

SUPPLEMENT**PRIORITY TOPICS IN THE DIAGNOSIS AND
MANAGEMENT OF CMV IN PREGNANCY****A TARGETED GUIDELINE**

ELENA COSTA, CÉLIA PRIMUS-DE JONG, LEEN VERLEYE, RENATE ZEEVAERT

Title:	Priority topics in the diagnosis and management of CMV in pregnancy – Supplement
Authors:	Elena Costa (KCE), Célia Primus-de Jong (KCE), Leen Verleye (KCE), Renate Zeevaert (KCE)
Project Manager:	Els Van Bruystegem (KCE)
Health Information Research Specialist:	Nicolas Fairon (KCE)
Guideline Development Group members:	Michael Aertsen (Universitair Ziekenhuis Leuven (UZ Leuven)), Mieke Cannie (Centre hospitalier universitaire Brugmann (CHU Brugmann)), (Universitair Ziekenhuis Brussel (UZ Brussel)), Michael Ceulemans (BELgian pregnancy REGistry (BELpREG)), Laura Chielens (Care4CMV), Caroline De Conninck (Hôpital Universitaire de Bruxelles, (H.U.B. Erasme)), Deborah De Geyter (UZ Brussel), Marie-Luce Delforge (Centre National de Référence des infections congénitales (CNR), (H.U.B. Erasme)), Ina Foulon (UZ Brussel), Annelies Keymeulen (UZ Gent), Clotilde Lamy (H.U.B. Erasme), Sophie Ripault (Citizen representative), Ellen Roets (UZ Gent), Oriane Stévar (CHU Saint-Pierre), Inge Tency (Odisee Hogeschool), Christine Van Linthout (Centre hospitalier universitaire de Liège (CHU de Liège)), Lennart Van der Veecken (Universitair Ziekenhuis Antwerpen (UZA), UZ Leuven))
Stakeholders:	Caroline Blain (Union Professionnelle des Sages-femmes Belges (UPSfB)), Gilles Ceysens (Collège Royal des Gynécologues Obstétriciens de Langue Française de Belgique (CRGOLFB)), Joël Daems (Institut national d'assurance maladie-invalidité – Rijksinstituut voor ziekte- en invaliditeitsverzekering (INAMI – RIZIV)), Els De Leenheer (Belgian Respiratory Society (BeRS)), Bart Demyttenaere (Union Nationale des Mutualités Socialistes (UNMS)), Jana Dierckens (Care4CMV), Catherine Donner (Association Belge Francophone d'Ultrasonologie Médicale (ABeFUM)), Benedicte Doods (Domus Medica), Marthe Knops (Société Scientifique de Médecine Générale (SSMG)), Hilde Logghe (Vlaamse Vereniging voor Obstetrie en Gynaecologie (VVOG)), Sabine Mortelmans (Vlaamse Beroepsorganisatie van Vroedvrouwen (VBOV)), Gunnar Naulaers (College van geneesheren voor de moeder en de pasgeborene – Collège de médecins pour la mère et le nouveau-né), Sien Ombelet (Belgische Vereniging voor Infectiologie en Klinische Microbiologie (BVIKM)), Chris Van haecht (Christelijke Mutualiteit (CM)), Astrid Van Hoyweghen (Belgian Society of Radiology (BSR)), Koen Vanden Driessche (Belgische Vereniging voor Kindergeneeskunde (BVK))
External validators:	Lisa Hui (The University of Melbourne, Australia), Frédéric Chantraine (CHU de Liège), Nele Brusselaers (Universiteit Antwerpen (UAntwerpen))
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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.

Membership of a stakeholder group on which the results of this report could have an impact:

Laura Chielens (Parent's Association Care4CMV), Deborah De Geyter (Clinical Biology Committee), Jana Dierckens (Parent's Association Care4CMV), Sabine Mortelmans (VBOV), Koen Vanden Driessche (Secretary of the Belgische Beroepsvereniging van Artsen-Specialisten in de Klinische infectiologie), Astrid Van Hoyweghen (Belgian Society of Radiology), Lennart Van der Veecken (TreatCMV study)

Participation in scientific or experimental research as an initiator, principal investigator or researcher:

Michael Aertsen (Researcher within the CMV Treat study in Flanders), Caroline De Coninck (Prenatal evaluation of the prognosis of congenital CMV infection: retrospective (cordocentesis, MRI) and prospective studies (gB antibodies)), Deborah De Geyter (Research into anti-Entamoeba histolytica antibodies in the MSM population. Research into the evolution of multi-residual bacteria in the intensive care unit post SARS-CoV-2), Marie-Luce Delforge (Ongoing research and supervision of a PhD thesis: Evaluation of prenatal prognostic factors for congenital cytomegalovirus infection), Ina Foulon (Several studies into the incidence of hearing loss in children with congenital CMV infection. The effects of cCMV on the development of children), Annelies Keymeulen (Principal investigator of the Flemish CMV registry), Ellen Roets (Fonds Wetenschappelijk Onderzoek Flanders - Applied Biomedical Research (FWO-TMB), co-investigator TreatCMV), Koen Vanden Driessche (We conducted and published a study showing that very few neonates in Flanders are tested for congenital CMV), Lennart Van der Veecken (Treat CMV study), Christine Van Linthout (Supervision of an assistant who retrospectively reviews all files of patients who received valacyclovir after seroconversion in the first trimester at the CHU de Liège)

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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

1 CURRENT PRACTICES IN CMV TESTING AND MANAGEMENT

We collected current practices by means of an online survey directed to midwives, general practitioners and gynaecologists who are involved in pregnancy follow-up and/or are specialists in foetal medicine. We report in this chapter the habits and opinions of the healthcare practitioners who participated in the survey.

1.1 Methods

We conducted 3 of explorative interviews with healthcare practitioners familiar with CMV management. From those interviews we designed an online survey addressed to gynaecologists, midwives and general practitioners, involved in pregnancy follow-up or in the specialist management of patients with a CMV infection during pregnancy. The survey was divided into two parts: in the first part we explored opinion and practices of first line healthcare practitioners on CMV counselling, screening and management (midwives, gynaecologists and general practitioners), in the second part we explored opinion and habits of specialists in foetal medicine on CMV counselling and management. If a gynaecologist involved in pregnancy follow-up was also a foetal medicine specialist, they answered both parts.

To reach a large number of healthcare professionals, we distributed the survey via social media posts and contacted professional organisations of gynaecologists, midwives and general practitioners as well as key stakeholders to disseminate it to their network. The survey was open from 19 September 2024 until 20 November 2024.

The quantitative analysis was done on complete questionnaires only. It was limited to descriptive statistics, such as frequency tables of the multiple-choice variables. The number of respondents varied between questions because some questions were addressed only to a subset of survey participants or were not compulsory. In addition, for some questions more than one answer could be selected.

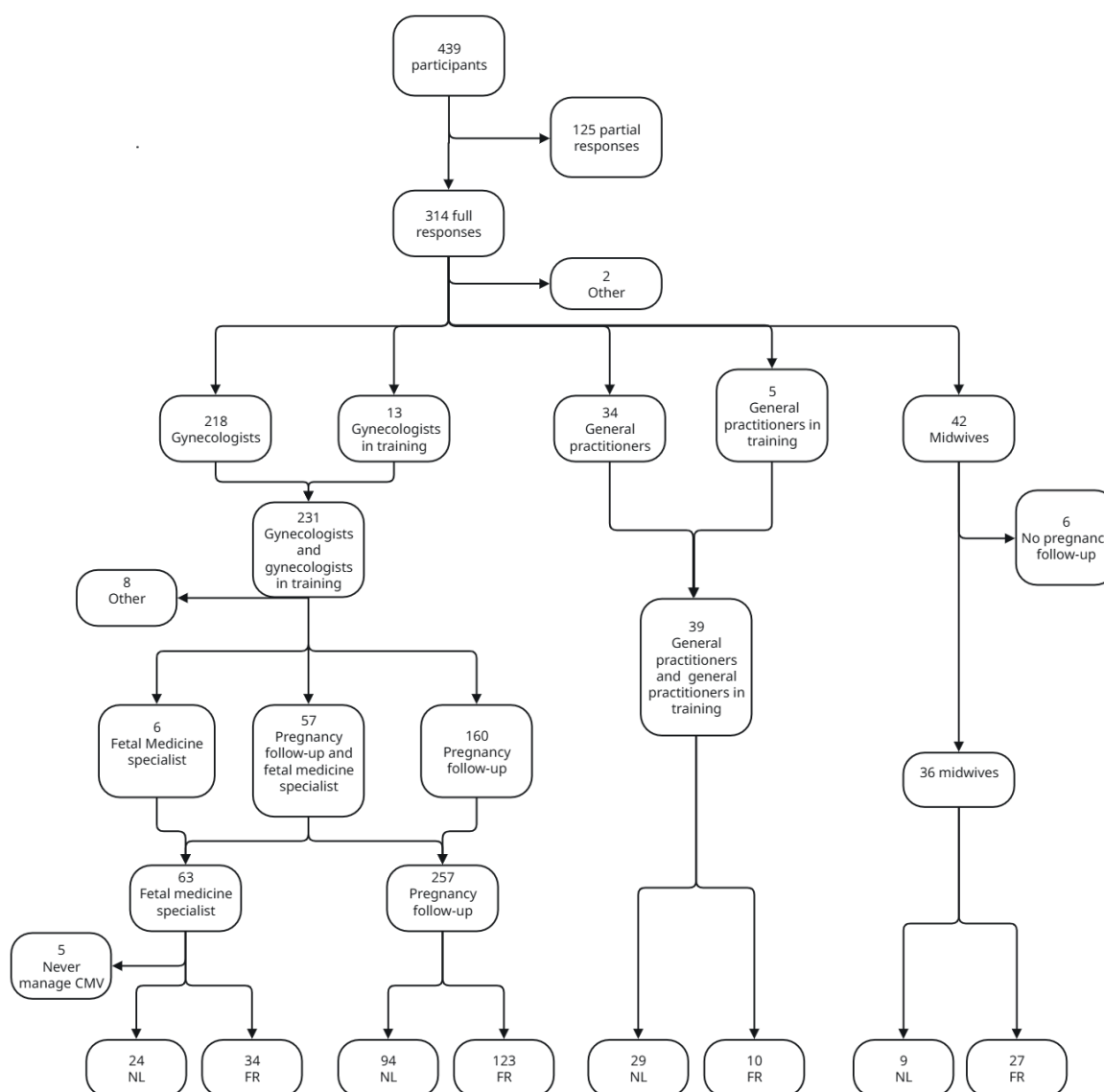
The entries in reply to the open questions of the survey, as well as any comments made by participants in the dedicated comment spaces, were analysed by means of thematic analysis. This consists of tagging text fragments with similar content with the same thematic label. In a next step, these themes are clustered into larger categories. The coding was carried out by one researcher, hence no investigator triangulation has been done.

1.2 Results

439 people responded to the survey, of which 314 completed it. Of these participants 16 were not part of our target population (gynaecologists, midwives or general practitioners involved in pregnancy follow-up or in the specialist management of CMV infection in pregnancy) and were excluded from analysis. A flow chart of the inclusion of survey participants can be found in Figure 1.



Figure 1 – Flowchart of survey participants



1.2.1 Participants characteristics

We analysed the results of 298 complete survey responses. Six participants were fetal medicine specialists not involved in the medical follow-up of pregnancies, 57 were both fetal medicine specialists and involved in the medical follow-up of pregnancies, 160 were gynaecologists involved in the medical follow-up of pregnancies, 39 were general practitioners and 36 were midwives. The full description of participant characteristics can be found in Table 1.

Overall, among our participants, a little over half ($n=164$; 55.03%) were French speaking and the rest were Dutch speaking. This proportion was inverted for general practitioners, of which there were significantly less French speaking ($n=10$; 25.64%) than Dutch speaking. Participants were homogeneously distributed among general hospitals, university hospitals and private practice. The biggest age group that was represented among our participants was the age bracket between 30 and 45. Most midwives practiced in the Brussels region most general practitioners practiced in the Flanders region (Table 1).



Table 1 – Survey participants' characteristics

		Fetal Medicine Specialist only (n=6)	Gynecologist (n=57)	Gynecologist and Fetal Medicine Specialist (n=160)	General Practitioner (n=39)	Midwife (n=36)	Total (n=298)
Language of Origin	French (FR)	4 (66.67%)	88 (55.00%)	35 (61.40%)	10 (25.64%)	27 (75.00%)	164 (55.03%)
	Dutch (NL)	2 (33.33%)	72 (45.00%)	22 (38.60%)	29 (74.36%)	9 (25.00%)	134 (44.97%)
Institution Type	General Hospital	1 (16.67%)	88 (55.00%)	17 (29.82%)	1 (2.56%)	5 (13.89%)	112 (37.58%)
	General Hospital with university beds	1 (16.67%)	11 (6.88%)	15 (26.32%)	0 (0.00%)	0 (0.00%)	27 (9.06%)
	Univ. Hospital	4 (66.67%)	27 (16.88%)	23 (40.35%)	0 (0.00%)	18 (50.00%)	72 (24.16%)
	Private Practice	0 (0.00%)	33 (20.63%)	1 (1.75%)	32 (82.05%)	9 (25.00%)	75 (25.17%)
	Other	0 (0.00%)	1 (0.63%)	1 (1.75%)	6 (15.38%)	4 (11.11%)	12 (4.03%)
Age Group	<30	0 (0.00%)	8 (5.00%)	1 (1.75%)	8 (20.51%)	2 (5.56%)	19 (6.38%)
	30-45	1 (16.67%)	84 (52.50%)	29 (50.88%)	23 (58.97%)	25 (69.44%)	162 (54.36%)
	46-65	4 (66.67%)	62 (38.75%)	24 (42.11%)	7 (17.95%)	9 (25.00%)	106 (35.57%)
	>65	1 (16.67%)	6 (3.75%)	3 (5.26%)	1 (2.56%)	0 (0.00%)	11 (3.69%)
Region of Practice	Brussels	3 (50.00%)	44 (27.50%)	14 (24.56%)	5 (12.82%)	27 (75.00%)	93 (31.21%)
	Flanders	2 (33.33%)	71 (44.38%)	23 (40.35%)	30 (76.92%)	5 (13.89%)	131 (43.96%)
	Wallonia	1 (16.67%)	45 (28.13%)	20 (35.09%)	4 (10.26%)	4 (11.11%)	74 (24.83%)



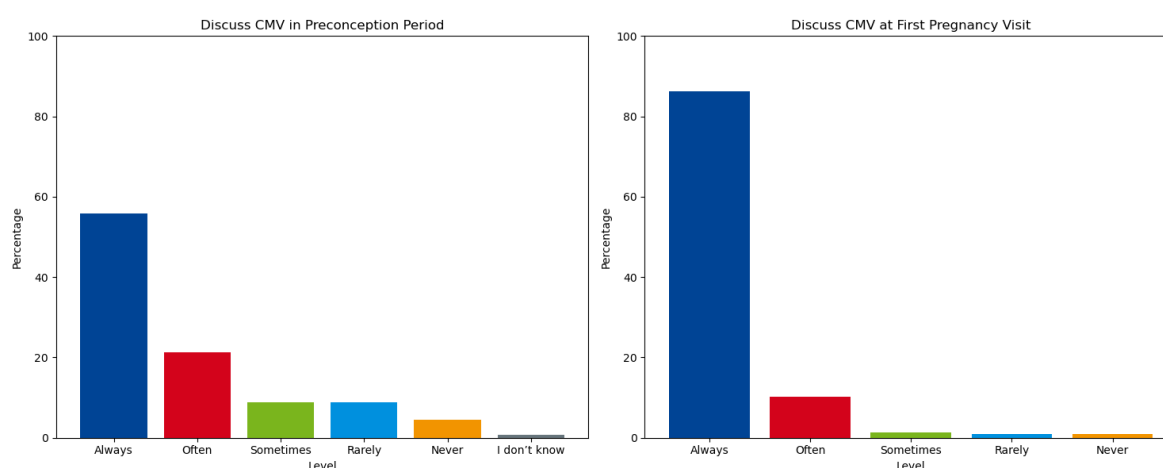
1.2.2 Attitudes and habits regarding antenatal CMV from practitioners involved in pregnancy follow-up

Discussion of CMV in pregnancy

Most respondents to our survey (n=163; 55.82%) stated that they always discuss CMV with their patients in the preconceptional period (see Figure 2). The lowest rate was among midwives with only 33.33% (n=12) always discussing CMV in the preconceptional period. Midwives pointed out that, although this is part of their competences, there is no reimbursement for preconceptional visits done by midwives, therefore they don't often patients in that period.

Most of our respondents reported they discuss CMV during the 1st pregnancy visit (n=252; 86.30%) (see Figure 2), with gynaecologists who are also fetal medicine specialists having the highest rate (n= 55; 96.49%) and general practitioners the lowest rate (n= 30; 76.92%).

Figure 2 – How often practitioners discuss CMV preconceptionally and at the first antenatal visit



When health professionals talk about CMV in the preconception period, they mostly talk about how the infection is transmitted and/or how to prevent infection. Preventive measures that were mentioned included hygienic measures, including regular hand washing and avoiding contact with (secretions of) (young) children. Sometimes additional information was provided on when preventive measures need to be followed (e.g. before conception or from confirmed pregnancy), and/or who needs to follow them (e.g. whether or not immunised, or including the partner). They also talk about screening for CMV and the potential risks of a CMV infection for the foetus or child. More general information about CMV (for example it being a virus or presenting symptoms is also sometimes mentioned. Professionals rarely reported talking also about possible reinfection, the non-existence of a vaccine, follow-up after infection, and the (non) existence of treatment in the preconceptional period. Some remarked they use supporting material like a website or a folder to explain what CMV is.

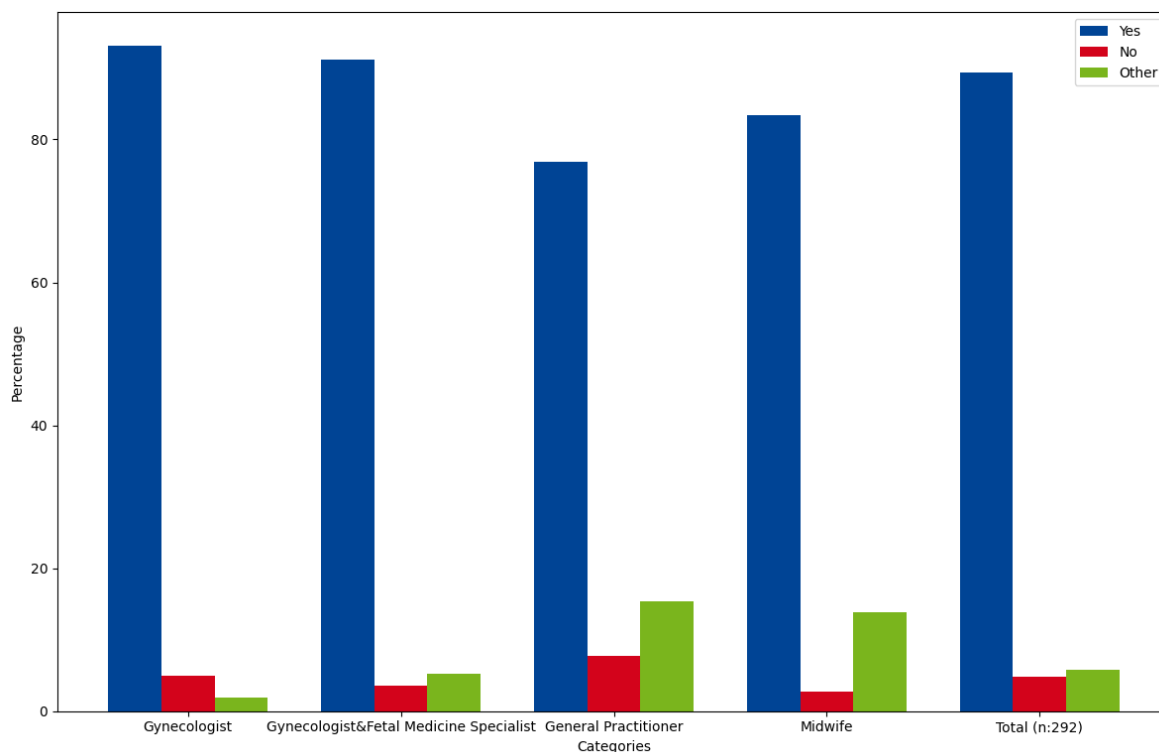
The themes discussed during the first visit during pregnancy were mostly the same as during preconception. No additional themes were added. Non-existence of a vaccine and follow-up after infection were not mentioned, but each of these themes was only mentioned once in the preconception period.

