9](#).

5.4 Evidence

5.4.1 Included studies and methodological quality

5.4.1.1 *Amniocentesis to predict the risk of CMV-related sequelae when cCMV is detected at birth*

We included 5 studies regarding the comparison between cCMV at birth following positive amniocentesis and cCMV at birth following negative amniocentesis.

Bilavsky 2016(49) was a retrospective cohort study including 45 children with negative amniocentesis and positive cCMV at birth, matched 1:2 with 93 children with positive amniocentesis and positive cCMV at birth, following primary maternal infection, in two separate centres (one in France and one in Israel). Timing of amniocentesis was at least 21 gestational weeks and 7 weeks after assumed date of maternal infection in Israel, and 20-21 gestational weeks and 6-7 weeks after maternal infection in France. The main limitations of the study were that there was no indication of the reason why the patients underwent amniocentesis (screening vs symptoms), or whether the reasons differed systematically between groups. The design (only live born children included) excluded any information on frequency and reason for TOP. The methodology used for collecting outcomes was insufficiently described; and there was no description in the results of loss to follow-up, although mentioned in the discussion. Newborns were treated with valganciclovir following two different schemes in the two centres, and the median follow-up was limited to 3.4 years of age in the negative amniocentesis group and 3 years in the positive amniocentesis group. Patients who terminated their pregnancy could not be included in the computation of the outcomes at birth or long term.

Keymeulen 2021(50) was a retrospective cohort study including 233 patients with positive amniocentesis and cCMV at birth, and 81 patients with negative amniocentesis and cCMV at birth, registered in the multicentre registry for CMV in Flanders. Amniocentesis was performed after 21 weeks of gestation. Similarly to Bilavsky 2016, there was no indication of the reason why the patients underwent amniocentesis (screening vs symptoms), or whether the reasons differed systematically between groups. The nature of the design excluded any information on frequency and reason of TOP. Collection of outcomes was limited to outcomes present at birth. It could not be excluded that some of the amniocenteses may have been performed for non-primary infections.

De Keersmaecker 2024(51) was a retrospective cohort including women who had a primary maternal infection before 14 weeks and underwent amniocentesis around 21 weeks of gestation. The cohort included 116 patients with positive amniocentesis and positive cCMV at birth, 7 patients with negative amniocentesis and positive cCMV at birth, and 220 patients with negative amniocentesis and negative cCMV at birth. Main limitations were that there is no indication of the reason why the patients underwent amniocentesis (screening vs symptoms), or whether the reasons differed systematically between groups. The study included only complete data collection cases and evaluation of severity of sequelae was only possible in those patients who did not undergo termination of pregnancy. Follow up was evaluated via a questionnaire to the parents, with only positive responses confirmed by documentation, while negative responses were not verified. Median follow-up for children with positive amniocentesis and positive cCMV at birth was limited to a median of 4.4 years, and to 4 years for children with negative amniocentesis and positive cCMV at birth, while the length of follow-up ranged between 1 and 8 years for both groups.

We also performed a post-hoc analysis of data coming from two studies that evaluated the efficacy of valganciclovir treatment : Shahar Nissan 2020 and Zammarchi 2023 (see 4.4.1.1), we report here a brief description relative to the current research question.

Shahar-Nissan 2020(10) was a randomized controlled trial that allowed analysis of 45 untreated and 47 treated children. In both arms (treated and untreated) we could compare patients with a positive cCMV at birth who had a negative vs a positive amniocentesis. Amniocentesis was performed at least 21 weeks of gestation and at least 7 weeks after seroconversion. The main limitation concerning the



current research question was that six participants, declined amniocentesis; in these cases, amniocentesis results were imputed using the results at birth. Although the study was randomised for the evaluation of valaciclovir, randomisation does not apply to the assessment of the prognostic value of amniocentesis results. Evaluation of severity of sequelae was only possible in those who did not undergo termination of pregnancy.

Zammarchi 2023(39) was a multicentre retrospective cohort study including 447 patients with maternal primary CMV infection before 24 weeks. Among these, 270 had amniocentesis performed: Amniocentesis was performed not before 20 weeks and 1 day of gestation and at least 8 weeks after seroconversion. Main limitations include that 12/116 (10.3%) of newborns of mothers treated with valaciclovir with a negative amniocentesis were lost to follow-up, and evaluation of severity of sequelae was only possible in those who did not undergo termination of pregnancy.