Screening

Of our participants 261 (89.38%) prescribe CMV screening in the first trimester, the highest percentage among gynecologists (93.13%), and lowest among general practitioners (76.92%) see Figure 3. The 17 participants (5.82%) that choose to respond "Other", mostly stated that the choice to screen was

not systematic, but done in concertation with the couple according to their wishes after counselling, and also that they do not prescribe it to patients who already have a known positive IgG serology.

Figure 3 – Prescription of CMV serologic screening in the first trimester of pregnancy, according to survey participants



Most practitioners reported that they test for CMV two times during the first trimester ($n=127$; 46.68%), but a large proportion only tests once ($n=124$; 44.6%), and 13 participants (4.68%) reported that they screen more than twice during the first trimester. The professional category most likely to test only once during the first trimester is general practitioners ($n=31$; 86.11%). The professional category most likely to test twice in the first trimester is gynecologists who are also fetal medicine specialists ($n=40$; 72.73%). Among the practitioners who responded “other” to the question how often they prescribe screening in the first trimester ($n=14$; 5.04%), some stated that they do it once except when patients opted for screening after counselling, in which case it is done twice. Some specified also that it is twice if the first serology was negative. Some stated that they do a second serology around 12 weeks so that the person can be enrolled in the TreatCMV study in case of seroconversion. And finally, some reported they do the last serology around 14-16 weeks, which can be the second or the third.

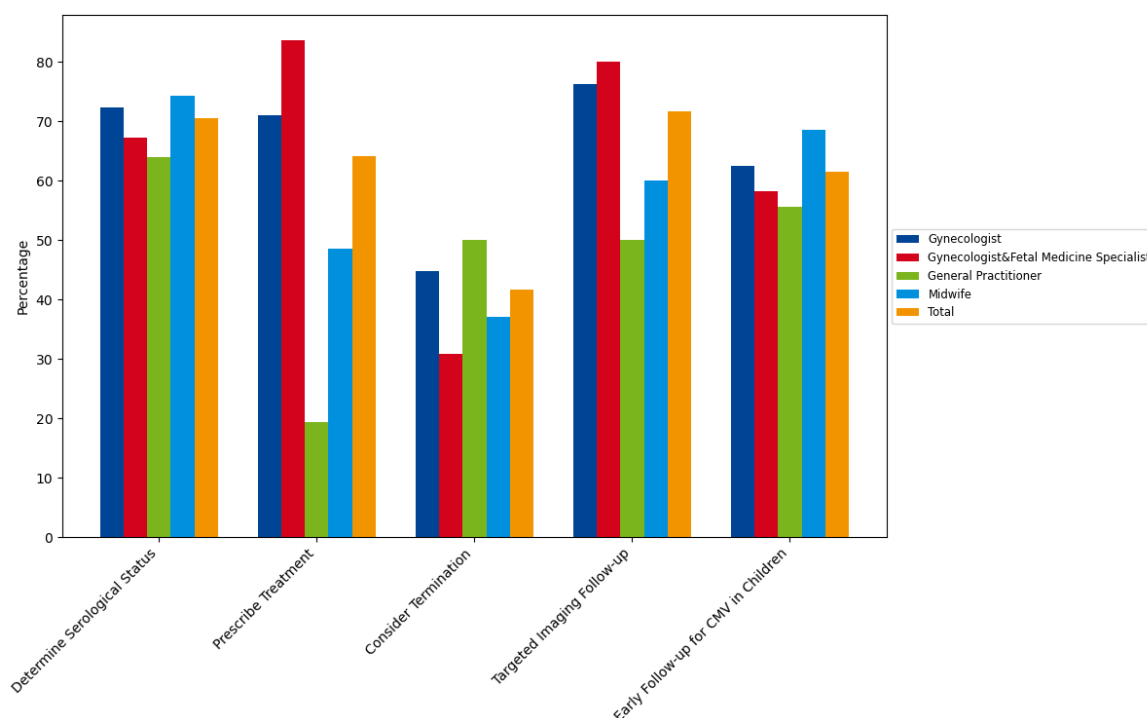
Regarding how CMV serology is billed during pregnancy, the vast majority ($n=223$; 80.22%) state that they bill the test as “screening during pregnancy” therefore not reimbursed. A few professionals stated that they believe that CMV serology is reimbursed once during pregnancy, and some twice. One professional remarked that the threshold of “symptoms” in pregnancy is quite low in order to justify a serology reimbursement. A few professionals stated that it is not acceptable that CMV serology is not reimbursed during pregnancy because they still have the obligation to follow protocols within institutions that may recommend screening, because there is no real choice for those who cannot afford it and because outside of pregnancy it is reimbursed.

The majority of practitioners do not propose CMV serology testing after the first trimester of pregnancy ($n=145$; 49.66%), with general practitioners having the highest rate of not proposing CMV serology after the first trimester ($n=27$; 69.23%), and gynaecologists who were also fetal medicine specialists having the lowest rate ($n=24$; 42.11%).

Professionals who test after the first trimester, most often reported testing once in the second trimester and less often once in each trimester or once in the third trimester. The end of second trimester was a popular timing for CMV testing (for example conveniently combined with an oral glucose tolerance test (OGTT)), whereas the beginning of the second trimester, mid pregnancy and the end of third trimester were less frequently mentioned.

The most cited objectives for CMV-screening during pregnancy was (see Figure 4) “to perform targeted imaging follow-up in case of seroconversion” (n=199; 71.58%), followed by “to determine serological status and target hygiene measures”(n=196; 70.5%), “to prescribe treatment for the prevention of vertical transmission” (n=178;64.03%), “to enable early follow up for congenital CMV in children” (n=171; 61.51%), and “to consider termination in case of seroconversion” (n=116; 41.73%). The aim with the highest discrepancy among practitioners was “to prescribe treatment for the prevention of vertical transmission” which was cited by 83.64% of gynaecologists who are also fetal medicine specialists and only 19.44% of general practitioners. A few of the gynaecologists, general practitioners and midwives who stated that their aim was “To determine serological status and target hygiene measures”, highlighted that they give hygiene measures regardless of serological status, and that they perform screening only if CMV status prior to pregnancy was unknown or negative. For those who stated that the aim was “To prescribe treatment for the prevention of vertical transmission”, some gynaecologists underscored that they enrolled patients in a clinical study currently ongoing in Belgium. As far as the aim “To consider termination in case of seroconversion”, practitioners highlighted the importance of the necessity of good clinical pathway to establish foetal risk and counselling. One GP stated that they did not test initially, but after concertation with local gynaecologists they were afraid of not testing for fear of litigation in case a couple had not had the possibility of TOP. When the aim is “To perform targeted imaging follow-up in case of seroconversion”, gynaecologists highlight the importance of good quality imaging in a foetal medicine centre.

Figure 4 – Frequency of the different aims, in screening CMV during pregnancy in the first trimester



As far as the screening acceptance is concerned, most participants stated that CMV screening in pregnancy is always accepted (n=200; 71.94%) or often accepted (n=71; 25.54%). The most cited reason for refusal of screening was the fact that the patient does not consider screening necessary (n=43; 55.13%).

Among the survey participants that stated that the screen for CMV beyond the first trimester the most cited objective was 'to perform targeted imaging follow-up in case of seroconversion' (n=116; 78.91%), followed by 'to enable early follow up for congenital CMV in children' (n=106; 72.11%), 'to prescribe treatment to prevent vertical transmission' (n=59; 40.14%) and finally 'to determine serological status and target hygiene measures' (n=46; 31.29%).

Most respondents reported always or often having time to discuss CMV during consultation (n= 173; 59.45%), but 35.05% (n=102) reported only rarely or sometimes having time and 4.12% (n=12) reported never having time. When asked about the frequency of prescription of CMV serology without the appropriate explanations, only 4.40% (n=15) said this happens often or always, compared to 57.91% rarely or sometimes and 35.25% (n=98) never.

Almost all professionals that stated they do not or rarely have sufficient time, they said nonetheless that they take the time even if it means delaying other consultations. This leads to more pressure and workload for the salaried professionals, and a loss of income for independent professionals. This view is shared by some of those who say they have sufficient time, as they say they "make" time through extra stress or loss of income. Reasons cited for not having enough time included language barriers, the number of questions from patients and the complexity of counselling. Quoted explanation for having sufficient time were spreading the counselling over several consultations or working in collaboration with another professional (e.g. midwife or gynaecologist) to share the counselling.

As far as prescribing CMV serology without explanation, practitioners indicated this happens because of language barriers. The fact that the test is not reimbursed by RIZIV/INAMI is a reason for some to be careful to give all appropriate explanations.

The content of counselling in case of a recent seroconversion, the answers differed between the type of health professional. The topics gynaecologists most frequently mentioned were risks of infection (vertical transmission and/or sequelae), followed by amniocentesis, treatment with valaciclovir, and imaging follow-up (ultrasound and/or MRI). The topics most frequently mentioned by midwives and general practitioners were referral, followed by risks of infection and imaging follow-up. In contrast, they rarely mentioned amniocentesis and sometimes valaciclovir. Termination of pregnancy is mentioned by some gynaecologists and general practitioners. Other themes occasionally mentioned were investigations or treatment in general, laboratory testing (repeat serology, avidity), and postnatal follow up,

The topics discussed in case of negative IgM and positive IgG were infection prevention and possible reinfection, followed by explanation of the blood result (being immunised, old/previous contact) and the risks. Preventive measures were generally considered necessary, regardless of whether the patient was considered being protected, partially protected or unprotected by the antibodies. Only few professionals stated no preventive measures were needed or measures could be less stringent. We are not sure what professionals mean if they tell their patients they are immunised. Do they mean they have had an old infection and/or they are protected against the virus? That is why we considered the topics separately. When IgG is positive and IgM negative, the risks of vertical transmission and sequelae were generally described as lower and some professionals even stated that there were no risks. If repeat serology was discussed with patients, they were usually told it is not necessary or useful. Only few professionals noted they would refer the patient, test the child after birth or check the avidity.

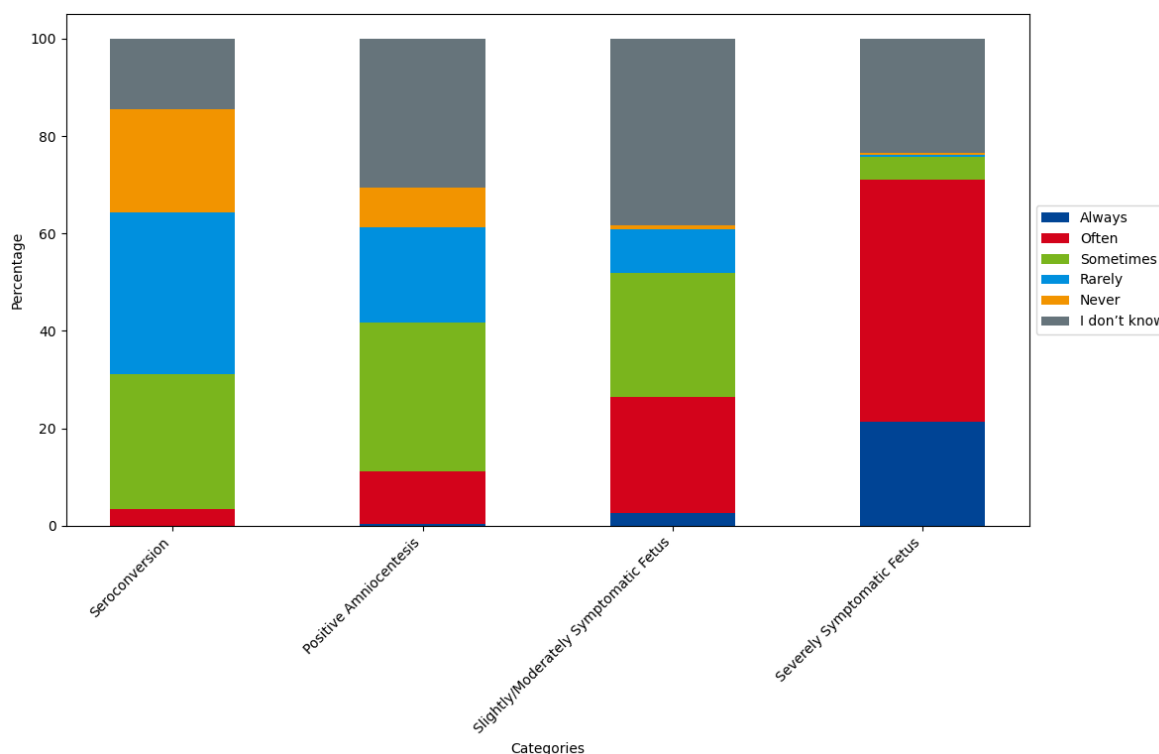
Termination of Pregnancy

Questions about termination of pregnancy in the survey were asked separately to foetal medicine specialists and to other practitioners. In this section we discuss the perception of gynaecologists who



are not also foetal medicine specialists, general practitioners and midwives. The perception of gynaecologists who are also foetal medicine specialists are reported in section 1.2.3.

Figure 5 – Perception of practitioners on the patients' uptake of Termination of Pregnancy at different stages after CMV seroconversion



Among our respondents, 21.28% (n=50) affirm that Termination of Pregnancy (TOP) for seroconversion alone is never chosen by parents (33% rarely, 27.7% sometimes, 3.4% often and 0% always) (see Figure 5). Professionals that provided comments on the question pertaining to TOP for seroconversion alone, stated that this is done mostly during the first trimester and that there is no active guiding towards it. TOP for seroconversion alone may be the result of bad counselling on behalf of a healthcare practitioner.

When amniocentesis is positive, 8.09% (n=19) of professionals affirm that TOP for positive amniocentesis alone is never chosen by patients (19.6% rarely, 30.6% sometimes, 10.6% often and 0.4% always, see Figure 5). Quite a few professional commented that TOP for positive amniocentesis is not allowed in Belgium since it won't be allowed by an ethical committee, and patients would have to go to the Netherlands to carry out the procedure. A few comments pointed out that TOP is not proposed by the professionals in this case and that they may be caused by suboptimal counselling by the healthcare practitioner.

In the case of mildly/moderately symptomatic fetuses, 0.85% (n=2) of professionals affirmed that TOP for mildly/moderately symptomatic fetuses is never chosen by patients (9% rarely, 25.5% sometimes, 24% often and 2.5% always). Occasional comments said that TOP for this indication is not allowed in Belgium, or they doubted whether it was allowed, and that TOP for this indication is not proposed. Gynaecologists point out that such conversations are held in tertiary centres and midwives say that they refer to gynaecologists.

Only one participant (GP) (0.4%) affirmed that parents never opt for TOP in case of a severely symptomatic foetus, and only 1 participant (gynaecologist) said they opt for TOP rarely (4.7% sometimes, 50% often, 21% always). Some respondents pointed out that religious beliefs were a reason for not choosing TOP.

An important proportion of the professionals did not know whether parents opt for TOP or not. The proportion was especially high for general practitioners, among which the proportion of “I don’t know” was consistently over 50%, for all TOP indications, and was highest among all respondents (38.3%) for mildly/moderately symptomatic fetuses.

Referral

Most professionals refer to a foetal medicine specialist when a seroconversion is discovered (183; 77.87%), with another 20%(47) prescribing valaciclovir prior to the referral.

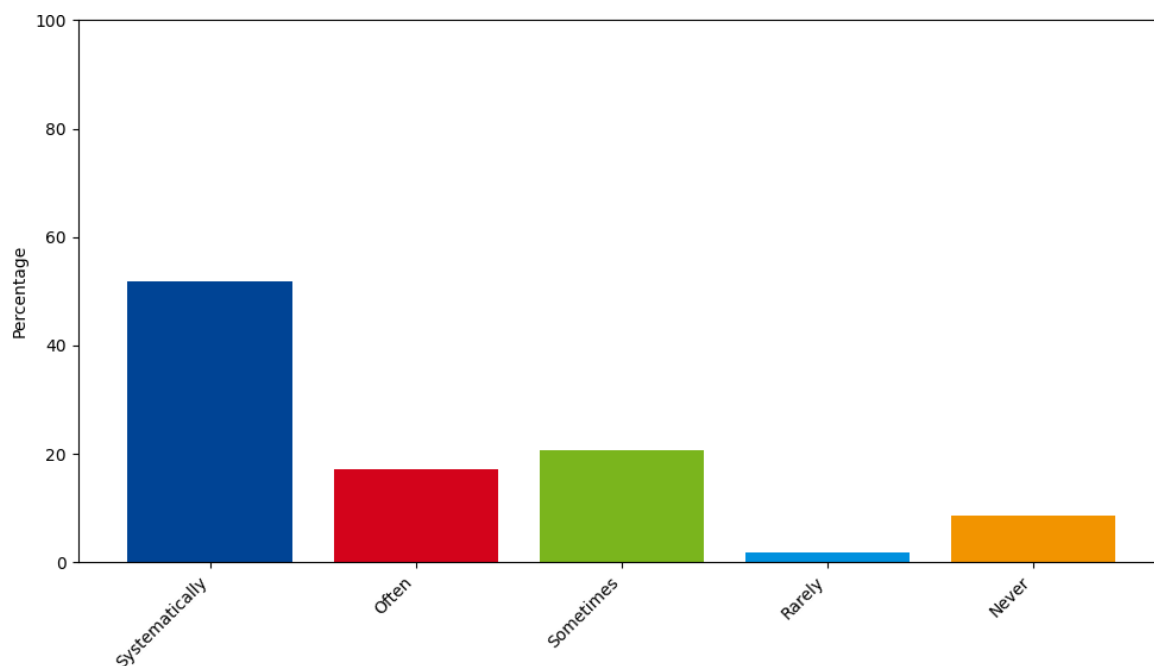
1.2.3 Attitudes and habits regarding antenatal CMV from fetal medicine specialists

Among our respondents, 63 were foetal medicine specialists. Of these, 5 (7.9%) stated that they never receive referrals for antenatal CMV and did not have to respond to further questions. Among the remaining 58 specialists, 36 (62.06%) get referrals for CMV frequently (11 systematically and 25 often) and 22 (37.94%) get referrals for CMV less frequently (17 sometimes and 5 rarely).

Treatment indications and duration

Among the fetal medicine specialists who receive referrals for antenatal CMV (n=58), 5 never propose valaciclovir for secondary prevention of CMV vertical transmission. The reason for not proposing valaciclovir treatment were that “valaciclovir is not reimbursed” (3/5), that “valaciclovir long-term consequences are not known” (3/5) and that “valaciclovir is not recommended” (1/5). There were 30 (51.72%) specialists who stated that they propose valaciclovir treatment systematically and 14 (38.93%), who propose it non-systematically (1 rarely, 12 sometimes and 10 often). A few practitioners who left further comments, indicated that the patient is informed of the existence of the treatment and that limitations and uncertainties are discussed, then the final choice is left to the patient. A relevant number of comments indicated that treatment is performed in the context of the TreatCMV study in Leuven, and some stated that treatment was not proposed before the beginning of this study. One participants pointed out that the high price of the treatment is a reason for a number of patients not to accept.