5.4.1.2 Follow up in case of negative amniocentesis

No studies were found regarding a comparison of more intensive versus less intensive follow up in case of negative amniocentesis.

5.4.2 Results

5.4.2.1 Amniocentesis to predict the risk of CMV-related sequelae when cCMV is detected at birth

We report below the results of the comparison of critical outcomes in positive amniocentesis with positive cCMV at birth compared to negative amniocentesis with positive cCMV at birth in treated(10,39,49–51) and untreated(10,39) patients.

Table 9 – Summary of findings table of outcomes in positive amniocentesis with positive cCMV at birth compared to negative amniocentesis with positive cCMV at birth in untreated patients

Outcomes	No of participants (studies) Follow-up	Certainty of the evidence (GRADE)	Relative effect (95% CI)	Anticipated absolute effects	
				Risk of Negative amniocentesis and positive cCMV at birth	Risk difference of Positive amniocentesis and positive cCMV at birth
<i>Severely symptomatic foetus</i>	not reported	-	-	-	-
<i>Termination of pregnancy</i>	182 (3 studies)	⊕○○○ Very low	OR 7.46 (1.32 to 42.31)	0 events	-
<i>Severely symptomatic newborn</i>	551 (5 studies)	⊕○○○ Very low	OR 2.75 (0.7 to 10.8)	133 per 1,000	297 more per 1,000 (from 36 fewer to 491 more)
<i>Motor developmental delay at follow-up</i>	57 (1 study)	⊕○○○ Very low	OR 2.90 (0.15 to 55.27)	0 events	-
<i>Cognitive developmental delay at follow-up</i>	138 (1 study)	⊕○○○ Very low	OR 2.51 (0.12 to 53.43)	0 events	-
<i>Cerebral palsy</i>	not reported	-	-	-	-
<i>Epilepsy</i>	not reported	-	-	-	-

Table 10 – Summary of findings table of outcomes in positive amniocentesis with positive cCMV at birth compared to negative amniocentesis with positive cCMV at birth in patients treated with valaciclovir.

Outcomes	No of participants (studies) Follow-up	Certainty of the evidence (GRADE)	Relative effect (95% CI)	Anticipated absolute effects	
				Risk of Negative amniocentesis and positive cCMV at birth	Risk difference of Positive amniocentesis and positive cCMV at birth
<i>Severely symptomatic fetus - not reported</i>	not reported	-	-	-	-
<i>Termination of pregnancy</i>	44 (2 non-randomised studies)	⊕○○○ Very low	OR 6.81 (0.71 to 65.06)	0 events	-
<i>Severely symptomatic newborn</i>	29 (2 non-randomised studies)	⊕○○○ Very low	OR 2.82 (0.22 to 36.79)	100 per 1,000	139 more per 1,000 (from 76 fewer to 703 more)
<i>Hearing loss at follow up - not reported</i>	not reported	-	-	-	-
<i>Motor developmental delay at follow-up - not reported</i>	not reported	-	-	-	-
<i>Cognitive developmental delay at follow-up - not reported</i>	not reported	-	-	-	-
<i>Cerebral palsy - not reported</i>	not reported	-	-	-	-

It is relevant to underline that since there were zero events for most of the reported outcomes in the negative amniocentesis group, a continuity correction was applied to allow estimation of the odds ratio. The use of this correction introduces uncertainty in the odds ratio estimate and is handled by downgrading for imprecision. By contrast, in zero-event settings, applying a continuity correction to absolute risks makes the entire absolute risk difference driven by the arbitrary correction and baseline risk choice; therefore, absolute risks are not reported, and only the fact that there were zero events is noted.

In patients that were not treated with valaciclovir, it appears that the risk profile is higher in those who had a positive amniocentesis compared to those with a negative amniocentesis across all reported outcomes. Nonetheless, for many of the outcomes, there were no events observed in the negative amniocentesis group, and the number of negative amniocenteses is generally limited. It is important to note that the absence of observed events does not imply total absence of risk, especially with small sample sizes.