Figure 6 – Frequency of Valaciclovir proposal by Fetal Medicine Specialists in case of seroconversion



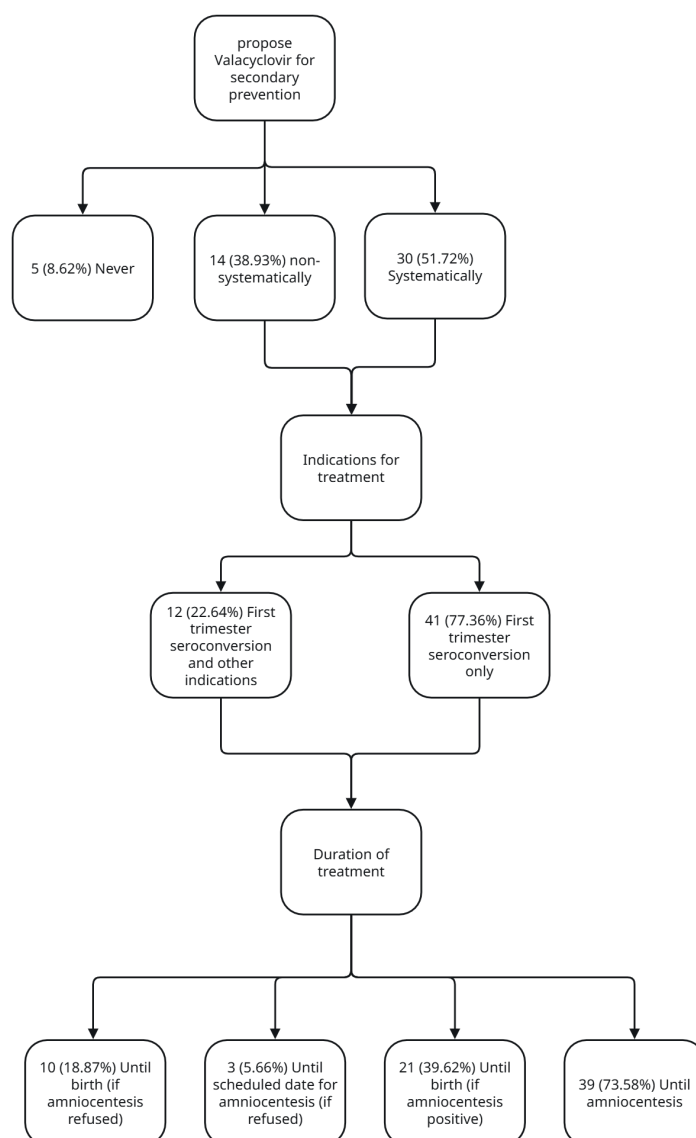


Among those who stated that they propose valaciclovir treatment, 41 (77.36%) only propose it if there was a first trimester seroconversion. In 12 cases (22.64%) practitioners stated that the indication for treatment was not limited to first trimester seroconversions. Other indications, as stated by the practitioners who chose to comment, included: periconceptional seroconversion, doubt on the timing of seroconversion, research programs and in one instance the comment was that it was prescribed for all seroconversions, regardless of timing.

The duration of treatment is 'until amniocentesis' for most foetal medicine specialists, but a significant proportion state that they prolong treatment 'until birth if amniocentesis is positive'. In case of refusal of amniocentesis, 3 practitioners stated that treatment is scheduled until the theoretical time for amniocentesis and 10 stated that it was continued until birth, but the vast majority of practitioners did not chose an option for patients refusing amniocentesis (see Figure 7). One practitioner pointed out that in the TreatCMV study, amniocentesis is a requirement.

The majority Foetal medicine specialists answered they prescribe the generic version of valaciclovir, and the largest possible packaging.

Figure 7 – Indications and duration of treatment with valaciclovir according to Fetal Medicine specialists



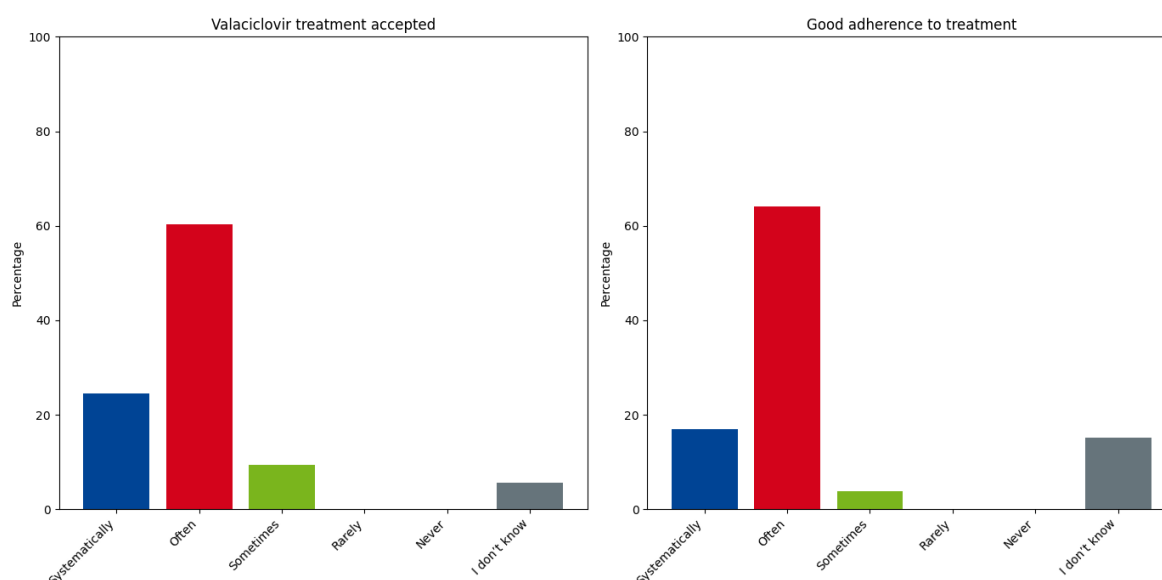
Treatment acceptance and adherence

There was only a minority of foetal medicine specialists ($n=13$; 24.53%), who stated that valaciclovir treatment is accepted in all circumstances, although most considered that it is often accepted ($n=32$; 60.38%) (see Figure 8). Practitioners pointed out that outside of a trial setting, cost is a problem for patients.

The practitioners that do not perceived acceptance of treatment as systematic, indicated as the main reasons for refusing treatment, the cost of treatment (22/37), maternal anxiety concerning potential long term side effects on the baby (17/37) and maternal anxiety regarding potential maternal side effects (13/37).

Adherence to treatment is perceived to be systematically good by a minority ($n=9$; 16.98%) of practitioners, but the majority ($n=34$; 64.15%) stated that that is often the case (see Figure 8). Major reasons of non-adherence are the number of pills per day (22/37) and maternal minor side effects (19/37).

Figure 8 – Perception on acceptance and adherence to treatment by fetal medicine specialists



Type of imaging follow-up

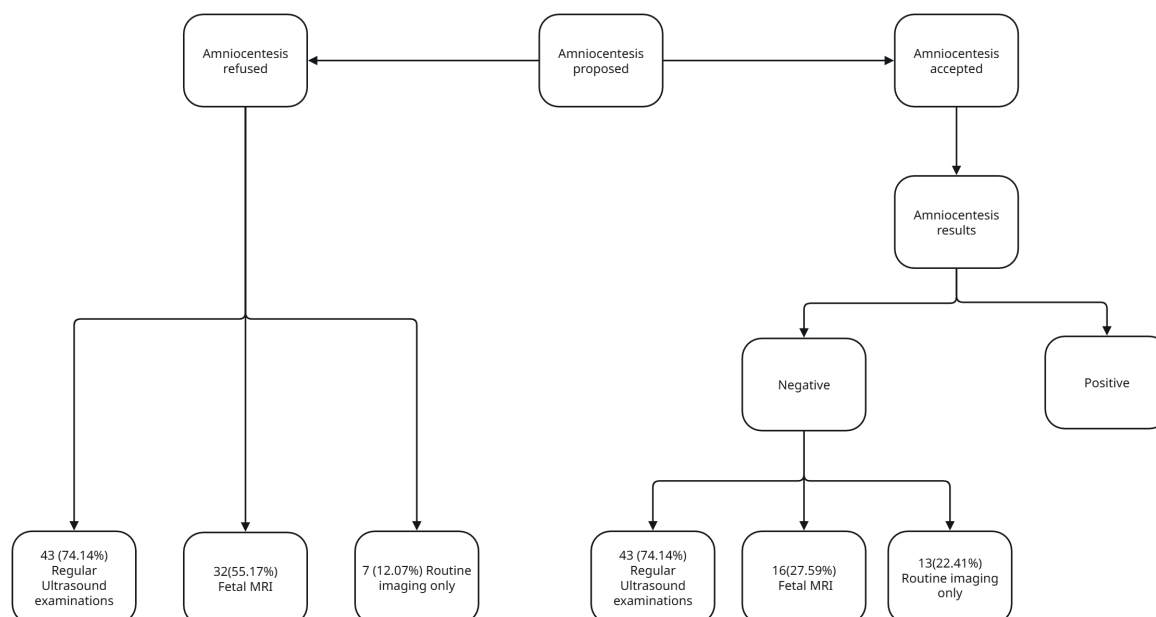
Imaging follow-up for patients with positive amniocentesis involves the use of regular ultrasound follow-up and foetal Magnetic Resonance Imaging (MRI).

In case of negative amniocentesis 43 (74.14%) respondents stated that they still perform regular ultrasound examinations. The most frequently cited frequency of regular ultrasound examinations was once every four weeks. foetal MRI is performed for amniocentesis negative patients who have a negative amniocentesis by 16 (22.41%) of practitioners. Some pointed out that MRI is only reactive to ultrasound anomalies. Only 7(12%) of our respondents chose only to perform routine imaging in case of negative amniocentesis (see Figure 9).

For patients who refuse amniocentesis, 50 (86.21%) of practitioners perform regular ultrasound examinations, in this case also the most cited frequency was once every 4 weeks, 32 (55.17%) perform foetal MRI,(although a few make a link between amniocentesis refusal and foetal MRI refusal), and 7(12.07%) only perform routine imaging (see Figure 9).



Figure 9 – Imaging follow-up according to amniocentesis status



Termination of Pregnancy

Foetal medicine specialists' perception of patient's choice on TOP in the first trimester was that, for most practitioners this choice is rarely ($n=28$; 48.28%) or sometimes ($n=18$; 31.03%) taken (see Figure 10). Those who provided additional comments pointed out that in the first trimester parents have a choice of electively terminating a pregnancy and that good counselling may decrease anxiety related to seroconversion.

Discussion on TOP based only on a positive amniocentesis was done by 41 (70.69%) of foetal medicine specialists. Some comments pointed out that the discussion is only carried out if the patients bring up the problem.

During counselling for TOP because of a positive amniocentesis, professionals reported mostly talking about the risk balance, including regularly remarking on high chances for a normal child. They also mentioned regularly that they would advise the patient to wait for the result of further imaging before making a decision. Sometimes they mentioned asking advice from a paediatrician. A few professionals reported the emotional difficulties or the need for emotional support in this context. The practitioners' perception of the choice patients make regarding TOP for a positive amniocentesis was, according to the majority, that it happens rarely ($n=28$; 48.28%), or sometimes ($n=17$; 29.31%) (see Figure 10). Some participants commented that TOP for this indication would be refused by healthcare practitioners, saying that the only option would be to go to a structure in the Netherlands to carry out the procedure.

Discussion of TOP for mildly/moderately symptomatic foetuses was carried out by 31 (53.45%) of foetal medicine specialists.

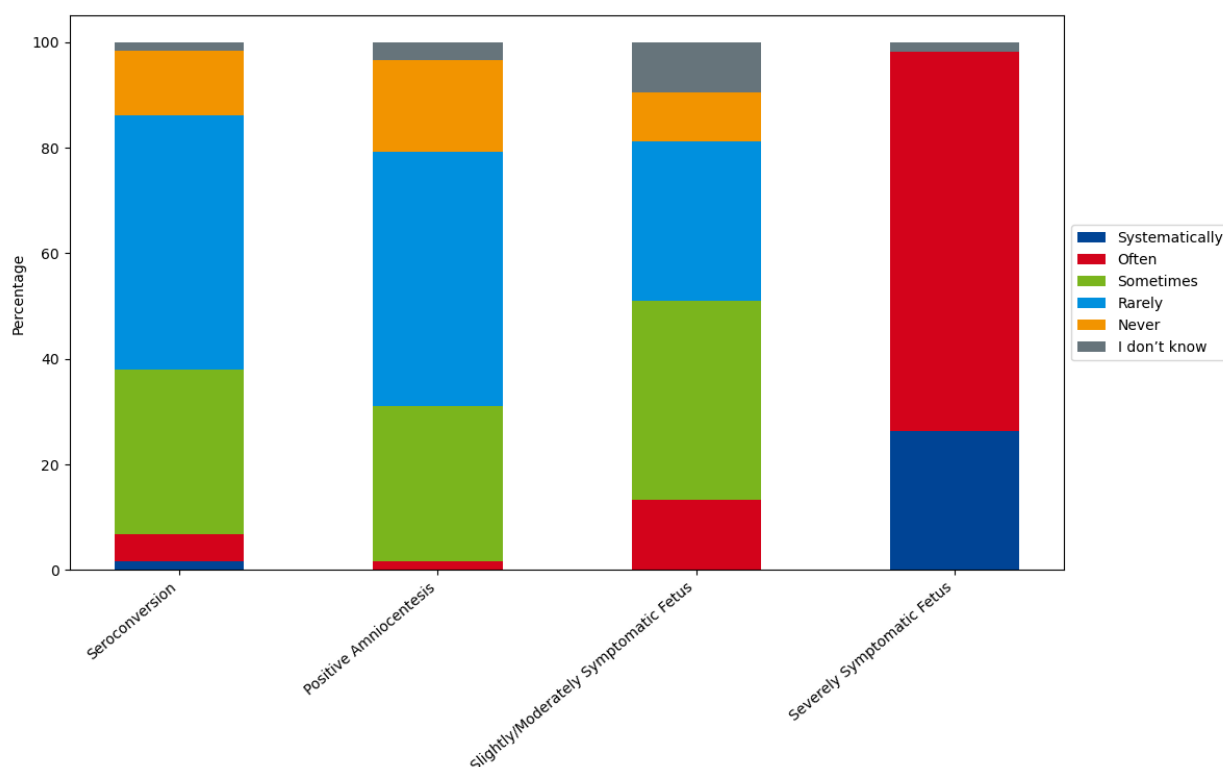
The most reported topic during counselling for termination of pregnancy in case of mild or moderate symptoms was the uncertainty about the clinical phenotype and/or risk balance. Many professionals remarked that they would ask advice from a paediatrician or multidisciplinary team/ethical committee or would refer to the tertiary level or to another hospital if the advice from ethical committee would be negative. Other remarks mentioned by few professionals included the symptoms not being severe enough to allow for a TOP, the suggestion to wait for result of the MRI, and the need for emotional support in this context.

The practitioners' perception of the choice patients make regarding TOP for mildly/moderately symptomatic foetuses was that it happened sometimes (n=20; 37.74%) or rarely (n=16; 30.19%) (see Figure 10).

A few participants commented that TOP for this indication was discussed only if the parents explicitly asked. Some pointed out that a mildly/moderately symptomatic foetus is not an indication for TOP.

In case of severely symptomatic foetuses TOP is perceived to be chosen often (n=38; 71.70%) or systematically (n=14; 26.42%) by parents.

Figure 10 – Perception of Fetal Medicine specialists on the patients' uptake of Termination of Pregnancy at different stages after CMV seroconversion



1.3 Discussion and conclusions

This survey aimed to gather insights into current opinions and practices among Belgian healthcare professionals regarding CMV screening and management during pregnancy.

Among respondents involved in pregnancy follow-up, most reported that they discuss CMV during the first pregnancy visit, and somewhat less frequently during preconception consultations. Midwives were less likely to address CMV preconceptionally, which they attributed mainly to rarely seeing patients at that stage due to the lack of reimbursement for preconceptional consultations.

A large majority of practitioners reported prescribing CMV serology in the first trimester. They were nearly evenly split between those who screen once and those who screen twice in this period. While nearly half do not screen beyond the first trimester, a substantial proportion do. Among those who continue testing, most do so once in the second trimester, with fewer testing once per trimester or in the third trimester.

CMV screening reimbursement was withdrawn in June 2017, though partial reimbursement remains for symptomatic cases—around 2 euros per serology. Despite this, some practitioners believe that serology is still reimbursed once or twice per pregnancy. Most report billing CMV screening as



“screening,” meaning the full cost is paid by patients. Some comments highlighted the perceived unfairness of the fact that CMV serology is still partially reimbursed outside of pregnancy.

The most commonly cited reason for CMV screening 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 fetal medicine specialists. In contrast, general practitioners (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.

Comments also indicated that the initiation of the TreatCMV study shifted attitudes toward screening, as it opened up the possibility for patients to access valaciclovir treatment, which is otherwise not reimbursed and must be paid out-of-pocket.

Respondents indicated that screening acceptance among patients is generally high. Although counselling about CMV is time-consuming, most practitioners stated they take or make time to provide proper information. Prescription of serology without adequate counselling was described as rare. However, many mentioned that counselling adds stress and may reduce income due to time constraints. Collaboration with other healthcare professionals was cited as helpful in sharing the counselling burden.

Termination of pregnancy (TOP) for a healthy foetus is one of the potential risks associated with CMV screening. Some practitioners expressed that seroconversion alone, a positive amniocentesis, or a mildly/moderately symptomatic foetus are not sufficient grounds for TOP. Nonetheless, many reported that patients do choose TOP in these scenarios—less frequently when only seroconversion is present, and more often with positive amniocentesis or symptomatic foetuses. A notable proportion of practitioners were unsure about what parents would decide in these scenarios, especially in cases of mild/moderate fetal symptoms, indicating the complexity and uncertainty involved.

Fetal 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, lack of official guidelines, and uncertainties regarding treatment effectiveness.

Practices regarding indications for treatment, duration, and follow-up imaging varied widely. While most offer treatment only in first-trimester infections many 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.

Practitioners reported variable patient acceptance and adherence to valaciclovir treatment. Most fetal medicine specialists said patients often accept and adhere to treatment, though not always. Common reasons for refusal included cost, concerns about side effects, and maternal anxiety about long-term effects on the baby. Valaciclovir treatment is burdensome, requiring 16 large pills daily and carrying a risk of side effects, including reversible acute renal failure.

Follow-up imaging approaches also varied in the context of a negative amniocentesis. A minority used routine imaging alone, while others conducted systematic MRI. Most opted for regular ultrasound examinations.

Fetal medicine specialists noted that TOP in the first trimester is rarely or sometimes chosen and emphasized the importance of effective counselling to alleviate anxiety. In cases of positive amniocentesis, TOP discussions focused on balancing risks and often advised waiting for further imaging. When foetuses showed mild or moderate symptoms, uncertainty about the prognosis was a central concern, prompting consultation with pediatricians or ethics committees. In cases of severe symptoms, TOP was often or systematically chosen by parents. Emotional support was frequently mentioned as critical throughout these discussions.

In conclusion, most practitioners involved in pregnancy follow-up prescribe at least one CMV serology for patients without documented positive serologic status, suggesting that many patients may need CMV-positive results managed.

The issue of TOP is complex. No respondents believed the option is never chosen by patients, though some indicated it is acceptable only in cases of severe fetal anomalies. The importance of appropriate counselling was emphasized across all responses.

Among fetal medicine specialists, treatment and follow-up strategies for CMV in pregnancy remain highly variable, underlining the need for standardized clinical management guidelines.

2 RATIONALE FOR EXCLUSION OF SCREENING FROM THE SCOPE OF THE GUIDELINE

WHY WAS UNIVERSAL PRENATAL CMV SCREENING NOT INCLUDED IN THE SCOPE OF THIS GUIDELINE?

KEY POINTS

Evidence base for universal prenatal CMV screening

The 2015 KCE guideline concluded that the available evidence did not support universal prenatal CMV screening and recommended, at most, a one-off serological test before or early in pregnancy, combined with informed consent and counselling on hygiene measures. An updated literature search indicates that randomised controlled trials or comparative observational studies evaluating universal CMV screening in pregnancy as an intervention have not been identified to date.

Limitations of economic evidence in the Belgian context

Economic considerations are a core component of the Evidence-to-Decision framework. Although recent cost-effectiveness analyses suggest that CMV screening combined with antiviral treatment may be cost-effective under specific assumptions, their applicability to Belgium is limited due to differences in healthcare organisation and reimbursement policies. In particular, the lack of reimbursement for antiviral treatment substantially affects the economic balance of a screening strategy.

Considerations related to access and equity

According to the Wilson and Jungner principles, a screening programme should only be implemented if effective treatment is available and accessible to all. In Belgium, routine prenatal CMV screening is not reimbursed and antiviral treatment with valaciclovir is associated with substantial out-of-pocket costs, raising concerns about equitable access and acceptability.

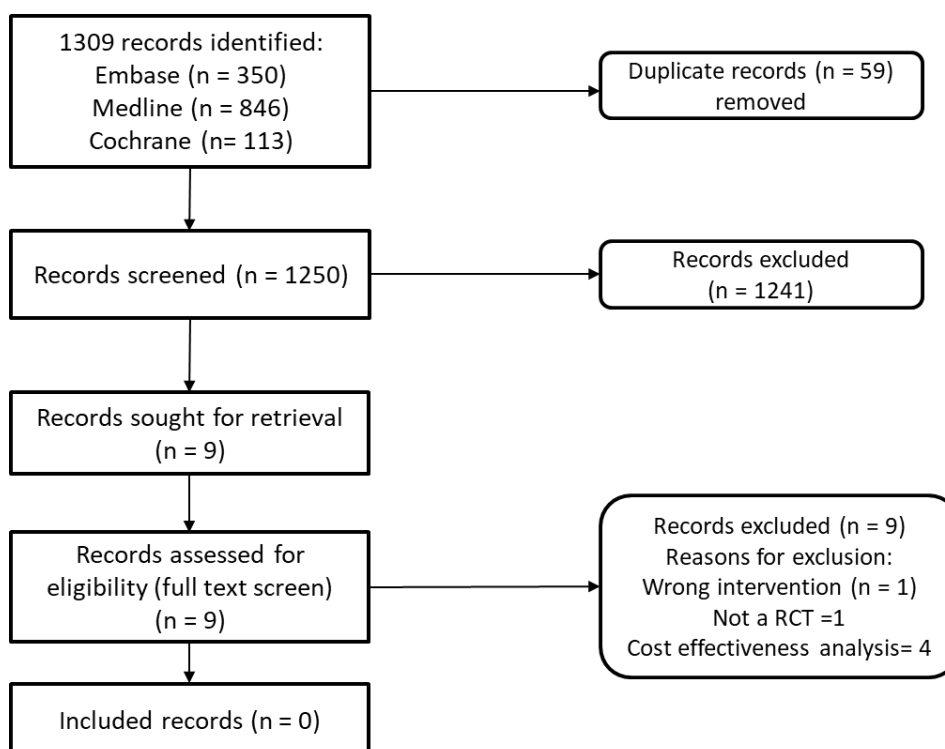
2.1 PICO and search strategy

PICO and search strategy are the same as in the KCE 2015 guidelines (1)

An update was performed from 2015.



2.2 PRISMA of the search for primary studies for screening



CMV guidelines elaborated by KCE in 2015, did not find sufficient evidence for universal CMV screening, and only recommend one-off testing, preferably before or at the beginning of pregnancy. Acknowledging the fact that many practitioners would want to test for CMV during pregnancy, guidelines recommend that full informed consent be asked from the (future) parent(s) in case CMV testing was offered. They also strongly recommend that all pregnant patients should receive information about the necessary hygiene measures to avoid CMV infection or reinfection.

Important novelties in the field of prenatal CMV, in particular the potential for treatment, have prompted a request for review of the guidelines of CMV screening and management in Belgium, that was accepted by KCE.

An update on the original 2015 systematic review on RCTs with universal CMV screening as an intervention, showed that no RCTs or observational studies with universal CMV screening in pregnancy as an intervention have been performed to this day.

2.3 Literature review on the cost-effectiveness of CMV screening

An initial literature search conducted in June 2024 identified four cost-effectiveness studies evaluating prenatal cytomegalovirus (CMV) screening (Séror 2021; Aoki 2022; Fisher 2022; Périllaud-Dubois 2023). An updated search performed in July 2025 via PubMed identified two additional publications on the cost-effectiveness of CMV screening (Lim 2025; Dzubay 2025).

Among the identified publications, two studies originated from France (Séror 2021; Périllaud-Dubois 2023) and two from the United States (Fisher 2022; Dzubay 2025). For each country, only the most recent publication was retained for detailed review, as these studies explicitly build on and update earlier analyses and are based on the most recent available evidence.

Table 2 provides an overview of the **four selected articles analysed**.

Table 2 – Overview of the cost-effectiveness studies analysed

Country Authors Year	Study design Model type	Population	Compared strategies (Sn)	Perspective Time horizon	Outcomes	Key assumptions	Authors conclusion	Limitations
Japan Aoki et al. 2022(2)	Cost-effectiveness analysis Decision tree model	811 604 live births cohort	S1: universal first trimester maternal antibody screening with prenatal valacyclovir Treatment S2: targeted newborn screening with postnatal valganciclovir treatment S3: universal newborn screening with postnatal valganciclovir treatment	Not specified	<u>Economic burden:</u> - medical costs (costs for screening test, antiviral treatment, investigations, outpatient follow-ups, hearing aid and cochlear implant surgery) - social costs for children with long-term sequelae of cCMV (educational expenses and parental productivity losses) <u>Quality-Adjusted Life Years (QALYs)</u> related health burden of each scenario calculated according to WHO recommendations (thresholds: highly cost-effective: < 1 GDP/capita, cost-	Rate of maternal CMV screening test: 95.4% Effectiveness of the maternal valacyclovir treatment: 63.3% Effectiveness of hygiene measures (54% reduction)	Both the prenatal and universal newborn screening programs may be cost-effective compared to the targeted newborn screening program Both below WHO threshold	Data partly from non-Japanese sources No probabilistic sensitivity analysis Very strong adherence to screening and treatment strategies Uncertainty regarding the effect of valaciclovir on vertical transmission No adverse effects of valaciclovir and valganciclovir Decision tree approach well suited to short-term analysis but less accurate for lifetime consequences (contrary to Markov models)



					effective: 1–3 GDP/capita)			
France Périllaud- Dubois et al. 2023(3)	Cost- effectiveness analysis Decision tree model	800 000 pregnant patients cohort	S1: no systematic screening (current public health recommendations in France) S2: screening of 25–50% of the pregnant population (current screening practice in France) S3: universal screening (current medical recommendations in France) S4: universal screening (as in Strategy S3) in conjunction with valacyclovir in case of T1 PI	Societal Child lifetime	<u>Cost:</u> • Direct medical lifetime costs associated with maternal CMV and cCMV screening • Diagnosis • Treatment for the entire cohort of pregnant patients with T1 PI infection • Direct medical costs for care of infected children during their lifetime <u>Effectiveness:</u> • Number of fetal cCMV infections, number of terminations of pregnancy (TOP), • Number of fetal diagnoses and number of infected children, with and without sequelae	Incidence: 0.46% Vertical transmission rate for strategies without treatment: 36.8% Vertical transmission rate for strategies with treatment: ref. Shahar-Nissan et al.	Universal screening in conjunction with valacyclovir treatment is cost-saving compared with current practice	Uncertainty regarding the estimation of the vertical transmission rate Uncertainty regarding the effect of valaciclovir on vertical transmission Uncertainty regarding the impact of treatment on the development of sequelae in the child Estimates of child management are based on expert opinion Assumes 100% uptake of amniocentesis among eligible women and 100% acceptance of antiviral treatment when offered (strategy 4) No adverse effects of valaciclovir



					Cost-effectiveness: Incremental Cost- Effectiveness Ratio (ICER) (€/QALY)			
USA Dzubay et al. 2025(4)	Decision analytic model	2 869 141 pregnant patients cohort	S1: screening for primary CMV in the first trimester of pregnancy S2: no screening.	Societal Lifetime	<u>Quality-Adjusted Life Years</u> (QALYs) <u>Societal costs:</u> - Screening tests for CMV - Valacyclovir treatment - Stillbirth - Excess cost of cCMV management in first y of life - CMV hearing loss - Neurodevelopmental disability	Probability of transmission in secondary CMV infection: 0,006 % Probability of treatment completion: 90% Probability of transmission to foetus after treatment: 11,06% Probability of transmission to foetus with no treatment: 25,50% Probability of Consenting to Amniocentesis: 93,33%	Universal screening relative to no screening reduced cases of adverse sequelae of congenital CMV, including stillbirth, neonatal death, hearing loss, and neurodevelopmental disability Universal screening is the strategy dominant: increase effectiveness and decrease costs	Very strong adherence to screening, treatment and amniocentesis Uncertainty regarding the effect of valaciclovir on vertical transmission No adverse effects of valaciclovir
Malaysia Lim et al. 2025(5)	Cost-utility analysis Markov model	100 000 pregnant patients cohort	S1: universal screening S2: routine ultrasound-directed testing	Healthcare payer Lifetime	<u>Direct medical costs:</u> - maternal CMV screening - diagnosis - treatment - management of complications arising from	CMV Seropositive in Singapore: 71,7% Transmission Rate after first trimester primary infection: 36,8%	Universal screening has the potential for averting childhood morbidity at birth and by 5-years-old	Small cohort compared to the other studies Very strong adherence to screening and treatment strategies



					cCMV infection in children <u>Quality-Adjusted Life Years (QALYs)</u> gained by preventing congenital CMV (cCMV) sequelae	Probability of symptomatic cCMV after first trimester primary infection: 28,8% Relative risk of vertical transmission after primary infection following valacyclovir treatment: 37,3% Relative risk of symptomatic infection after primary infection following valacyclovir treatment: 31,6% Propensity to proceed with amniocentesis with primary maternal infection: 64% No contra-indication or adverse reactions to valacyclovir	Uncertainty regarding the effect of valaciclovir on vertical transmission No adverse effects of valaciclovir
--	--	--	--	--	---	---	--

The four selected studies, conducted in Japan, France, the United States, and Malaysia, evaluated the cost-effectiveness of various CMV screening strategies during pregnancy, but differed substantially in design, assumptions, and modelling approaches. Aoki et al. (2022) and Périllaud-Dubois et al. (2023) employed decision tree models, appropriate for short-term evaluations but less reliable for lifetime outcomes, whereas Lim et al. (2025) used a Markov model, more suitable for capturing long-term trajectories.

Despite methodological diversity, all studies converged on the finding that maternal serological screening combined with valaciclovir treatment could be cost-effective, or even cost-saving, compared with current practice or no screening. This result was primarily driven by the avoidance of long-term sequelae and associated costs. However, **these conclusions rely on strong assumptions, including very high acceptance rates of amniocentesis and antiviral therapy, and optimistic estimates of treatment efficacy, which limit their generalisability.**

Incremental cost-effectiveness ratios (ICERs) in all four studies proved highly sensitive to a narrow set of parameters. Chief among these were the effectiveness of valaciclovir in reducing vertical transmission, the prevalence of primary maternal CMV infection, the probability of symptomatic congenital CMV following maternal infection, and the unit costs of serological screening. Although additional elements, such as treatment adherence and the inclusion of long-term societal costs, contributed to variability, these four parameters consistently shaped the direction and magnitude of ICERs. This underscores the central role of epidemiological assumptions and drug efficacy estimates in determining the robustness and transferability of economic evaluations.

Nonetheless, **important limitations persist. Considerable uncertainty remains regarding the true impact of valaciclovir on vertical transmission and long-term child outcomes.** None of the studies, with the partial exception of Lim et al. (2025), incorporated adverse effects of antiviral therapy into their modelling. While Lim's analysis drew on systematic review data suggesting a significantly reduced risk of congenital CMV following valaciclovir and a low, reversible risk of maternal adverse events, the remaining studies simply assumed the absence of harms. This methodological choice tends to bias results towards cost-effectiveness. Moreover, the scope of costs and outcomes varied widely: while some analyses integrated societal costs such as productivity losses and educational expenditures, others restricted their focus to direct medical costs. Combined with differences in national willingness-to-pay thresholds and healthcare organisation, this heterogeneity complicates cross-country comparisons.

In the Belgian context, the external validity of these findings is particularly constrained. The absence of reimbursement for valaciclovir not only alters the cost structure but also undermines the feasibility of achieving the treatment uptake rates assumed in the models.

2.4 Ethical considerations

From an ethical perspective, the implementation of a prenatal CMV screening programme cannot be justified unless effective treatment is both available and accessible to all eligible patients. According to the Wilson & Jungner's principles of screening, a fundamental criterion for any screening programme is that an accepted treatment for the identified condition should exist and be readily accessible.

In the Belgian context, the absence of reimbursement for valaciclovir means that treatment, although potentially effective, is associated with high out-of-pocket costs (up to 851,84€ for a 12-week course – see Table 3) which can represent a prohibitive financial barrier for many patients.

Introducing a screening program without ensuring equitable access to treatment would therefore conflict with these foundational screening principles, raising serious ethical concerns about fairness, patient autonomy, and the responsible allocation of healthcare resources.

**Table 3 – Detailed treatment cost**

	Valaciclovir AB	Valaciclovir Sandoz
Daily dose	8g	
Duration	8 weeks (56 days) – 12 weeks (84 days)	
Tablet per day	16 tablets	
Total tablets	896 – 1 344 tablets	
Tablets per box	42 tablets	
Number of boxes	22 – 32 boxes	
Price per box	25.57	26.62
Total cost	562.54€ - 818.24€	585.64€ - 851.84€

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 a CMV in pregnancy

3 SELECTION AND APPRAISAL OF CURRENT GUIDELINES

3.1 Search strategies

3.1.1 Medline through Pubmed

Database: Ovid MEDLINE(R) and Epub Ahead of Print, In-Process, In-Data-Review & Other Non-Indexed Citations, Daily and Versions <1946 to February 14, 2025>

Search Strategy:

- ```

1 exp Cytomegalovirus Infections/ (29594)
2 Cytomegalovirus/ (24078)
3 1 or 2 (39917)
4 CMV.ab,ti,kf. (31586)
5 (saliva* adj2 gland* adj2 virus*).ti,ab,kf. (217)
6 (hhv5 or hhv 5).ti,ab,kf. (68)
7 (herpesvirus 5 or herpes virus 5).ti,ab,kf. (211)
8 cytomegal*.ab,ti,kf,ot. (51282)
9 hcmv.ab,ti,kf. (7413)
10 (inclusion* adj2 disease*).ti,ab,kf. (2111)
11 (saliva* adj2 gland* adj2 disease*).ti,ab,kf. (1025)
12 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 (64164)
13 3 or 12 (68967)

```



- 14 Prenatal Care/ (34300)
- 15 exp Pregnancy/ (1052191)
- 16 exp Pregnancy complications/ (495233)
- 17 perinatal care/ (5649)
- 18 pregnant patients / (16250)
- 19 14 or 15 or 16 or 17 or 18 (1091001)
- 20 (antenatal\* or ante-natal\* or prenatal\* or pre-natal\* or perinatal\* or peri-natal\* or pregnan\* or antepartum or ante-partum or peripartum or peri-partum).ti,ab,kf. (804969)
- 21 pregnant.ab,ti,kf,jw. (239192)
- 22 pregnanc\*.ab,ti,kf,jw. (558892)
- 23 20 or 21 or 22 (807336)
- 24 19 or 23 (1306518)
- 25 13 and 24 (5193)
- 26 exp clinical pathway/ (8236)
- 27 exp clinical protocol/ (199082)
- 28 exp consensus/ (24652)
- 29 exp consensus development conference/ (13022)
- 30 exp consensus development conferences as TOPic/ (3014)
- 31 critical pathways/ (8236)
- 32 exp guideline/ (40144)
- 33 guidelines as TOPic/ (42486)
- 34 exp practice guideline/ (33080)
- 35 practice guidelines as TOPic/ (131412)
- 36 health planning guidelines/ (4169)
- 37 (guideline or practice guideline or consensus development conference or consensus development conference, NIH).pt. (50294)
- 38 (position statement\* or policy statement\* or practice parameter\* or best practice\*).ti,ab,kf,kw. (54041)
- 39 (standards or guideline or guidelines).ti,kf,kw. (149685)
- 40 ((practice or treatment\* or clinical) adj guideline\*).ab. (60827)
- 41 (CPG or CPGs).ti. (6733)
- 42 consensus\*.ti,kf,kw. (40266)
- 43 consensus\*.ab. /freq=2 (39947)
- 44 ((critical or clinical or practice) adj2 (path or paths or pathway or pathways or protocol\*).ti,ab,kf,kw. (30736)
- 45 recommendat\*.ti,kf,kw. (60365)
- 46 (care adj2 (standard or path or paths or pathway or pathways or map or maps or plan or plans)).ti,ab,kf,kw. (98689)



47 (algorithm\* adj2 (screening or examination or test or tested or testing or assessment\* or diagnosis or diagnoses or diagnosed or diagnosing)).ti,ab,kf,kw. (11771)

48 (algorithm\* adj2 (pharmacotherap\* or chemotherap\* or chemotreatment\* or therap\* or treatment\* or intervention\*)).ti,ab,kf,kw. (14560)

49 or/26-48 (815414)

50 25 and 49 (115)

51 limit 50 to yr="2015-2025" (58)

### 3.1.2 Embase (17 Feb 2025)

#1 'cytomegalovirus infection'/exp (51054)

#2 'cytomegalovirus'/exp (50709)

#3 #1 OR #2 (88087)

#4 cmv:ab,ti,kw (55512)

#5 (saliva\* NEAR/2 gland\* NEAR/2 virus\*):ab,ti,kw (161)

#6 hhv5:ab,ti,kw OR 'hhv 5':ab,ti,kw (103)

#7 'herpesvirus 5':ab,ti,kw OR 'herpes virus 5':ab,ti,kw (254)

#8 cytomegal\*:ab,ti,kw (65883)

#9 hcmv:ab,ti,kw (8555)

#10 (inclusion\* NEAR/2 disease\*):ti,ab,kw (2995)

#11 (saliva\* NEAR/2 gland\* NEAR/2 disease\*):ti,ab,kw (957)

#12 #4 OR #5 OR #6 OR #7 OR #8 OR #9 OR #10 OR #11

#13 #3 OR #12 (120596)

#14 'prenatal care'/exp (195966)

#15 'pregnancy'/exp (931524)

#16 'perinatal care'/exp (79800)

#17 'pregnant woman'/de (128163)

#18 'pregnancy complication'/exp (690697)

#19 #14 OR #15 OR #16 OR #17 OR #18 (1440515)

#20 antenatal\*:ti,ab,kw (73787)

#21 'ante natal\*':ti,ab,kw (1076)

#22 prenatal\*:ti,ab,kw (172594)

#23 'pre natal\*':ti,ab,kw (2140)

#24 perinatal\*:ti,ab,kw (135095)

#25 'peri natal\*':ti,ab,kw (456)

#26 pregnan\*:ti,ab,kw,jt (881710)

#27 antepartum:ti,ab,kw (10698)

#28 'ante partum':ti,ab,kw (657)

#29 peripartum:ti,ab,kw (11333)  
#30 'peri partum':ti,ab,kw (582)  
#31 #20 OR #21 OR #22 OR #23 OR #24 OR #25 OR #26 OR #27 OR #28 OR #29 OR #30 (1070028)  
#32 #19 OR #31 (1697866)  
#33 #13 AND #32 (9649)  
#34 'clinical pathway'/de (10759)  
#35 'clinical protocol'/exp (131495)  
#36 'consensus'/exp (110256)  
#37 'consensus development'/de (30374)  
#38 'practice guideline'/exp (783942)  
#39 'guideline'/de (142)  
#40 'health care planning'/de (118109)  
#41 'position statement\*':ab,ti,kw OR 'policy statement\*':ab,ti,kw OR 'practice parameter\*':ab,ti,kw  
OR 'best practice\*':ab,ti,kw (76672)  
#42 standards:ti,kw OR guideline:ti,kw OR guidelines:ti,kw (203820)  
#43 ((practice OR treatment\*) NEAR/3 guideline\*):ab (94738)  
#44 cpg:ti,kw OR cpgs:ti,kw (11454)  
#45 consensus\*:ti,kw (49979)  
#46 ((critical OR clinical OR practice) NEAR/3 (path OR paths OR pathway OR pathways OR  
protocol\*)):ab,ti,kw (63213)  
#47 recommendat\*:ti,kw (75647)  
#48 (care NEAR/3 (standard OR path OR paths OR pathway OR pathways OR map OR maps OR  
plan OR plans)):ab,ti,kw (184977)  
#49 (algorithm\* NEAR/3 (screening OR examination OR test OR tested OR testing OR assessment\*  
OR diagnosis OR diagnoses OR diagnosed OR diagnosing)):ab,ti,kw (24576)  
#50 (algorithm\* NEAR/3 (pharmacotherap\* OR chemotherap\* OR chemotreatment\* OR therap\* OR  
treatment\* OR intervention\*)):ab,ti,kw (25030)  
#51 #34 OR #35 OR #36 OR #37 OR #38 OR #39 OR #40 OR #41 OR #42 OR #43 OR #44 OR #45  
OR #46 OR #47 OR #48 OR #49 OR #50 (1470139)  
#52 #33 AND #51 (407)  
#53 #52 NOT ('conference abstract'/it OR 'conference paper'/it OR 'conference review'/it) (329)  
#54 #53 NOT [medline]/lim (79)  
#55 #54 AND [2015-2025]/py (52)