The estimate of the frequency of adverse outcomes at birth and long term follow up is influenced by the fact that pregnancies that were terminated were not observed, so the natural history of the disease could not be accurately described since the group with the highest risk of sequelae cannot be observed. Hence, the risk of sequelae may be underestimated, especially in the group after positive amniocentesis where the frequency of TOP is higher.



The most widely accepted theory is currently that when CMV is diagnosed at birth despite a negative amniocentesis, it is the consequence of a transmission of the virus after amniocentesis (in the case of our review around 20-21 weeks and 7-8 weeks after maternal seroconversion) (49,52). The risk profile would then reflect the fact that infection in a later stage of pregnancy carries a better prognosis. Bilavsky 2016(49) highlights the fact that when vertical transmission occurs long after the moment of infection a mature maternal immunity may be sufficient to avoid foetal disease.

In patients treated with valaciclovir the evidence is less clear, as we have only two low-quality studies(10,39) including a limited number of patients that decided to undergo amniocentesis. The only outcomes available are TOP and severely symptomatic newborns (largely driven by hearing loss). It is worth noting, that no TOP for CMV related anomalies has been reported in the studies in patients with a negative amniocentesis. Crucially, severe antenatal imaging anomalies has not been reported as an outcome.

In treated patients, mechanisms that explain a negative amniocentesis with a subsequent cCMV at birth, other than late viral transmission cannot be excluded. In addition, in treated patients, late viral transmission may not be accompanied by maturation of maternal immunity, and rebound viraemia after interruption of treatment has been observed(53,54).

5.4.2.2 Follow up in case of negative amniocentesis

No studies were identified in which a more, or less intensive antenatal post-amniocentesis follow-up was applied to pregnancies with a negative amniocentesis (see [Appendix 9.7](#)).

Nonetheless, among the studies included for the question on CMV-related sequelae in cases with negative amniocentesis and cCMV detected at birth, three studies reported sufficiently detailed follow-up of children both positive and negative for cCMV at birth to allow for post-hoc calculation of prognostic accuracy measures in untreated and treated patients (10,39,51). However, differential follow-up between cCMV-positive and cCMV-negative cases cannot be excluded, particularly in retrospective studies.

For the purpose of this analysis, prevalences are calculated as the global pooled prevalences reported in the studies used to calculate accuracy statistics (i.e. prevalences in the whole cohort, not limited to those undergoing amniocentesis). Since, globally, the prevalence of critical outcomes is low in the overall population, negative predictive values for the prognostic accuracy of amniocentesis are high in both treated and untreated patients.

Nonetheless, especially in treated patients, the high level of bias in the considered studies, the small number of patients included, and the even lower number of included patients who accepted amniocentesis, renders the evidence very uncertain.

CONCLUSIONS

Without treatment during pregnancy

In children with cCMV at birth of mothers with a primary CMV infection that are not treated with valaciclovir and underwent amniocentesis around 20-21 weeks and 7-8 weeks after seroconversion:

- The risk of termination of pregnancy, severely symptomatic newborns, hearing loss at follow up, motor developmental delay at follow up, and cognitive developmental delay at follow up, may be higher in those who had a positive amniocentesis compared to those who have a negative amniocentesis, but the evidence remains very uncertain (very low level of evidence).
- There is no reported evidence on the risk for a severely symptomatic foetus and cerebral palsy in those who have a positive amniocentesis compared to those who have a negative amniocentesis.
- The negative predictive value of amniocentesis for the prognosis of termination of pregnancy, severely symptomatic newborns, hearing loss at follow up, motor developmental delay at follow up may be high, but the evidence remains very uncertain (very low level of evidence).
- The negative predictive value for the prognosis of a severely symptomatic foetus, cerebral palsy and cognitive developmental delay at follow up are unknown.