Figure 11 – Flow chart of guidelines' selection

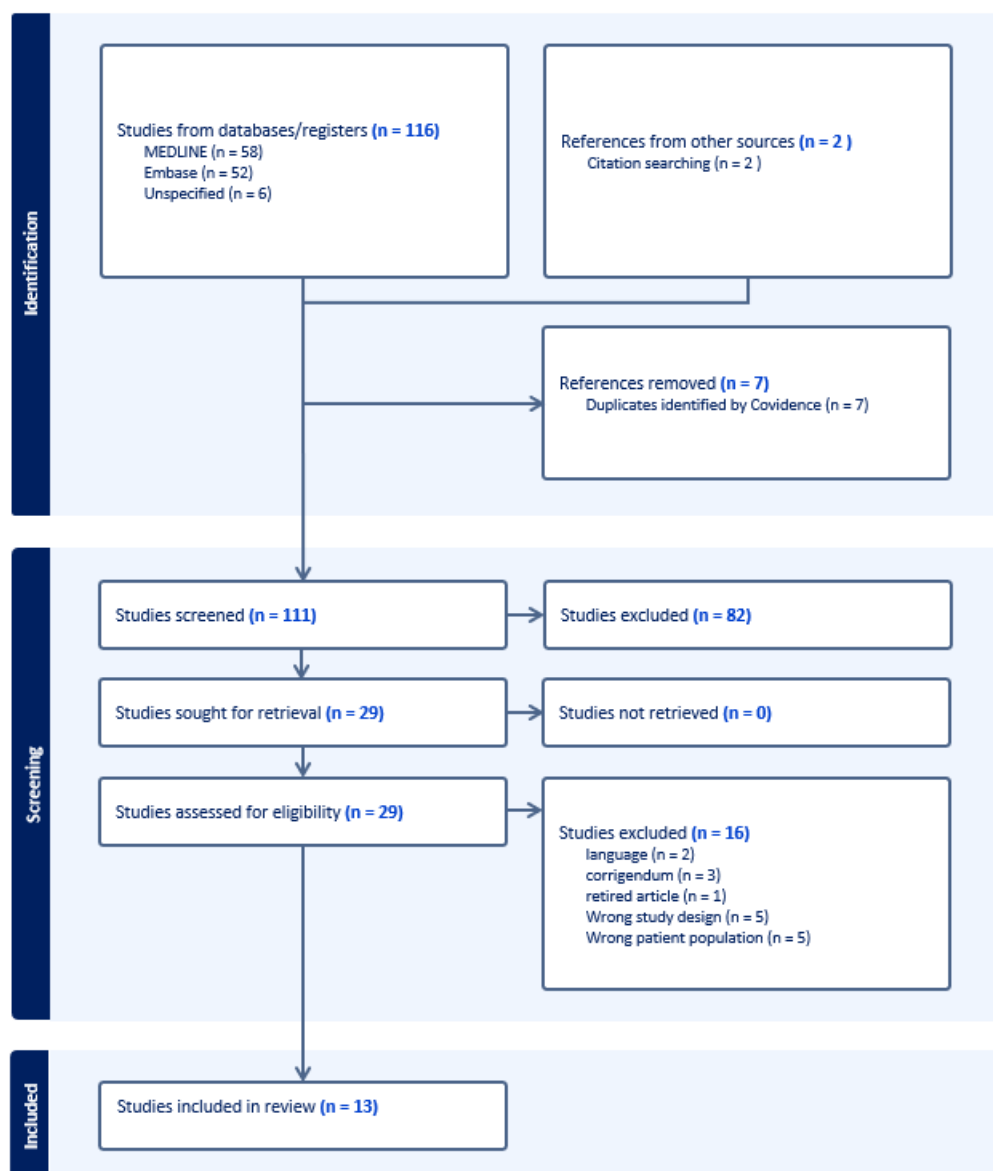


Table 4 – AGREE II tables for existing guidelines on CMV

| Country/<br>Organisation  | Title                                                                                                                    | Standardised Score/100 |                         |                       |         |               |                        |
|---------------------------|--------------------------------------------------------------------------------------------------------------------------|------------------------|-------------------------|-----------------------|---------|---------------|------------------------|
|                           |                                                                                                                          | Scope                  | Stakeholder involvement | Rigour of development | Clarity | Applicability | Editorial independence |
| South Australia (6)       | Perinatal practice guideline cytomegalovirus                                                                             | 67                     | 39                      | 24.5                  | 50      | 46            | 25                     |
| ACOG <sup>a</sup>         | Cytomegalovirus, Parvo B19, varicella zoster, and toxoplasmosis in Pregnancy                                             | 72                     | 33                      | 37.5                  | 39      | 8             | 0 no info              |
| SOGC (Boucoiran 2021) (7) | Guideline No. 420: cytomegalovirus infection in pregnancy                                                                | 67                     | 83                      | 62.5                  | 72      | 29            | 50                     |
| WAPM Coll 2009 (8)        | Guidelines on CMV congenital infection                                                                                   | 39                     | 22                      | 19                    | 50      | 25            | 50                     |
| SMFM Hughes 2016 (9)      | Diagnosis and antenatal management of congenital cytomegalovirus infection                                               | 72                     | 6                       | 27                    | 78      | ND            | 100                    |
| Japan Ito 2024 (10)       | Clinical Practice guidelines for the management of congenital cytomegalovirus infection in Japan 2023: executive summary | 78                     | 11                      | 60                    | 56      | 8             | 83                     |
| RCOG Khalil 2025 (11)     | Congenital cytomegalovirus infection: update on screening, diagnosis and treatment                                       | 55                     | 39                      | 33                    | 50      | 25            | 75                     |

<sup>a</sup> <https://www.acog.org/clinical-information/physician-faqs/cytomegalovirus-in-pregnancy>



|                                                                                     |                                                                                                                                                                    |    |    |    |    |      |    |
|-------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------|----|----|----|----|------|----|
| ISUOG<br>Khalil 2020 (12)                                                           | Practice guidelines: role of ultrasound in congenital infection                                                                                                    | 61 | 39 | 60 | 78 | 12.5 | ND |
| ECCI<br>Leruez-Ville 2024 (13)                                                      | Consensus recommendation for prenatal, neonatal and postnatal management of congenital cytomegalovirus infection from the European congenital infection initiative | 69 | 36 | 41 | 88 | 25   | 64 |
| International congenital cytomegalovirus recommendation group<br>Rawlinson 2017(14) | Congenital cytomegalovirus infection in pregnancy and the neonate: consensus recommendations for prevention, diagnosis and therapy                                 | 83 | 67 | 77 | 78 | 25   | 50 |
| Chili<br>Izquierdo 2021 (15)                                                        | Recommendations for the diagnosis and management of cytomegalovirus infection in pregnant woman and newborn infant                                                 | 61 | 33 | 23 | 78 | 33   | 75 |
| ASID <sup>b</sup><br>Palasanthiran 2022                                             | Management of perinatal infections                                                                                                                                 | 67 | 33 | 29 | 72 | 21   | 25 |
| RANZCOG <sup>c</sup><br>2023                                                        | Prevention of congenital cytomegalovirus infection statement                                                                                                       | 61 | 61 | 29 | 83 | 12.5 | 75 |

<sup>b</sup> <https://asid.net.au/publications>

<sup>c</sup> <https://ranzcoг.edu.au/wp-content/uploads/2022/05/Prevention-of-congenital-cytomegalovirus-CMV-infection-C-Obs-64.pdf>

ACOG The American College of Obstetricians and Gynaecologists

SOGC Society of Obstetricians and Gynaecologists of Canada

WAPM World Association of Perinatal Medicine

SMFM Society for Maternal-Foetal Medicine

ISUOG International Society of Ultrasound in Obstetrics and Gynaecology

RCOG Royal College of Obstetricians and Gynaecologists

How AGREE-II Standardised Scores Are Calculated

1. **Item Ratings:** Assessors rate each item on the AGREE II tool using a 7-point Likert scale, from 1 ("Strongly Disagree") to 7 ("Strongly Agree").
2. **Domain Scoring:** The scores for individual items within each of the six domains are summed.

## 4 GUIDELINE DEVELOPMENT GROUP AND STAKEHOLDERS

Two groups contributed to the development of this guideline. The first group consisted of health professionals forming the Guideline Development Group (GDG). The second group comprised stakeholders, including representatives of relevant professional societies, patient representatives, and other end users of the guideline. While each group has a distinct role (see below).

### 4.1 Guideline development group (GDG)

#### Role of the GDG

The GDG, working in close collaboration with the KCE Expert Team and stakeholders, was responsible for providing clinical expertise throughout the guideline development process. Methodological guidance, literature review support, and facilitation were provided by the KCE Expert Team.

The roles assigned to the GDG include:

- Definition of the clinical questions in collaboration with KCE and stakeholders.
- Contribution to the literature review, including:
  - Providing feedback on outcomes and thresholds for clinical relevance;
  - Reviewing the selection of studies and identifying relevant publications that may have been missed;
  - Providing input on the quality of the evidence, including issues of indirectness (applicability).
- Review of the guideline content and recommendations, including:
  - Providing feedback on expected health benefits and harms, certainty of the best available evidence, costs, feasibility, and their potential impact on the final recommendations;
  - Adapting the formulation of recommendations to the local context,

#### Composition of the GDG

The GDG was constituted through a structured process to ensure methodological rigor, multidisciplinary expertise, linguistic balance, and representation of patient values.

In February 2025, we identified members by contacting healthcare professionals and patient representatives through targeted outreach. Selection criteria emphasized documented expertise in



CMV during pregnancy or congenital CMV, as evidenced by published literature and peer recognition, or methodological competence in evidence synthesis.

We sought linguistic balance between French- and Dutch-speaking stakeholders to reflect Belgium's bilingual context.

To ensure appropriate multidisciplinary input, the following categories of healthcare professionals were identified as relevant for the GDG; however, participation depended on response and availability:

- *Gynecologists*: Individuals with recognized expertise in CMV in pregnancy based on published research or peer recommendations, or with methodological knowledge in evidence synthesis.
- *Radiologists, Clinical Biologists, and Pediatricians*: Professionals with known expertise in CMV in pregnancy or congenital CMV according to published literature and their peers.
- *Midwives*: We contacted Belgian midwifery associations to nominate members with clinical experience in CMV during pregnancy or methodological expertise.
- *General Practitioners (GPs)*: Recruitment was conducted through *Domus Medica* and *SSMG (Société Scientifique de Médecine Générale)*, requesting members with expertise in CMV in pregnancy or evidence synthesis.
- *Pharmacologist*

No general practitioners were able to participate into the Guideline development group despite targeted outreach.

To incorporate patient values and preferences, we included two representatives: one representing the general population and one representative of Care4CMV, a Belgian patient association composed of parents of children affected by congenital cytomegalovirus (CMV) infection, which aims to support families, raise awareness, and improve information and care related to congenital CMV.

All members declared potential conflicts of interest prior to participation. These were reviewed and managed according to established procedures to maintain transparency.

In total, the GDG comprised 16 members (see Table 5) and met five times between June 2025 and January 2026.

**Table 5 – Nominative composition of GDG members**

| Institution/Organisation | First Name | Last Name    | Profession                                 |
|--------------------------|------------|--------------|--------------------------------------------|
| CHU Liège                | Christine  | Van Linthout | Foetal Medicine specialist                 |
| CNR – HUB Erasme         | Marie-Luce | Delforge     | Clinical Biologist                         |
| HUB Erasme               | Caroline   | De Conninck  | Obstetrician and Fetal Medicine specialist |
| UZ Gent                  | Annelies   | Keymeulen    | Pediatrician                               |
| CHU St Pierre            | Oriane     | Steevaert    | Pediatrician                               |
| CHU Brugmann/ UZBrussels | Mieke      | Cannie       | Radiologist                                |
| HUB Erasme               | Clotilde   | Lamy         | Obstetrician                               |
| UZ Brussel               | Ina        | Fulon        | Otorhinolaryngologist                      |
| Odisee                   | Inge       | Tency        | Midwife                                    |
| UZA/Leuven               | Lennart    | Vanderveken  | Fetal Medicine specialist                  |
| UZ Brussel               | Deborah    | De Geyter    | Clinical Biologist                         |
| Leuven                   | Michael    | Aertsen      | Radiologist                                |

|                                                   |         |           |                                            |
|---------------------------------------------------|---------|-----------|--------------------------------------------|
| UZ Gent                                           | Ellen   | Roets     | Obstetrician and Fetal medicine specialist |
| Coordinator BELpREG pregnancy registry in Belgium | Michael | Ceulemans | Pharmacist                                 |
| General population                                | Sophie  | Ripault   | Citizen representative                     |
| Care4CMV                                          | Laura   | Chielens  | Patient association representative         |

## 4.2 Stakeholders' group

### Role of the stakeholders' group

Stakeholders are individuals or groups who are affected by a guideline's development and implementation. The stakeholders' advice is independent from the GDG.

The stakeholder group was tasked with:

- Prioritisation of research questions,
- providing feedback on draft recommendations,
- and ensuring that the guideline reflects clinical realities and patient values.

### Composition of the stakeholder group

The stakeholder group was constituted through a structured process to ensure broad representation and transparency. It included representatives from clinical disciplines relevant to the guideline, similar to those represented in the GDG, and was complemented by representatives from health insurance and public institutions.

In February 2025, we established this group by contacting relevant professional associations, institutions, and the patient association.

We also sought linguistic balance between French- and Dutch-speaking stakeholders to reflect Belgium's bilingual context.

Invitations were sent to the following organisations to nominate a representative, resulting in stakeholder representation across the following groups (participation depended on response and availability):

- Medical and clinical professional societies
  - Gynaecology and obstetrics: CRGOLFB (Collège Royal des Gynécologues-Obstétriciens de Langue Française de Belgique), VVOG (Vlaamse Vereniging voor Obstetrie en Gynaecologie)
  - Infectious diseases and clinical microbiology: BVIKM/SBIMC (Belgische Vereniging voor Infectiologie en Klinische Microbiologie / Société Belge d'Infectiologie et de Microbiologie Clinique)
  - Paediatrics: BVK/SBP (Belgische Vereniging voor Kindergeneeskunde / Société Belge de Pédiatrie)
  - Radiology: BeRS (Belgian Radiology Society)
- Midwifery organisations
  - VBOV (Vlaamse Beroepsorganisatie van Vroedvrouwen)
  - UPSfB (Union Professionnelle des Sages-femmes Belges)
  - LCM-ANMC (Ligue des Sages-femmes Catholiques – Association Nationale des Sages-femmes Catholiques)



- Primary care organisations
  - SSMG (Société Scientifique de Médecine Générale)
  - Domus Medica
- Health insurance and public institutions
  - UNMlibres-LOZ (Union Nationale des Mutualités Libres – Landsbond van Onafhankelijke Ziekenfondsen)
  - UNMS (Union Nationale des Mutualités Socialistes)
  - UNMN (Union Nationale des Mutualités Neutres)
  - INAMI (Institut National d'Assurance Maladie-Invalidité)

All members declared potential conflicts of interest prior to participation. These were reviewed and managed according to established procedures to maintain transparency.

In total, the stakeholder group comprised 15 members (see Table 6) and met twice between June 2025 and December 2025.

**Table 6 – Nominative composition of stakeholder group**

| Institution/Organisation                                                                                       | First Name | Last Name        |
|----------------------------------------------------------------------------------------------------------------|------------|------------------|
| CRGOLFB                                                                                                        | Gilles     | Ceysens          |
| VVOG                                                                                                           | Hilde      | Logghe           |
| Collège de médecins pour la mère et le nouveau-né/<br>College van geneesheren voor de moeder en de pasgeborene | Gunnar     | Naulaers         |
| BVIKM/SBIMC                                                                                                    | Sien       | Ombelet          |
| BVK/SBP                                                                                                        | Koen       | Vanden Driessche |
| BeRS                                                                                                           | Els        | De Leenheer      |
| VBOV                                                                                                           | Sabine     | Mortelmans       |
| UPSfB                                                                                                          | Caroline   | Blain            |
| LCM - ANMC                                                                                                     | Chris      | Van haecht       |
| UNMS                                                                                                           | Bart       | Demyttenaere     |
| SSMG                                                                                                           | Marthe     | Knops            |
| Domus Medica                                                                                                   | Benedicte  | Dooms            |
| INAMI                                                                                                          | Joel       | Daems            |
| ABeFUM                                                                                                         | Catherine  | Donner           |
| BRS                                                                                                            | Astrid     | Van Hoyweghen    |

## 5 DEVELOPMENT AND PRIORITISATION OF RESEARCH QUESTIONS

The five research questions addressed in this clinical practice guideline are the result of a structured and transparent prioritisation process involving the Guideline Development Group (GDG) and the stakeholders. This process was designed to ensure that the guideline focuses on the most relevant and practice-oriented questions related to the management of cytomegalovirus (CMV) infection acquired during pregnancy in the Belgian context.

### Identification of candidate research questions

To support the prioritisation process, a survey was developed. The survey compiled and refined candidate research questions derived from existing international consensus guidelines on CMV infection during pregnancy. The initial list aimed to capture the breadth of potentially relevant clinical issues while allowing subsequent narrowing of scope based on prioritisation (see Box 1).

The survey was organised into four thematic sections reflecting the main stages of CMV management:

1. Diagnosis of maternal CMV infection
2. Therapeutic interventions during pregnancy
3. Management of maternal and foetal CMV infections
4. Neonatal assessment and follow-up

At the end of each section, participants were invited to propose additional research questions and to briefly justify their relevance.

### **Box 1 – questions submitted in the survey**

#### **How should maternal CMV infection be diagnosed?**

1. What is the diagnostic accuracy of IgG IgM and avidity in the diagnosis of primary CMV infection in pregnant patients?
2. Do you have any further comments on the question above?
3. What is the diagnostic accuracy of CMV DNA PCR on maternal blood in the diagnosis of primary CMV infection in pregnant patients?
4. Do you have any further comments on the question above?
5. What is the diagnostic accuracy of CMV DNA PCR on maternal urine in the diagnosis of primary CMV infection in pregnant patients?
6. Do you have any further comments on the question above?
7. What is the diagnostic accuracy of IgG kinetics in the diagnosis of non-primary CMV infection in pregnant patients?
8. Do you have any further comments on the question above?
9. What is the diagnostic accuracy of IgM in the diagnosis of non-primary CMV infection in pregnant patients?
10. Do you have any further comments on the question above?
11. What is the diagnostic accuracy of CMV DNA PCR on blood in the diagnosis of non-primary CMV infection in pregnant patients?
12. Do you have any further comments on the question above?



13. What is the diagnostic accuracy of CMV DNA PCR on urine in the diagnosis of non-primary CMV infection in pregnant patients?

14. Do you have any further comments on the question above?

15. Is there any other research question that should or could be included for this section? If yes, please write it in the box below and explain shortly why this new question is important.

**What therapies should be recommended for CMV infection in pregnant patients?**

16. In pregnant patients with primary CMV infection, should antiviral drugs be used compared to no antiviral drugs to reduce the risk of vertical transmission, adverse foetal outcomes and long-term sequelae?

17. Do you have any further comments on the question above?

18. In pregnant patients with primary CMV infection, should CMV-specific hyperimmune globulin be used compared to no immunoglobulin treatment to reduce the risk of vertical transmission, adverse foetal outcomes and long-term sequelae?

19. Do you have any further comments on the question above?

20. In pregnant patients with confirmed foetal CMV infection (positive amniocentesis), should antiviral drugs be used compared to no antiviral drugs to reduce the severity of sequelae?

21. Do you have any further comments on the question above?

22. Is there any other research question that should or could be included for this section? If yes, please write it in the box below and explain shortly why this new question is important.

**How should maternal and foetal CMV infections be managed?**

23. In pregnant patients with primary CMV infection, should amniocentesis be performed at  $\geq 17+0$  weeks and  $\geq 6$  weeks after seroconversion, compared to later timing, for detecting foetal cCMV infection?

24. Do you have any further comments on the question above?

25. In pregnancies with confirmed foetal CMV infection (positive amniocentesis), should the quantification of CMV viral load (in amniotic fluid) be used to improve prognostic accuracy compared to standard evaluation without viral load measurement?

26. Do you have any further comments on the question above?

27. In pregnancies with confirmed foetal CMV infection (positive amniocentesis), should foetal blood sampling (cordocentesis) be used to improve prognostic accuracy compared to no foetal blood sampling?