With treatment with valaciclovir during pregnancy

In children with cCMV at birth of mothers with a primary CMV infection that are treated with valaciclovir and underwent amniocentesis around 20-21 weeks and 7-8 weeks after seroconversion :

- The risk of termination of pregnancy and severely symptomatic newborns may be higher in those who had a positive amniocentesis compared to those who had a negative amniocentesis, but the evidence remains extremely uncertain (very low level of evidence).
- There is no reported evidence on the risk of a severely symptomatic foetus, hearing loss at follow up, motor developmental delay at follow up, cognitive developmental delay at follow up, cerebral palsy and epilepsy in those who had a positive amniocentesis compared to those who had a negative amniocentesis.
- The negative predictive value of amniocentesis for the prognosis of termination of pregnancy and severely symptomatic newborns may be high but the evidence remains extremely uncertain (very low level of evidence).
- There is no evidence on the negative predictive value of amniocentesis for the prognosis of severely symptomatic foetus, motor developmental delay at follow up, cognitive developmental delay at follow-up, and cerebral palsy at follow up.



5.5 Evidence to recommendations

5.5.1 *Balance between clinical benefits and harms*

In untreated patients, the combination of (a) evidence that the risk profile of patients with positive amniocentesis and cCMV at birth may be higher than that of those with negative amniocentesis, and (b) the apparent rarity of cCMV at birth despite negative amniocentesis, may allow for reassurance of patients.

Enhanced follow-up after negative amniocentesis would likely burden patients with extra testing and risk of false positives without a clearly apparent benefit.

For patients that are treated with valganciclovir, the frequency of cCMV at birth in spite of negative amniocentesis appears to be higher. There is currently insufficient evidence on its significance for short- and long-term outcomes.

Nonetheless, the frequency of sequelae in treated patients appears to be lower than in untreated patients, and in the published literature there is no report of a TOP for CMV related anomalies occurring in a patient who had a negative amniocentesis. The significance of imaging anomalies described antenatally, and their correlation with functional anomalies later on, in this group is not described. So, the risk of false-positive findings and the potential for uncertain counselling must be acknowledged.

One group member reported the recent case of one treated child with a negative amniocentesis at 18 weeks and cCMV at birth, that needed treatment with valganciclovir at birth because of brain imaging anomalies discovered postnatally, in spite of a negative antenatal MRI (data not published).

5.5.2 *Quality of evidence*

Overall, the evidence base is heterogeneous and limited by study design (all but one study is retrospective) and heterogeneity of outcome definition and collection.

In most retrospective studies it was never investigated whether the indication for amniocentesis had a bearing on the risk profile of the patients, and if it differed between those with positive and negative results (with maternal or foetal symptoms potentially more frequent among positives).

Methods of follow-up are sometimes poorly described and, in most cases, limited to outcomes at birth. Asymmetrical outcome ascertainment between negative and positive amniocentesis could not be excluded in most cases. Only three studies have some clinical information on children with negative cCMV at birth, to allow the calculation of accurate prognostic values.

Definition of outcomes is variable between studies. Loss to follow-up is not clearly addressed in most studies. Additionally, in the only non-retrospective study, the results of amniocentesis were imputed, in patients who refused amniocentesis, from the results at birth.

Overall, the number of patients included is low, especially in the group with CMV at birth and negative amniocentesis.

5.5.3 *Patient preferences*

After the introduction of treatment, there is a concern on behalf of the patients regarding the value of performing amniocentesis at all.

Amniocentesis carries a small risk of miscarriage (estimated at 0.3%)(55). Given the reduced sensitivity for CMV detection at the time of the procedure, the uncertainty around the risk profile when a negative amniocentesis is followed by cCMV detected at birth, the increasing number of such discordant cases, and the fact that no routine intervention is currently offered after a positive amniocentesis, some patients may choose to decline the procedure altogether. More intensive follow up after a negative amniocentesis may be chosen by certain parents that value avoiding at all costs

having an affected child. Not performing the appropriate imaging follow up may deprive parents of the only choice they have available, notably TOP.

On the other hand, a more intensive follow-up may be considered an unnecessary burden by other patients, knowing that the risk of sequelae in case of a negative amniocentesis is low, and that imaging anomalies may turn out to be false positives.

Our patient representative pointed out that if imaging is performed in a peripheral centre, after a negative amniocentesis, patients may be deprived of fast access to appropriate counselling, further imaging and TOP, should imaging show alarm signs, or there may not be sufficient expertise to detect such alarm signs.

5.5.4 Cost and resource allocation

There is no evidence available on cost-effectiveness of management including enhanced imaging (regular ultrasound, MRI) compared with no enhanced imaging (only routine imaging) in patients with negative amniocentesis.

Antenatal imaging in case of CMV in pregnancy is reimbursed for patients.

Unnecessary extra imaging in pregnant patients with a negative amniocentesis, may lead to extra cost and resource use, and extra potentially unnecessary interventions especially considering that the prevalence of critical adverse outcomes may be low. A guideline development group member pointed out that organising a foetal MRI for all patients who seroconvert, regardless of amniocentesis results, may prove organisationally challenging.

6 IN PREGNANT PATIENTS WITH A CONFIRMED PRIMARY CMV INFECTION, SHOULD AMNIOCENTESIS BE PERFORMED AT 17 WEEKS OF GESTATION?

6.1 Recommendations

	Strength of recommendation	Level of evidence
Recommendations for pregnant patients with a primary CMV infection not treated with valaciclovir		
Practitioners should not perform amniocentesis before 18 weeks of gestation	Strong	Very low
Practitioners should not perform amniocentesis before 8 weeks after the timing of seroconversion	Strong	Very low
Recommendations for pregnant patients with a primary CMV infection treated with valaciclovir		
In the context of research, high quality evidence should be gathered on the diagnostic and prognostic value of amniocentesis performed before 21 weeks. They should also gather evidence on the efficacy of shortening treatment duration until the timing of earlier amniocentesis.	Research recommendation	



CLINICAL PRACTICE GUIDANCE

Outside the context of research, The Guideline Development Group suggests:

In treated patients

To offer amniocentesis at least at 21 weeks of gestation and at least 7 weeks after seroconversion.

6.2 Introduction

In the published literature, amniocentesis for suspected foetal infection following primary maternal CMV seroconversion has most commonly been performed around 21 weeks of gestation(56).

If cCMV is detected at birth despite a negative amniocentesis, the risk of adverse clinical outcomes may be considered low in untreated patients. However, limited data is available to establish this risk in patients treated with valaciclovir (see 5.1)

In the event of a positive amniocentesis, pregnant patients have closer follow-up, including serial targeted prenatal ultrasound and foetal MRI around 32 weeks(14).

Recently, consensus guidelines have recommended earlier timing (from 17 weeks of gestation and 8 weeks after seroconversion) for amniocentesis to allow earlier reassurance, on the rationale that accuracy of amniocentesis to detect cCMV at birth starting from 17 weeks is comparable to accuracy after 21 weeks when a sufficient interval has elapsed since seroconversion(57).

6.3 Research questions and search

A search was made for the following research question:

“In pregnant patients with primary CMV infection, should amniocentesis be performed at $\geq 17+0$ weeks and ≥ 8 weeks after seroconversion, compared to later timing, for detecting cCMV at birth?”

The focus was on accuracy outcomes.

Only studies directly comparing amniocentesis performed in the 17th week of gestation with procedures undertaken at later gestational ages were included.

The PIRTS developed for the search and all details related to the analysis of the evidence can be found in [Appendix 10](#).

6.3.1 Study identification and selection

Two publications were identified.

The evidence for this chapter draws on two linked publications by Enders et al. Enders 2017a(58) is a retrospective cohort study reporting the largest sample, but it is presented as a research letter with limited methodological detail. Enders 2017b(59) analyses a subset of the same population with a different analytical focus and provides a more complete description of methods. For consistency and interpretability across both reports, we assume that the methodology described in Enders 2017b also applies to Enders 2017a.

6.4 Evidence

6.4.1 Included studies and methodological quality

Enders 2017b(59) was a retrospective, single-centre diagnostic accuracy cohort from Germany (2010–2014) including pregnancies with primary CMV infection that underwent invasive prenatal diagnosis. The sample size was 132 (all patients undergoing prenatal diagnosis for primary CMV infection), of which 111 were analysed, since they had known virological outcome at birth. The key

analytic factor was the interval from estimated maternal seroconversion to amniocentesis (≤ 8 weeks vs > 8 weeks), with gestational-age strata of $\geq 17+0$ and $\geq 20+0$ weeks. Methodological limitations included the retrospective, single-centre design and the absence of systematic collection of outcomes beyond accuracy statistics. Also, the reason for performing amniocentesis (screening results vs warning signs at imaging) was not described in the cohort, although we know that abnormal ultrasound findings were observed in 22% of patients at initial invasive prenatal diagnosis.