28. Do you have any further comments on the question above?

29. In fetuses of pregnant patients who had a primary CMV infection, and had a negative amniocentesis, is systematic additional imaging (serial ultrasound every 2 to 4 weeks) more effective than routine imaging alone in identifying foetal abnormalities and predicting postnatal outcomes?

30. Do you have any further comments on the question above?

31. In fetuses of pregnant patients who had a primary CMV infection, and had a negative amniocentesis, is systematic additional imaging with foetal Magnetic Resonance more effective than imaging without Magnetic Resonance in identifying foetal abnormalities and predicting postnatal outcomes?

32. Do you have any further comments on the question above?

33. In fetuses of pregnant patients who had a primary CMV infection, and had a positive amniocentesis, is systematic additional imaging (ultrasound every 2 weeks) more effective than less frequent imaging (ultrasound every 4 weeks) in identifying foetal abnormalities and predicting postnatal outcomes?
34. Do you have any further comments on the question above?
35. In fetuses of pregnant patients who had a primary CMV infection, and had a positive amniocentesis, is systematic additional imaging with foetal Magnetic Resonance more effective than imaging without Magnetic Resonance in identifying foetal abnormalities and predicting postnatal outcomes?
36. Do you have any further comments on the question above?
37. Is there any other research question that should or could be included for this section? If yes, please write it in the box below and explain shortly why this new question is important.
- How should neonates be assessed?**
38. Should all neonates born from mothers with CMV seroconversion be screened for CMV within the first three weeks of life to diagnose cCMV?
39. Do you have any further comments on the question above?
40. In neonates born from mothers with CMV seroconversion, does performing CMV DNA PCR on urine, compared to other methods (saliva, blood spot, blood...), have better diagnostic accuracy for detecting cCMV infection?
41. Do you have any further comments on the question above?
42. In neonates with cCMV born after a negative maternal amniocentesis (false negative amniocentesis), should parents receive the same counselling (prognosis comparable) to neonates with cCMV born after a positive amniocentesis?
43. Do you have any further comments on the question above?
44. Is there any other research question that should or could be included for this section? If yes, please write it in the box below and explain shortly why this new question is important.

### Prioritisation criteria and survey process

Participants were asked to prioritise each proposed research question using a predefined scale reflecting its importance for inclusion in the guideline:

- TOP priority: should be included
- Moderate priority: can be included
- Some priority: inclusion unsure
- Little priority: likely not to be included
- No priority: should not be included
- I have no expertise to judge the priority

To ensure focus, respondents were encouraged to assign higher priority to questions addressing areas where:

- practice has recently changed,
- there is an ongoing debate in the clinical community
- or there is a large variability in clinical practice.



Questions that were consistently rated as high priority and considered feasible within the scope of the guideline were retained. Questions receiving lower priority ratings or deemed outside the scope were not included.

### **Selection of final research questions**

The results of the survey were reviewed and discussed during the first GDG, held on June 2025. During this meeting, the GDG examined the prioritisation results, considered qualitative feedback, and selected the final set of five research questions to be addressed in the guideline. This selection was submitted to the stakeholders group members in a second place also in June 2025.

## **6 PRIORITISATION OF OUTCOMES**

Outcome prioritisation was conducted through a structured process involving two patient representatives, both from the Guideline Development Group (GDG). The process was based on the five research questions validated by both the GDG and the stakeholder group:

### **On diagnosis**

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

### **On treatment**

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

### **On management**

- Negative amniocentesis: frequency of CMV-associated sequelae and follow-up:
  - In pregnant patients with a confirmed primary CMV infection, what is the prognostic accuracy of amniocentesis for detecting CMV-related sequelae in children with cCMV detected at birth?
  - 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?
- In pregnant patients with a confirmed primary CMV infection, should amniocentesis be performed at 17 weeks of gestation compared to a later gestational age?

For each research question, relevant outcomes were identified based on the available literature. Outcome prioritisation was conducted from a patient perspective; therefore, patient representatives were responsible for rating the importance of the identified outcomes.

Each outcome was independently rated by both patient representatives using a numerical scale from 1 to 9:

- scores of 7–9 indicated outcomes considered critical for decision-making,
- scores of 4–6 indicated important but not critical outcomes,
- and scores of 1–3 indicated outcomes of limited importance.

The addition of outcomes was also possible.

For each outcome, the mean of the two ratings was calculated and used as the prioritisation score. Outcomes were subsequently classified into three categories based on this average score: critical, important but not critical, or of limited importance.

A second round of discussion was held to resolve any remaining uncertainties or discrepancies between the ratings. The final prioritised list of outcomes was then reviewed and discussed during the second GDG meeting.

Table 7 – Table of outcomes prioritisation by each patient representative

| Outcome                                           | Evaluation              | Patient1 | Patient2 | Comment by patients                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          | Definitions                                                                                                                                                                                                                                                                                                                                                                 | Thresholds <sup>a</sup>                                                                                       |
|---------------------------------------------------|-------------------------|----------|----------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------|
| <i>cCMV at the moment of amniocentesis</i>        | IMPORTANT/NOT IMPORTANT | 6/3      | 6        | A negative amniocentesis result provides reassurance, but patients need clarity on its prognostic value. From a patient's perspective, a false negative is the worst possible outcome—you believe your baby is healthy, but CMV is detected at birth, leading to a cascade of tests and uncertainty. Ultimately, what matters most is how the child develops, not the test result itself. Even with a negative result, worry may persist. For patients, the result of the amniocentesis is not particularly important; it may be more relevant for research purposes. The guilt associated with transmitting CMV is significant, and if a negative result is later contradicted, it can intensify feelings of guilt and fear |                                                                                                                                                                                                                                                                                                                                                                             |                                                                                                               |
| <i>Slightly or moderately symptomatic foetus*</i> | IMPORTANT               | 6        | 6        | If imaging is reassuring, it provides comfort to the mother and helps reduce stress during the third trimester.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | *A slightly or moderately symptomatic foetus presents isolated ultrasound anomalies such as echogenic bowel, mild ventriculomegaly, or isolated calcifications. Alternatively, it may have isolated biological anomalies detected via fetal blood sampling, without significant cerebral abnormalities on ultrasound. (Ref: Khalil et al., 2020, ISUOG Practice Guidelines) |                                                                                                               |
| <i>Severely symptomatic foetus *</i>              | CRITICAL                | 8        | 8        | If imaging is reassuring, it provides comfort to the mother and helps reduce stress during the third trimester.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | *A severely symptomatic foetus presents severe cerebral abnormalities on ultrasound, such as microcephaly, ventriculomegaly, white matter abnormalities, cavitations, intracerebral                                                                                                                                                                                         | 12 events per 1000 individuals<br>distinguishing trivial from small effects<br>34 events per 1000 individuals |



|                                       |                 |   |   |                                                                                                                                                       |                                                                                                                                                               |                                                                                                                                                                                                                                             |
|---------------------------------------|-----------------|---|---|-------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                                       |                 |   |   |                                                                                                                                                       | hemorrhages, or cortical development delay, often associated with thrombocytopenia. (Ref: Khalil et al., 2020, ISUOG Practice Guidelines)(12)                 | distinguishing small from moderate effects<br>67 events per 1000 individuals<br>distinguishing moderate from large effects                                                                                                                  |
| <b>Termination of pregnancy</b>       | <b>CRITICAL</b> | 7 | 7 | The feeling of guilt associated with the choice is very traumatic                                                                                     |                                                                                                                                                               | 12 events per 1000 individuals<br>distinguishing trivial from small effects<br>34 events per 1000 individuals<br>distinguishing small from moderate effects<br>67 events per 1000 individuals<br>distinguishing moderate from large effects |
| <b>Miscarriage or stillbirth</b>      | IMPORTANT       | 4 | 4 | Are we going to allow babies to live, who would have died from the natural course of CMV infection, but now survive with potentially severe sequelae? |                                                                                                                                                               |                                                                                                                                                                                                                                             |
| <b>cCMV at birth</b>                  | IMPORTANT       | 5 | 5 | The reassurance that comes at birth is important for parents, even if it may not hold significant medical value.                                      |                                                                                                                                                               |                                                                                                                                                                                                                                             |
| <b>Mildly symptomatic newborns **</b> | NOT IMPORTANT   | 1 | 1 | Clinically nonsignificant, transient findings                                                                                                         | **Mild symptoms at birth: Up to 2 non-significant or transient findings (e.g., IUGR, petechiae, mild liver/spleen enlargement, mild lab abnormalities)(16,17) |                                                                                                                                                                                                                                             |

|                                           |           |   |   |                                                                                                                               |                                                                                                                                                                                                                                                       |                                                                                                                                                                                                                                    |
|-------------------------------------------|-----------|---|---|-------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <i>Moderately symptomatic newborns</i> ** | IMPORTANT | 4 | 4 | In the majority of cases this is not very impactful for the mother                                                            | **Moderate symptoms at birth: More than 2 mild symptoms or persistent abnormalities (>2 weeks), or mild brain imaging findings(16,17)                                                                                                                 |                                                                                                                                                                                                                                    |
| <i>Severely symptomatic newborns</i> **   | CRITICAL  | 9 | 9 | Severe symptoms at birth may have long-term consequences; in some cases, they can lead to multi-organ failure and even death. | **Severely symptomatic newborns: CNS involvement (e.g., seizures, chorioretinitis, microcephaly, significant brain abnormalities), severe single or multi-organ involvement, life-threatening disease, or isolated sensorineural hearing loss.(16,17) | 12 events per 1000 individuals distinguishing trivial from small effects<br>34 events per 1000 individuals distinguishing small from moderate effects<br>67 events per 1000 individuals distinguishing moderate from large effects |
| <i>Hearing loss</i>                       | IMPORTANT | 4 | 4 | Hearing aids can improve functional ability in children with hearing loss.                                                    |                                                                                                                                                                                                                                                       |                                                                                                                                                                                                                                    |
| <i>Visual impairment</i>                  | IMPORTANT | 4 | 4 | It's difficult to assess visual impairment across the spectrum                                                                |                                                                                                                                                                                                                                                       |                                                                                                                                                                                                                                    |
| <i>Epilepsy</i>                           | IMPORTANT | 5 | 5 | There are medications available for epilepsy, and in most cases, it is not refractory.                                        |                                                                                                                                                                                                                                                       |                                                                                                                                                                                                                                    |
| <i>Cerebral palsy</i>                     | CRITICAL  | 8 | 8 | Cerebral palsy has no cure; while care can help make the child more comfortable, it significantly limits life opportunities.  |                                                                                                                                                                                                                                                       | 12 events per 1000 individuals distinguishing trivial from small effects<br>34 events per 1000 individuals distinguishing small from moderate effects                                                                              |



|                                      |               |   |   |                                                                                                                                                   |  |                                                                                                                                                                                                                                    |
|--------------------------------------|---------------|---|---|---------------------------------------------------------------------------------------------------------------------------------------------------|--|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                                      |               |   |   |                                                                                                                                                   |  | 67 events per 1000 individuals distinguishing moderate from large effects                                                                                                                                                          |
| <i>Motor developmental delay</i>     | CRITICAL      | 7 | 7 | Motor developmental delay is similar to cerebral palsy, but generally less severe in its impact.                                                  |  |                                                                                                                                                                                                                                    |
| <i>Cognitive developmental delay</i> | CRITICAL      | 9 | 9 | Cognitive developmental delay is considered the most significant and frightening outcome.                                                         |  | 12 events per 1000 individuals distinguishing trivial from small effects<br>34 events per 1000 individuals distinguishing small from moderate effects<br>67 events per 1000 individuals distinguishing moderate from large effects |
| <i>Learning difficulties</i>         | NOT IMPORTANT | 2 | 4 | Many children experience learning disabilities.                                                                                                   |  |                                                                                                                                                                                                                                    |
| <i>Behavioural problems</i>          | IMPORTANT     | 4 | 4 | Many children experience behavioural problems, but these are often not very different from those seen in typically developing children.           |  |                                                                                                                                                                                                                                    |
| <i>Vestibular dysfunction</i>        | IMPORTANT     | 6 | 6 | Vestibular dysfunction can be debilitating, often requiring years of physical therapy before a child is able to do activities like riding a bike. |  |                                                                                                                                                                                                                                    |
|                                      |               |   |   |                                                                                                                                                   |  |                                                                                                                                                                                                                                    |

|                                |               |   |   |                                                                                                                                                                                                                                       |                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |  |
|--------------------------------|---------------|---|---|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--|
| <b>AE treatment child***</b>   | IMPORTANT     | 6 | 6 | Adverse events affecting the child are more significant to mothers than those affecting themselves. It is emotionally difficult for a mother to witness her child's discomfort, especially when the child is too young to understand. | *** Adverse Event : Any untoward or unfavorable medical occurrence in a human study participant, including any abnormal sign (e.g. abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the participants' involvement in the research, whether or not considered related to participation in the research.(18)                                                                                                                      |  |
| <b>SAE treatment child***</b>  | CRITICAL      | 8 | 8 | When taking treatment to prevent CMV-related consequences, it's important to also understand whether the treatment itself may cause adverse effects in the child.                                                                     | ***Serious Adverse Event : Serious Adverse Event (SAE):<br>Any adverse event that:<br>• Results in death<br>• Is life threatening, or places the participant at immediate risk of death from the event as it occurred<br>• Requires or prolongs hospitalization<br>• Causes persistent or significant disability or incapacity<br>• Results in congenital anomalies or birth defects<br>• Is another condition which investigators judge to represent significant hazards(18) |  |
| <b>AE treatment mother</b>     | NOT IMPORTANT | 2 | 2 | If a mother has to endure discomfort for the benefit of her child, it is considered acceptable.                                                                                                                                       |                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |  |
| <b>SAE treatment mother***</b> | CRITICAL      | 8 | 8 | A severe adverse event is just as important to avoid in the mother as it is in the child.                                                                                                                                             |                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |  |

<sup>a</sup> Following methodology described in GRADE Guidance 42 (ref), and after discussion with the guideline development group, minimal important difference thresholds corresponding to a utility value of 0 were used for critical outcomes.



## 7 SHOULD PRACTITIONERS USE SEROLOGIC TESTS TO DIAGNOSE NON-PRIMARY MATERNAL CMV INFECTION IN PREGNANCY?

### 7.1 PIRTS

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

**Table 8 – PIRTS diagnostic accuracy review of timing of amniocentesis for cCMV at birth**

| Question                  | Should practitioners use serologic test to diagnose non-primary CMV infection in pregnant patients?                                                                                                                                                                                                                                                                                                        |
|---------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Objective</b>          | To determine the appropriateness of using serologic tests in the diagnosis cCMV in children following non-primary CMV infection in pregnant patients                                                                                                                                                                                                                                                       |
| <b>Review Question</b>    | 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?                                                                                                                                                                                                                                      |
| <b>Population</b>         | Pregnant patients with a known previous positive CMV serological status                                                                                                                                                                                                                                                                                                                                    |
| <b>Index test</b>         | IgG kinetics and/or IgM detection                                                                                                                                                                                                                                                                                                                                                                          |
| <b>Reference standard</b> | Detection of cCMV at birth                                                                                                                                                                                                                                                                                                                                                                                 |
| <b>Target condition</b>   | cCMV                                                                                                                                                                                                                                                                                                                                                                                                       |
| <b>Outcomes</b>           | Critical: Diagnostic accuracy<br>Important: Any test burden, complications<br>Other: clinical management after amniocentesis, congenital CMV disease                                                                                                                                                                                                                                                       |
| <b>Study design</b>       | Good quality systematic reviews, diagnostic randomised controlled trials, prospective and retrospective diagnostic cohort studies, in which the index test and the reference standard are applied to the same patients in a cross-sectional design, and case-control studies<br>Exclusion: Narrative reviews; Case reports; Studies presented as conference abstract only or if no full text was available |
| <b>Analysis</b>           | No particular stratification                                                                                                                                                                                                                                                                                                                                                                               |

### 7.2 Search strategies

All searches were performed on October 28<sup>th</sup>, 2025

#### 7.2.1 Medline through Ovid

- 1 exp Cytomegalovirus Infections/ (30056)
- 2 Cytomegalovirus/ (24481)
- 3 1 or 2 (40425)
- 4 CMV.ab.ti.kf. (32395)
- 5 (saliva\* adj2 gland\* adj2 virus\*).ti.ab.kf. (220)

- 6 (hhv5 or hhv 5).ti,ab,kf. (72)
- 7 (herpesvirus 5 or herpes virus 5).ti,ab,kf. (215)
- 8 cytomegal\*.ab,ti,kf,ot. (52435)
- 9 hcmv.ab,ti,kf. (7568)
- 10 (inclusion\* adj2 disease\*).ti,ab,kf. (2173)
- 11 (saliva\* adj2 gland\* adj2 disease\*).ti,ab,kf. (1054)
- 12 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 (65610)
- 13 3 or 12 (70413)
- 14 Prenatal Care/ (35100)
- 15 exp Pregnancy/ (1070211)
- 16 exp Pregnancy complications/ (503964)
- 17 perinatal care/ (5824)
- 18 pregnant patients / (17123)
- 19 exp prenatal diagnosis/ (85493)
- 20 14 or 15 or 16 or 17 or 18 or 19 (1116382)
- 21 (antenatal\* or ante-natal\* or prenatal\* or pre-natal\* or perinatal\* or peri-natal\* or pregnan\* or antepartum or ante-partum or peripartum or peri-partum).ti,ab,kf. (830986)
- 22 20 or 21 (1339118)
- 23 13 and 22 (5321)
- 24 cCMV.ab,ti,kf. (843)
- 25 23 or 24 (5917)
- 26 Reinfection/ (1462)
- 27 recurrence/ (208395)
- 28 26 or 27 (209774)
- 29 reinfection?.ab,ti,kf. (12689)
- 30 re-infection?.ab,ti,kf. (3404)
- 31 recurrence.ab,ti,kf. (422778)
- 32 recurrent infection?.ab,ti,kf. (9457)
- 33 relapse?.ab,ti,kf. (221285)
- 34 secondary.ab,ti,kf. (1014858)
- 35 nonprimary.ab,ti,kf. (739)
- 36 non primary.ab,ti,kf. (1099)
- 37 "not primary".ab,ti,kf. (1066)
- 38 reactivation.ab,ti,kf. (41115)
- 39 preexisting immunity.ab,ti,kf. (478)
- 40 pre-existing immunity.ab,ti,kf. (898)
- 41 seroprevalent.ab,ti,kf. (152)



- 42 29 or 30 or 31 or 32 or 33 or 34 or 35 or 36 or 37 or 38 or 39 or 40 or 41 (1658612)
- 43 28 or 42 (1768978)
- 44 25 and 43 (673)
- 45 NPMI.ab,ti,kf. (28)
- 46 44 or 45 (701)
- 47 ((systematic adj2 review).ti. not comment.pt. not (protocol or protocols).ti. not medline.st.) or (Cochrane Database Syst Rev.ja. and review.pt.) or systematic review.pt. (370790)
- 48 meta-analys\*.ti. (243741)
- 49 meta-analysis.pt. (218254)
- 50 exp Technology Assessment, Biomedical/ (12858)
- 51 health technology assessment.ti,kf. (3483)
- 52 HtA.ti,kf. (1087)
- 53 47 or 48 or 49 or 50 or 51 or 52 (508019)
- 54 46 and 53 (15)
- 55 randomized controlled trial.pt. (647813)
- 56 controlled clinical trial.pt. (95743)
- 57 randomized.ti,ab. (779148)
- 58 placebo.ti,ab. (270130)
- 59 clinical trials as TOPic/ (205961)
- 60 randomly.ti,ab. (472353)
- 61 trial?.ti. (458157)
- 62 55 or 56 or 57 or 58 or 59 or 60 or 61 (1772050)
- 63 exp animal/ not humans/ (5385479)
- 64 62 not 63 (1636978)
- 65 46 and 64 (32)
- 66 65 not 54 (28)
- 67 46 not (54 or 66) (658)

### 7.2.2 Embase

- #1 'cytomegalovirus infection'/exp
- #2 'cytomegalovirus'/exp
- #3 #1 OR #2
- #4 cmv:ab,ti,kw
- #5 (saliva\* NEAR/2 gland\* NEAR/2 virus\*):ab,ti,kw
- #6 hhv5:ab,ti,kw OR 'hhv 5':ab,ti,kw
- #7 'herpesvirus 5':ab,ti,kw OR 'herpes virus 5':ab,ti,kw
- #8 cytomegal\*:ab,ti,kw