Enders 2017a(58) is a retrospective, single-centre cohort (research letter) from a German centre (2001–2016) expanding on the analysis of Enders 2017b. The cohort comprised 338 pregnant patients with known virological outcomes who underwent amniocentesis after primary CMV infection. Analysis was limited to the 264 patients who had amniocentesis more than eight weeks after seroconversion. These were drawn from a population of 2,706 patients who underwent amniocentesis overall, including 531 with known primary infection. The key analytic factor was the comparison of amniocentesis performed between 17 and 20+6 weeks or between 21 and 22+6 weeks. Methodological limitations were its single-centre, retrospective design and the fact that the reason for performing amniocentesis (screening results vs warning signs at imaging) was not described in the cohort. Bias may also arise from systematic differences between patients with virological outcome data at birth and those without.

6.4.2 Results

The main results of our analysis can be found in the table below. Since specificity (and consequently positive predictive value) for amniocentesis to detect cCMV at birth is 100%, we report only sensitivity and negative predictive value. The prevalences are those reported in the studies.

Table 13 – Summary of findings table for accuracy statistics for timing of amniocentesis in patients not treated with valaciclovir

Test	No. of studies ^a (No. of patients)	Prevalence %	Sensitivity (95% CI)*	Negative predictive value (95% CI)*	GRADE LoE
<i>Amniocentesis at $\geq$ to 17 GW and $\leq$ 8 weeks after seroconversion</i>	1(25)	39.6	45.45 (16.75 to 76.62)	73.66 (61.98 to 82.75)	Very Low
<i>Amniocentesis at $\geq$ to 17 GW and $>$ 8 weeks after seroconversion</i>	1(52)	39.6	90.91 (70.84 to 98.88)	94.38% (81.74 to 98.43)	Very low
<i>Amniocentesis at 17 weeks and $>$ 8 weeks after seroconversion</i>	1(18)	32	50 (6.76 to 93.24)	80.95 (61.47 to 91.89)	Very low
<i>Amniocentesis at 18 weeks $>$ 8 weeks after seroconversion</i>	1(27)	32	85.71 (42.13 to 99.64)	93.7 (70.79 to 98.92)	Very low
<i>Amniocentesis at 19 weeks $>$ 8 weeks after seroconversion</i>	1(24)	32	100 (63.06 to 100)	100 (79.41 to 100)	Very low
<i>Amniocentesis at 20 weeks $>$ 8 weeks after seroconversion</i>	1(66)	32	89.29 (71.77 to 97.73)	95.2 (87.19 to 98.3)	Very low



Amniocentesis at 21 weeks > 8 weeks after seroconversion	1(93)	32	92 (73.97 to 99.02)	96.37 (87.55 to 99.01)	Very Low
Amniocentesis at 22 weeks > 8 weeks after seroconversion	1(36)	32	92.31 (63.97 to 99.81)	96.51 (80.78 to 99.45)	Very low

According to the results of Enders 2017b, sensitivity and negative predictive value appear lower when amniocentesis is performed within eight weeks of estimated maternal seroconversion compared to after 8 weeks of seroconversion. This analysis is not replicated in the expanded cohort (Enders 2017a).

Although Enders 2017a reports no statistically significant difference between amniocentesis performed between 17 weeks and 20 weeks and 6 days of gestation, and between 21 weeks and 22 weeks and 6 days of gestation, our post-hoc week-by-week stratification shows that only 18 procedures were performed in the 17th week. In this stratum the sensitivity point estimate is 50% (95% CI 6.76–93.24). From 18 weeks onwards, sensitivity point estimates appear to be consistently higher, although confidence intervals remain wide.