#9 hcmv:ab,ti,kw

#10 (inclusion\* NEAR/2 disease\*):ab,ti,kw

#11 (saliva\* NEAR/2 gland\* NEAR/2 disease\*):ab,ti,kw

#12 #4 OR #5 OR #6 OR #7 OR #8 OR #9 OR #10 OR #11

#13 #3 OR #12

#14 'prenatal care'/exp

#15 'pregnancy'/exp

#16 'pregnancy complication'/exp

#17 'perinatal care'/exp

#18 'pregnant woman'/de

#19 'prenatal diagnosis'/exp

#20 #14 OR #15 OR #16 OR #17 OR #18 OR #19

#21 antenatal\*:ab,ti,kw OR 'ante natal\*':ab,ti,kw OR prenatal\*:ab,ti,kw OR 'pre natal\*':ab,ti,kw OR perinatal\*:ab,ti,kw OR 'peri natal\*':ab,ti,kw OR pregnan\*:ab,ti,kw OR antepartum:ab,ti,kw OR 'ante partum':ab,ti,kw OR peripartum:ab,ti,kw OR 'peri partum':ab,ti,kw

#22 #20 OR #21

#23 #13 AND #22

#24 ccmv:ab,ti,kw

#25 #23 OR #24

#26 'reinfection'/exp

#27 'recurrent disease'/de

#28 'secondary'/exp

#29 #26 OR #27 OR #28

#30 reinfection\*:ab,ti,kw

#31 're-infection\*':ab,ti,kw

#32 recurrence:ab,ti,kw

#33 'recurrent infection\*':ab,ti,kw

#34 relapse\*:ab,ti,kw

#35 secondary:ab,ti,kw

#36 nonprimary:ab,ti,kw

#37 'non primary':ab,ti,kw

#38 'not primary':ab,ti,kw

#39 reactivation:ab,ti,kw

#40 'preexisting immunity':ab,ti,kw

#41 'pre existing immunity':ab,ti,kw

#42 seroprevalent:ab,ti,kw



#43 #30 OR #31 OR #32 OR #33 OR #34 OR #35 OR #36 OR #37 OR #38 OR #39 OR #40 OR #41 OR #42

#44 #29 OR #43

#45 #25 AND #44

#46 npmi:ab,ti,kw

#47 #45 OR #46

#48 #47 NOT [medline]/lim

#49 #48 NOT ('conference abstract'/it OR 'conference paper'/it OR 'conference review'/it)

#50 #49 AND ('meta-analysis'/exp OR 'meta-analysis' OR 'systematic review'/exp OR 'systematic review')

#51 #49 AND (random\*:ab,ti OR placebo\*:de,ab,ti OR ((double NEXT/1 blind\*):ab,ti))

#52 #51 NOT #50

#53 #49 NOT (#50 OR #52)

### 7.2.3 Cochrane

Date Run: 20/10/2025 23:41:28

Comment:

| ID  | Search Hits                                    |       |
|-----|------------------------------------------------|-------|
| #1  | [mh "Cytomegalovirus Infections"]              | 1060  |
| #2  | [mh ^"Cytomegalovirus"]                        | 460   |
| #3  | #1 or #2                                       | 1127  |
| #4  | cmv:ab,ti                                      | 2340  |
| #5  | (saliva* NEAR/2 gland* NEAR/2 virus*):ti,ab    | 2     |
| #6  | (hhv5 OR "hhv 5"):ti,ab                        | 0     |
| #7  | ("herpesvirus 5" OR "herpes virus 5"):ti,ab    | 1     |
| #8  | cytomegal*:ab,ti                               | 2055  |
| #9  | hcmv:ab,ti                                     | 78    |
| #10 | (inclusion* NEAR/2 disease*):ti,ab             | 1352  |
| #11 | (saliva* NEAR/2 gland* NEAR/2 disease*):ti,ab  | 29    |
| #12 | #4 OR #5 OR #6 OR #7 OR #8 OR #9 OR #10 OR #11 | 4471  |
| #13 | #3 OR #12                                      | 4564  |
| #14 | [mh ^"Prenatal Care"]                          | 2361  |
| #15 | [mh "Pregnancy"]                               | 33888 |
| #16 | [mh "Pregnancy Complications"]                 | 18220 |
| #17 | [mh ^"Perinatal Care"]                         | 255   |
| #18 | [mh ^"Pregnant patients "]                     | 1069  |
| #19 | [mh "Prenatal Diagnosis"]                      | 1240  |

#20 #14 OR #15 OR #16 OR #17 OR #18 OR #19 38701

#21 (antenatal\* OR ante-natal\* OR prenatal\* OR pre-natal\* OR perinatal\* OR peri-natal\* OR pregnan\* OR antepartum OR ante-partum OR peripartum OR peri-partum):ti,ab 84809

#22 #20 OR #21 99095

#23 #13 AND #22 293

#24 cCMV:ab,ti 12

#25 #23 OR #24 298

#26 [mh ^"Reinfection"] 39

#27 [mh ^"Recurrence"] 16442

#28 #26 OR #27 16475

#29 reinfection?:ab,ti 1348

#30 re-infection?:ab,ti 404

#31 recurrence:ab,ti 45221

#32 (recurrent NEAR/1 infection?):ab,ti 546

#33 relapse?:ab,ti 44482

#34 secondary:ab,ti 468022

#35 nonprimary:ab,ti 126

#36 "non primary":ab,ti 113

#37 "not primary":ab,ti 105

#38 reactivation:ab,ti 1636

#39 "preexisting immunity":ab,ti 78

#40 "pre-existing immunity":ab,ti 54

#41 seroprevalent:ab,ti 4

#42 #29 OR #30 OR #31 OR #32 OR #33 OR #34 OR #35 OR #36 OR #37 OR #38 OR #39 OR #40 OR #41 529660

#43 #28 OR #42 535941

#44 #25 AND #43 164

#45 npmi:ab,ti 1

#46 #44 OR #45 165

#47 #46 in Cochrane Reviews 0

#48 #46 with Cochrane Library publication date Between Jan 2012 and Oct 2025 164



### 7.3 Study selection

Figure 12 – Study flow selection of search for non-primary maternal infection

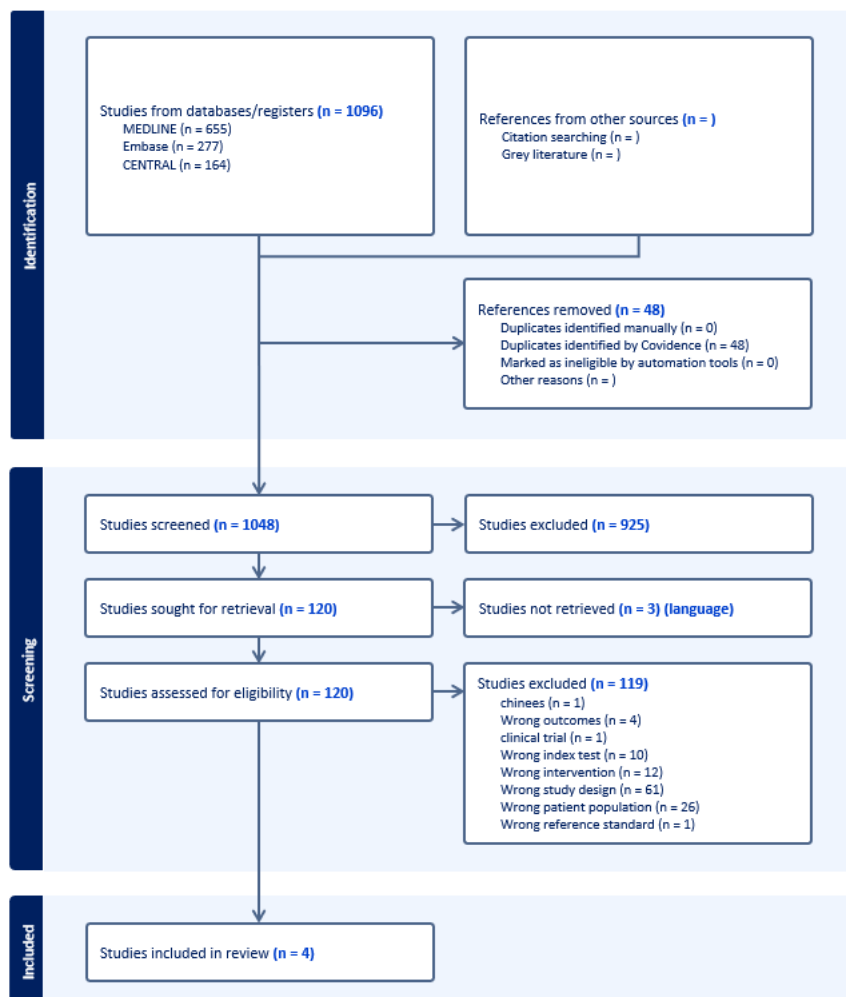


Figure provided by Covidence

#### 7.3.1 Studies excluded with reason

Table 9 – Excluded primary studies for non-primary maternal infection

| Study and year       | Exclusion reasons                    |
|----------------------|--------------------------------------|
| Kalogeropoulou 2025  | Exclusion reason: Wrong study design |
| Schattner 2024       | Exclusion reason: Wrong study design |
| Clinicaltrials.gov   | Exclusion reason: Clinical trial     |
| Iijima 2022          | Exclusion reason: Wrong study design |
| Scaramuzzino 2022    | Exclusion reason: Wrong intervention |
| Talavera-Barber 2022 | Exclusion reason: Wrong outcomes     |
| Salih 2020           | Exclusion reason: Wrong study design |



|                      |                                            |
|----------------------|--------------------------------------------|
| Román-Soto 2020      | Exclusion reason: Wrong study design       |
| Lazzarotto 2020      | Exclusion reason: Wrong study design       |
| Paixão 2019          | Exclusion reason: Wrong patient population |
|                      | Exclusion reason: Wrong index test         |
| Huang 2019           | Exclusion reason: Wrong study design       |
| Jahan 2017           | Exclusion reason: Wrong study design       |
| Joseph 2014          | Exclusion reason: Wrong study design       |
| Janan 2013           | Exclusion reason: Wrong reference standard |
| Nataša 2009          | Exclusion reason: Wrong patient population |
| Devi 2008            | Exclusion reason: Wrong study design       |
| Tejerizo-García 2006 | Exclusion reason: Wrong study design       |
| Salmaso 2006         | Exclusion reason: Wrong study design       |
| Bodéus 2003          | Exclusion reason: Wrong study design       |
| Numazaki 1995        | Exclusion reason: Wrong study design       |
| Xercavins 1994       | Exclusion reason: no full text - language  |
| Salzman 1992         | Exclusion reason: Wrong study design       |
| Vodopivec 2022       | Exclusion reason: Wrong study design       |
| Gerna 2020           | Exclusion reason: Wrong intervention       |
| Puhakka 2017         | Exclusion reason: Wrong study design       |
| Rouse 2022           | Exclusion reason: Wrong patient population |
| Leruez-Ville 2020    | Exclusion reason: Wrong study design       |
| Ahlfors 1983         | Exclusion reason: Wrong patient population |
| Ahlfors 1982         | Exclusion reason: Wrong index test         |
| Ara 2025             | Exclusion reason: Wrong intervention       |
| Barbosa 2018         | Exclusion reason: Wrong index test         |
| BenShoham 2023       | Exclusion reason: Wrong index test         |
| Blackburn 1991       | Exclusion reason: Wrong patient population |
| Bodeus 2001          | Exclusion reason: Wrong outcomes           |
| Bodeus 1998          | Exclusion reason: Wrong intervention       |
| Boppana 1999         | Exclusion reason: Wrong index test         |
| Boppana 2001         | Exclusion reason: Wrong index test         |
| Casteels 1999        | Exclusion reason: Wrong patient population |
| Chakravarti 2007     | Exclusion reason: Wrong patient population |
| Chan 2024            | Exclusion reason: Wrong study design       |
| Cinar 2025           | Exclusion reason: Wrong study design       |



|                         |                                            |
|-------------------------|--------------------------------------------|
| Coscia 2020             | Exclusion reason: Wrong patient population |
| Coskun 2025             | Exclusion reason: Wrong study design       |
| Coste-Mazeau 2024       | Exclusion reason: Wrong patient population |
| Daiminger 1998          | Exclusion reason: Wrong outcomes           |
| deVries 2013            | Exclusion reason: Wrong patient population |
| Demmler-Harrison 2020   | Exclusion reason: Wrong patient population |
| Dorfman 2021            | Exclusion reason: Wrong study design       |
| Eggers 2000             | Exclusion reason: Wrong patient population |
| Eggers 1998             | Exclusion reason: Wrong patient population |
| Erenel 2024             | Exclusion reason: Wrong study design       |
| Förner 2016             | Exclusion reason: Wrong study design       |
| Fowler 1992             | Exclusion reason: Wrong study design       |
| Furione 2018            | Exclusion reason: Wrong study design       |
| Gartner 1979            | Exclusion reason: Wrong study design       |
| Gatta 2022              | Exclusion reason: Wrong study design       |
| Gibert 1987             | Exclusion reason: Wrong study design       |
| Griffiths 1991          | Exclusion reason: Wrong index test         |
| Gunkel 2017             | Exclusion reason: Wrong study design       |
| Hazell 2007             | Exclusion reason: Wrong study design       |
| Ikuta 2013              | Exclusion reason: Wrong index test         |
| Kaneko 2023             | Exclusion reason: Wrong study design       |
| Karageorgou 2022        | Exclusion reason: Wrong index test         |
| Kekkou 2021             | Exclusion reason: Wrong patient population |
| Khalil 2025             | Exclusion reason: Wrong study design       |
| Kobas 2018              | Exclusion reason: Wrong patient population |
| Kouri 2010              | Exclusion reason: Wrong index test         |
| Kyriazopoulou 1996      | Exclusion reason: Wrong study design       |
| Lawrence 2024           | Exclusion reason: Wrong study design       |
| Lazzarotto 2004         | Exclusion reason: Wrong intervention       |
| Leruez-Ville 2017       | Exclusion reason: Wrong study design       |
| Letamendia-Richard 2022 | Exclusion reason: Wrong patient population |
| Li 2017                 | Exclusion reason: Wrong patient population |
| Lilleri 2023            | Exclusion reason: Wrong intervention       |
| Malherbe 2024           | Exclusion reason: Wrong study design       |
| Mocanu 2024             | Exclusion reason: Wrong patient population |

|                         |                                            |
|-------------------------|--------------------------------------------|
| Mussi-Pinhata 2020      | Exclusion reason: Wrong study design       |
| Natali 1997             | Exclusion reason: Wrong study design       |
| Nigro 2003              | Exclusion reason: Wrong patient population |
| Palav 2024              | Exclusion reason: Wrong patient population |
| Papaevangelou 2019      | Exclusion reason: Wrong study design       |
| Paris 2023              | Exclusion reason: Wrong patient population |
| Peckham 1983            | Exclusion reason: Wrong study design       |
| Picone 2017             | Exclusion reason: Wrong study design       |
| Portolani 1995          | Exclusion reason: Wrong patient population |
| Preece 1984             | Exclusion reason: Wrong study design       |
| Prince 2014             | Exclusion reason: Wrong patient population |
| Puhakka 2022            | Exclusion reason: Wrong patient population |
| Rahav 2007              | Exclusion reason: Wrong study design       |
| Revello 2014            | Exclusion reason: Wrong intervention       |
| Ross 2006               | Exclusion reason: Wrong intervention       |
| Rothe 2001              | Exclusion reason: Wrong intervention       |
| Rousseau 2000           | Exclusion reason: Wrong study design       |
| Sahiner 2015            | Exclusion reason: Wrong study design       |
| Saldan 2015             | Exclusion reason: Wrong study design       |
| Saldan 2017             | Exclusion reason: Wrong patient population |
| Satilmis 2007           | Exclusion reason: Wrong study design       |
| Schoub 1993             | Exclusion reason: Wrong study design       |
| Seo 2009                | Exclusion reason: Wrong patient population |
| Seror 2022              | Exclusion reason: Wrong study design       |
| Serra 2025              | Exclusion reason: Wrong study design       |
| Simonazzi 2018          | Exclusion reason: Wrong study design       |
| Sorrenti 2025           | Exclusion reason: Wrong study design       |
| St-Georges 2025         | Exclusion reason: Wrong outcomes           |
| Stagno 1977             | Exclusion reason: Wrong study design       |
| Swiatkowska-Freund 2025 | Exclusion reason: Wrong intervention       |
| Tanimura 2017           | Exclusion reason: Wrong study design       |
| Tanimura 2021           | Exclusion reason: Wrong study design       |
| Tokodi 2004             | Exclusion reason: Wrong study design       |
| Uchida 2020             | Exclusion reason: Wrong intervention       |
| Wang 2011               | Exclusion reason: Wrong study design       |



|                |                                      |
|----------------|--------------------------------------|
| Yamada 2018    | Exclusion reason: Wrong intervention |
| Yamamoto 2010  | Exclusion reason: Wrong study design |
| Zavattoni 2018 | Exclusion reason: Wrong study design |
| Zelini 2022    | Exclusion reason: Wrong study design |

## 7.4 Appraisal

The selected studies were assessed using QUADAS 2 (Quality Assessment for Studies of Diagnostic Accuracy, 2nd version)(19)

**Table 10 – QUADAS-2 assessment for non-primary maternal infection**

| Question                                                                                                        | Hadar 2017(20) | Huang 2024a(21)                   | Périllaud-Dubois 2022(22) | Tang 2025(23)  |
|-----------------------------------------------------------------------------------------------------------------|----------------|-----------------------------------|---------------------------|----------------|
| <b>Selection of participants</b>                                                                                |                |                                   |                           |                |
| 1. Was a consecutive or random sample of <b>participants</b> enrolled?                                          | No             | Yes                               | No                        | Unclear        |
| 2. Was a case-control design avoided?                                                                           | Yes            | No                                | Yes                       | Yes            |
| 3. Did the study avoid inappropriate exclusions?                                                                | No             | Unclear                           | Unclear                   | Yes            |
| <b>Could selection of participants have introduced bias?</b>                                                    | <b>High</b>    | <b>High</b>                       | <b>High</b>               | <b>Unclear</b> |
| <b>Is there concern that the included participants do not match the review question?</b>                        | <b>High</b>    | <b>Low</b>                        | <b>High</b>               | <b>High</b>    |
| <b>Index test</b>                                                                                               |                |                                   |                           |                |
| 1. Were the index test results interpreted without knowledge of the results of the reference standard?          | No             | No, it was tested retrospectively | No                        | Unclear        |
| 2. If a threshold was used, was it pre-specified?                                                               | No             | Yes                               | No                        | Yes            |
| <b>Could the conduct or interpretation of the index test have introduced bias?</b>                              | <b>High</b>    | <b>Unclear</b>                    | <b>High</b>               | <b>Unclear</b> |
| <b>Are there concerns that the conduct or interpretation of the index test differ from the review question?</b> | <b>Low</b>     | <b>Low</b>                        | <b>Low</b>                | <b>Low</b>     |
| <b>Reference standard</b>                                                                                       |                |                                   |                           |                |
| 1. Is the reference standard likely to correctly classify the target condition?                                 | No             | Yes                               | Yes                       | Yes            |



|                                                                                                                        |             |             |             |                |
|------------------------------------------------------------------------------------------------------------------------|-------------|-------------|-------------|----------------|
| 2. Were the reference standard results interpreted without knowledge of the results of the index test?                 | Unclear     | Yes         | No          | Unclear        |
| <b>Could the reference standard, its conduct, or its interpretation have introduced bias?</b>                          | <b>High</b> | <b>Low</b>  | <b>High</b> | <b>Unclear</b> |
| <b>Are there concerns that the reference standard, its conduct, or interpretation differ from the review question?</b> | <b>High</b> | <b>Low</b>  | <b>Low</b>  | <b>High</b>    |
| <b>Flow and timing</b>                                                                                                 |             |             |             |                |
| 1. Was there an appropriate interval between index test(s) and reference standard?                                     | Unclear     | Yes         | No          | Yes            |
| 2. Did all participants receive the same reference standard?                                                           | No          | Yes         | Yes         | Yes            |
| 3. Were all participants included in the analysis?                                                                     | No          | No          | Yes         | No             |
| <b>Could the participant flow have introduced bias?</b>                                                                | <b>High</b> | <b>High</b> | <b>High</b> | <b>High</b>    |