In addition, there is no available evidence comparing the diagnostic accuracy of amniocentesis across different gestational weeks in patients treated with valaciclovir. In this population, the reported pooled sensitivity for amniocentesis performed at 20–21 weeks and 7–8 weeks after estimated maternal seroconversion is of 55% (95% CI 40–68%) (see 5.4.2.2).

In our systematic review, we did not find any study comparing amniocentesis performed at 17 weeks to amniocentesis performed at a later date in patients treated with valaciclovir.

CONCLUSIONS

In patients not treated with valaciclovir

- The sensitivity and negative predictive value of amniocentesis performed within 8 weeks after seroconversion may be lower than the sensitivity and negative predictive value performed more than 8 weeks after seroconversion, but the evidence remains very uncertain (very low level of evidence).
- The sensitivity and negative predictive value of amniocentesis performed between 17 and 17 weeks and 6 days of gestation may be lower than amniocentesis done at or more than 18 weeks, but the evidence remains very uncertain (very low level of evidence).

In patients treated with valaciclovir

- There are no data comparing the sensitivity or negative predictive value of amniocentesis performed at 17 weeks and 8 weeks after seroconversion with that of amniocentesis performed later in pregnancy.

6.5 Evidence to recommendations

6.5.1 Balance between clinical benefits and harms

In untreated patients, performing amniocentesis earlier than 21 weeks (while maintaining an interval of at least eight weeks between seroconversion and the procedure) may offer earlier reassurance about the low likelihood of major sequelae.

However, studies assessing the prognosis of a negative amniocentesis with positive cCMV at birth, have evaluated procedures conducted at 20–21 weeks and 7–8 weeks after seroconversion (10,39,49–51).

Undertaking the procedure earlier, when sensitivity is markedly lower than the one we described in 5.4.2.2 (91%, with a 95% CI of 84% to 95%), may deprive patients of the opportunity for more intensive follow-up and timely counselling. This applies particularly to procedures carried out in the 17th week, where the only available sensitivity point estimate is 50%.

In treated patients, prognostic accuracy statistics for negative amniocentesis could be calculated post hoc for amniocentesis performed either at least 21 weeks and 7 weeks after estimated time of seroconversion (RCT)(10) or at least 20 weeks and 8 weeks after seroconversion (cohort)(39) (see 5.4.2.2)

Additionally, in treated patients, earlier amniocentesis may be linked to a shorter duration of treatment with valaciclovir because clinicians report discontinuing treatment thereafter (see [Appendix 1](#)).

The clinical benefits of shortening treatment length are not described in the published literature. In studies reporting critical outcomes, treatment is mostly stopped either at least 21 weeks and 7 weeks after estimated time of seroconversion (RCT)(10) or at least 20 weeks and 8 weeks after seroconversion (cohort)(39), especially when amniocentesis was negative (see 4.4.1.1).

Therefore, the prognostic value of an earlier negative amniocentesis, alongside a reduced treatment duration, compared to a later negative amniocentesis seems, at the moment of our literature review, unknown.

6.5.2 *Quality of evidence*

The quality of the evidence is very low, driven by the high risk of bias in the included studies (which can be considered, in effect, a single study) and by the small sample sizes across the different strata. Notably, only 18 amniocentesis procedures were performed at 17 weeks of gestation.

We cannot draw any conclusion for treated patients from this body of evidence.

6.5.3 *Patient preferences*

As discussed previously (see 5.5.3) after the introduction of treatment, there is a concern on behalf of the patients regarding the value of performing amniocentesis at all, given its small associated risk of miscarriage(55).

When amniocentesis is performed, some patients may prioritise more robust results obtained later over less robust results obtained earlier, while others, anxious after CMV seroconversion, may seek reassurance as soon as possible.

Shortening the duration of treatment by up to four weeks may, on the other hand, be less acceptable once the associated uncertainty is explained. Although shorter antiviral courses may reduce out-of-pocket costs and side effects, most expectant mothers are likely to prioritise the baby's outcome over other considerations.

6.5.4 *Cost and resource allocation*

The direct costs of amniocentesis do not vary with its timing.

Conversely, interrupting treatment too early may reduce immediate costs but, if efficacy is hindered, could increase the proportion of infants requiring postnatal valganciclovir, potentially offsetting savings.



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