## 7.5 Evidence tables

**Hadar E, Dorfman E, Bardin R, Gabbay-Benziv R, Amir J, Pardo J. Symptomatic congenital cytomegalovirus disease following non-primary maternal infection: a retrospective cohort study. BMC Infect Dis. 2017 Jan 5;17(1):31.(20)**

|                                                     |                                                                                                                                                                                                                                                                                                                                                             |
|-----------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Design                                              | Retrospective cohort study                                                                                                                                                                                                                                                                                                                                  |
| Source of funding and competing interest            | No conflicts of interest declared. No funding requested.                                                                                                                                                                                                                                                                                                    |
| Setting                                             | Referral center in Israel from June 2006 to December 2013.                                                                                                                                                                                                                                                                                                  |
| Sample size                                         | 12 non-primary maternal CMV infections with congenital symptomatic infection (the children following primary maternal infections are not considered)                                                                                                                                                                                                        |
| Time interval between tests                         | Not discussed                                                                                                                                                                                                                                                                                                                                               |
| Statistical analysis                                | Categorical variables were compared using Fischer's exact or Pearson Chi-square tests. Continuous variables were analyzed by One Way Analysis of Variance (ANOVA). A probability value below 0.05 was considered significant.                                                                                                                               |
| Patients                                            | Symptomatic neonates with cCMV following either primary or non-primary symptomatic infection                                                                                                                                                                                                                                                                |
| Eligibility criteria                                | Not further described                                                                                                                                                                                                                                                                                                                                       |
| Exclusion criteria                                  | Insufficient data on antenatal/neonatal care and serological data. Congenital asymptomatic infection                                                                                                                                                                                                                                                        |
| Index test                                          | Complete maternal serologic data (IgG, IgM, IgG avidity)                                                                                                                                                                                                                                                                                                    |
| Target condition                                    | Symptomatic cCMV                                                                                                                                                                                                                                                                                                                                            |
| Reference standard                                  | CMV positive urinary shell vial culture or PCR, performed within 48h after birth and up to 2 weeks following delivery.                                                                                                                                                                                                                                      |
| Key methodological features                         | Retrospective cohort of 107 neonates with congenital symptomatic CMV infection, following either primary (n = 95) or non-primary (n = 12) maternal CMV infection. We compared the groups for the manifestations and severity of congenial CMV disease, as well as for possible factors associated with the risk of developing CMV related infant morbidity. |
| Results                                             |                                                                                                                                                                                                                                                                                                                                                             |
| n patients with symptomatic cCMV                    | 12                                                                                                                                                                                                                                                                                                                                                          |
| n patients with IgM in symptomatic cCMV             | 3/12 (25%)                                                                                                                                                                                                                                                                                                                                                  |
| n patients with significant increase in IgG in cCMV | /                                                                                                                                                                                                                                                                                                                                                           |
| n patients without cCMV                             | Data not collected                                                                                                                                                                                                                                                                                                                                          |



|                                                        |                                                                |
|--------------------------------------------------------|----------------------------------------------------------------|
| n patients with IgM + in no cCMV                       | /                                                              |
| n patients with significant increase in IgG in no cCMV | /                                                              |
| Observations                                           | It is unclear if all NPI mothers were tested with IgM serology |

**Périllaud-Dubois C, Letamendia E, Bouthry E, Rafek R, Thouard I, Vieux-Combe C, Picone O, Cordier AG, Vauloup-Fellous C. Cytomegalovirus Specific Serological and Molecular Markers in a Series of Pregnant patients With Cytomegalovirus Non Primary Infection. Viruses. 2022 Oct 31;14(11):2425.(22)**

|                                                     |                                                                                                                                                                                |
|-----------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Design                                              | Retrospective cohort study                                                                                                                                                     |
| Source of funding and competing interest            | The authors declare no conflict of interest.                                                                                                                                   |
| Setting                                             | One level III maternity hospital in France, another non described French maternity, between 2016 and 2021                                                                      |
| Sample size                                         | n=53                                                                                                                                                                           |
| Time interval between tests                         | Serum samples collected during pregnancy were retrospectively analyzed if patients fulfilled inclusion criteria                                                                |
| Statistical analysis                                | No information                                                                                                                                                                 |
| Patients                                            | Documented maternal immunity before pregnancy, confirmed cCMV infection in foetus/neonate                                                                                      |
| Eligibility criteria                                | Not further discussed                                                                                                                                                          |
| Exclusion criteria                                  | Patient without established contact with CMV prior to pregnancy. Patients who did not deliver a cMV child                                                                      |
| Index test                                          | CMV-IgG and CMV-IgM and IgG avidity. First measure before 10 weeks to establish serostatus. Significant rise of IgG is doubling of antibody titer                              |
| Target condition                                    | cCMV                                                                                                                                                                           |
| Reference standard                                  | positive CMV PCR in urine samples.                                                                                                                                             |
| Key methodological features                         | 195 serum samples collected antenatally, median 4 sera per patient, in first, second and/or third trimester. In the sera IgG, IgM and CMVDA are investigated as markers of NPI |
| Results                                             |                                                                                                                                                                                |
| n patients with cCMV                                | 53                                                                                                                                                                             |
| n patients with IgM in at least one serum + in cCMV | not extractable, but not more than 21                                                                                                                                          |



|                                                                           |                     |
|---------------------------------------------------------------------------|---------------------|
| n patients with significant increase in IgG in at least one serum in cCMV | 3/53 (5.6%)         |
| n patients without cCMV                                                   | excluded from study |
| n patients with IgM + in no cCMV                                          | /                   |
| n patients with significant increase in IgG in no cCMV                    | /                   |
| Other results                                                             |                     |
| Stable IgG titer, negative IgM, negative PCR                              | 29/53               |
| Rising IgG titer, negative IgM, negative PCR                              | n= 3/53             |
| Stable IgG titer with either positive IgM or positive PCR                 | 14/53               |
| Rising IgG titer with either positive IgM or positive PCR                 | n=7/53              |

**Huang Y, Tang J, Yu H, Song Q, Hao M, Wang H, Liu J, Dong Y, Liang M, Zhuang S, Li C, Wang J, Liang C, Su Y, Li T, Wu T, Ge S, Zhang J, Xia N. Reconsideration of Maternal Serological Testing for Predicting Congenital CMV Infection. J Infect Dis. 2024 Jun 14;229(6):1817-1822.(21)**

|                                          |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
|------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Design                                   | Nested case-control study                                                                                                                                                                                                                                                                                                                                                                                                                                                       |
| Source of funding and competing interest | The authors declare no conflict of interest.                                                                                                                                                                                                                                                                                                                                                                                                                                    |
| Setting                                  | 3 county-level maternal and child health hospitals located in Xinmi, Zhongmu, and Jiaxian, Henan Province, China, June 2015 to May 2018                                                                                                                                                                                                                                                                                                                                         |
| Sample size                              | 48 cases and 144 controls taken from the 6602 enrolled pregnant patients who had seropositive results in early pregnancy (before 23 gestational weeks), 6228 had live newborns who were followed up with screening and confirmation of cCMV infection by CMV PCR tests for both saliva and urine samples.                                                                                                                                                                       |
| Time interval between tests              | Not defined.                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
| Statistical analysis                     | Based on the hypothesis that the proportions of CMV DNA-positive results in the maternal samples of the cCMV group and uninfected group would be 20% and 5%, respectively, the statistical power increased with the ratio. With a ratio of 3, the statistical power reached 0.9. Pearson $\chi^2$ or Fisher exact test was used to analyze categorical variables. Conditional logistic regression was applied to analyze the odds ratio (OR) of maternal DNAemia or urinary CMV |



|                                                        |                                                                                                                                                                                                                                                                                                                                                                                                 |
|--------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                                                        | shedding with infant cCMV infection status; the adjusted OR was attained by a multivariable model involving maternal age and the gestational week at which the sample was taken.                                                                                                                                                                                                                |
| Patients                                               | Patients with seropositive results early in pregnancy (previous contact with CMV). Patients who had a newborn tested positive for CMV DNA. Newborns with negative saliva and urine test results were deemed newborns without cCMV infection. Subjects without CMV infection who had a complete series of maternal tests during pregnancy were selected as the control group to match the cases. |
| Eligibility criteria                                   | Pregnant patients were consecutively enrolled at their first antenatal visit in a mother-child cohort.<br>For the case-control study newborns with congenital CMV infection were included, and each of them was matched to 3 newborns without cCMV infection.                                                                                                                                   |
| Exclusion criteria                                     | Not specified.                                                                                                                                                                                                                                                                                                                                                                                  |
| Index test                                             | Retrospective samples were tested for IgG avidity and IgM antibodies in maternal serum and for CMV DNA in maternal blood and urine.                                                                                                                                                                                                                                                             |
| Target condition                                       | cCMV                                                                                                                                                                                                                                                                                                                                                                                            |
| Reference standard                                     | CMV DNA in saliva and urine samples within 13 days of birth; for those with positive saliva and/or urine screening results, subsequent saliva and urine samples would typically be collected within 21 days of birth to confirm the result                                                                                                                                                      |
| Key methodological features                            | Newborns with cCMV were included, and each of them was matched to 3 newborns without cCMV. Retrospective samples were tested for IgG, avidity and IgM in maternal serum in middle and late pregnancy, and CMV DNA in maternal blood and urine. IgG increase was defined as a 4-fold increase in IgG titers.                                                                                     |
| Results                                                |                                                                                                                                                                                                                                                                                                                                                                                                 |
| n patients with cCMV                                   | 48                                                                                                                                                                                                                                                                                                                                                                                              |
| n patients with IgM in cCMV                            | 1/48(2.1%)                                                                                                                                                                                                                                                                                                                                                                                      |
| n patients with significant increase in IgG in cCMV    | 4/48 (8.5%)                                                                                                                                                                                                                                                                                                                                                                                     |
| n patients without cCMV                                | 142                                                                                                                                                                                                                                                                                                                                                                                             |
| n patients with IgM + in no cCMV                       | 6/142 (4.2%)                                                                                                                                                                                                                                                                                                                                                                                    |
| n patients with significant increase in IgG in no cCMV | 2 (1.4%)                                                                                                                                                                                                                                                                                                                                                                                        |
| Other results                                          |                                                                                                                                                                                                                                                                                                                                                                                                 |
| CMV DNA in maternal blood in cCMV                      | 18 (40.9%)                                                                                                                                                                                                                                                                                                                                                                                      |



|                                      |            |
|--------------------------------------|------------|
| CMV DNA in maternal blood in no cCMV | 16(11.6%)  |
| CMV DNA in maternal urine in cCMV    | 8 (17%)    |
| CMV DNA in maternal urine in cCMV    | 50 (35.2%) |

**Tang J, Wei H, Dai Y, Luo Y, Hu Y, Zhou YH, Lin N, Liu A. Transplacental Transmission of Cytomegalovirus (CMV) in Pregnant patients with Positive Anti-CMV IgG and Negative Anti-CMV IgM in Highly CMV Seropositive Region. Pathogens. 2025 Sep 5;14(9):894(23)**

|                                          |                                                                                                                                                                                                                                                                           |
|------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Design                                   | Prospective cohort study                                                                                                                                                                                                                                                  |
| Source of funding and competing interest | This research was funded by grants from the Science and Technology Department of Changzhou City (CJ20220009) and from the Science and Technology Department of Jiangsu Province (BK20221169), Jiangsu Province, China. The authors declare no conflicts of interest.      |
| Setting                                  | Two tertiary hospitals in Jiangsu province of China                                                                                                                                                                                                                       |
| Sample size                              | 695 pregnant patients were enrolled between April 2019 and February 2023. Of them, 567 (81.6%) were single pregnancies and 128 (18.4%) were twin pregnancies                                                                                                              |
| Time interval between tests              | Both tests were performed at the moment of cesarean section                                                                                                                                                                                                               |
| Statistical analysis                     | Continuous data were presented as mean $\pm$ standard deviation (SD). Categorical variables were reported as number and percentage and compared by the $\chi^2$ test or Fisher's exact test where appropriate. A two-sided p-value of $<0.05$ was considered significant. |
| Patients                                 | Pregnant patients with positive anti-CMV IgG and negative anti-CMV IgM during the first or second trimester who delivered by Cesarean section                                                                                                                             |
| Eligibility criteria                     | Not further discussed                                                                                                                                                                                                                                                     |
| Exclusion criteria                       | HIV positive or received immunosuppression therapy                                                                                                                                                                                                                        |
| Index test                               | CMV IgG and IgM were measured with enzyme-linked immunosorbent assay                                                                                                                                                                                                      |
| Target condition                         | cCMV                                                                                                                                                                                                                                                                      |
| Reference standard                       | CMV DNA positive in amniotic fluid                                                                                                                                                                                                                                        |
| Key methodological features              | At the moment of cesarean section maternal serum was tested for anti-CMV IgG and IgM, amniotic fluid was tested for CMV-DNA. The proportion of positive serology in CMVDNA positive and negative (in amniotic fluid) patients is reported.                                |
| Results                                  |                                                                                                                                                                                                                                                                           |
| n patients with cCMV                     | 5                                                                                                                                                                                                                                                                         |



|                                                        |               |
|--------------------------------------------------------|---------------|
| n patients with IgM in cCMV                            | 0/5 (0%)      |
| n patients with significant increase in IgG in cCMV    | Not analysed  |
| n patients without cCMV                                | 690           |
| n patients with IgM + in no cCMV                       | 11/690 (1.6%) |
| n patients with significant increase in IgG in no cCMV | Not analysed  |

## 7.6 Summary tables for GRADE

Effect estimates (e.g., sensitivity/specificity) cannot be computed because the included studies lack consistent diseased vs. non-diseased comparators with uniform application of the index test and reference standard, use heterogeneous thresholds/timing for IgG/IgM, and have very few events.

**Table 11 – Summary GRADE table for non-primary maternal infection**

| Marker                                | Studies & participants                                                                         | Patients cCMV                                                                 | with               | Patients without cCMV | Effect estimate                                                                                                           | Certainty of the evidence | Reasons                                                                            |
|---------------------------------------|------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------|--------------------|-----------------------|---------------------------------------------------------------------------------------------------------------------------|---------------------------|------------------------------------------------------------------------------------|
| IgM positivity                        | Hadar 2017 (n=12) <sup>1</sup> ; Huang 2024 (n=48) <sup>3</sup> ; Tang 2025 (n=5) <sup>4</sup> | Hadar: 3/12 (25%);<br>Huang: 2.1% (1/48) <sup>3</sup>                         | /                  | Huang: 4.2% (6/142)   | OR 0.49 (95%CI 0.05 to 4.19)                                                                                              | Very low                  | Risk of bias; inconsistency; indirectness; imprecision <sup>67</sup>               |
|                                       |                                                                                                | Tang: 0% (0/5) <sup>4</sup>                                                   | Tang 1.6% (11/690) |                       | OR 5.45 (95%CI 0.28 to 104.62)<br>The OR is driven by the continuity correction due to the 0 events in patients with cCMV |                           |                                                                                    |
| Significant IgG rise among cCMV cases | Périllaud-Dubois 2022 (n=53) <sup>2</sup> ; Huang 2024 (n=48) <sup>3</sup>                     | Périllaud-Dubois: 5.7% (3/53) <sup>2</sup><br>Huang: 8.5% (4/48) <sup>3</sup> | /                  | Huang: 1.4% (2/142)   | OR 5.91 (95%CI 1.05 to 33.32)                                                                                             | Very low                  | Risk of bias; inconsistency in thresholds; indirectness; imprecision <sup>67</sup> |

1. Hadar E. et al. (2017): Retrospective cohort of symptomatic cCMV after non-primary maternal infection; small sample; neonatal PCR/culture within 48h–2 weeks after birth; IgM+ 25%; no non-cCMV comparator.

2. Périllaud-Dubois C. et al. (2022): Retrospective cohort including only cCMV; IgM positivity not fully extractable; significant IgG rise defined as doubling; potential selection bias.

3. Huang Y. et al. (2024): Nested case–control with neonatal saliva/urine PCR reference; IgG rise threshold  $\geq 4$ -fold

4. Tang J. et al. (2025): Prospective cohort; maternal tests at cesarean; fetal infection defined by amniotic fluid CMV DNA; only 5 cCMV cases; IgM 0%.

6. GRADE: Risk of bias (retrospective designs, unclear blinding, incomplete or selective data).

7. GRADE: Inconsistency (heterogeneous IgG thresholds: doubling vs  $\geq 4$ -fold; varied sampling times; differing laboratory platforms).

8. GRADE: Indirectness and imprecision (different reference standards—neonatal vs amniotic fluid; small numbers of cCMV cases; wide or non-estimable CIs).



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

### 8.1 PICO

Table 12 – PICO

|                 | Should high dose Valaciclovir be used in pregnant patients with a primary CMV infection?                                                                                                                                                                                                                                                                                                                                                                                      |
|-----------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Objective       | To determine whether pregnant patients with a primary CMV infection should be treated with high dose valaciclovir for secondary or tertiary prevention of congenital CMV                                                                                                                                                                                                                                                                                                      |
| Review Question | In pregnant patients with a primary CMV infection, should valaciclovir(8g/day) be used compared to placebo or no treatment, to reduce the risk of fetal or neonatal CMV infection, adverse pregnancy outcomes, and long-term sequelae in the child?                                                                                                                                                                                                                           |
| Population      | Pregnant patients with a primary CMV infection                                                                                                                                                                                                                                                                                                                                                                                                                                |
| Intervention    | Valaciclovir 8g/day                                                                                                                                                                                                                                                                                                                                                                                                                                                           |
| Comparator      | Placebo /No treatment                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
| Outcomes        | Critical: Severe antenatal imaging anomalies, termination of pregnancy, severely symptomatic newborns, cerebral palsy, motor developmental delay, cognitive developmental delay, severe adverse events for mother and child<br>Important: fetal or neonatal CMV infection, mild antenatal imaging abnormalities, miscarriage, moderate symptoms at birth, hearing loss, visual impairment, epilepsy, behavioral difficulties, vestibular dysfunction, adverse event for child |
| Study design    | Good quality systematic reviews, diagnostic randomised controlled trials, prospective and retrospective diagnostic cohort studies, in which the index test and the reference standard are applied to the same patients.<br>Excluded: Narrative reviews. Case reports. Studies presented as conference abstract only or if no full text was available                                                                                                                          |
| Analysis        | By trimester of seroconversion if available                                                                                                                                                                                                                                                                                                                                                                                                                                   |

### 8.2 Search strategies

All searches were performed on April 1<sup>st</sup>, 2025

#### 8.2.1 Medline through Pubmed

Database: Ovid MEDLINE(R) and Epub Ahead of Print, In-Process, In-Data-Review & Other Non-Indexed Citations, Daily and Versions <1946 to February 14, 2025>

Search Strategy:

- 
- 1 exp Cytomegalovirus Infections/ (29594)
  - 2 Cytomegalovirus/ (24078)
  - 3 1 or 2 (39917)
  - 4 CMV.ab,ti,kf. (31586)



- 5 (saliva\* adj2 gland\* adj2 virus\*).ti,ab,kf. (217)
- 6 (hhv5 or hhv 5).ti,ab,kf. (68)
- 7 (herpesvirus 5 or herpes virus 5).ti,ab,kf. (211)
- 8 cytomegal\*.ab,ti,kf,ot. (51282)
- 9 hcmv.ab,ti,kf. (7413)
- 10 (inclusion\* adj2 disease\*).ti,ab,kf. (2111)
- 11 (saliva\* adj2 gland\* adj2 disease\*).ti,ab,kf. (1025)
- 12 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 (64164)
- 13 3 or 12 (68967)
- 14 Prenatal Care/ (34300)
- 15 exp Pregnancy/ (1052191)
- 16 exp Pregnancy complications/ (495233)
- 17 perinatal care/ (5649)
- 18 pregnant women/ (16250)
- 19 14 or 15 or 16 or 17 or 18 (1091001)
- 20 (antenatal\* or ante-natal\* or prenatal\* or pre-natal\* or perinatal\* or peri-natal\* or pregnan\* or antepartum or ante-partum or peripartum or peri-partum).ti,ab,kf. (804969)
- 21 pregnant.ab,ti,kf,jw. (239192)
- 22 pregnanc\*.ab,ti,kf,jw. (558892)
- 23 20 or 21 or 22 (807336)
- 24 19 or 23 (1306518)
- 25 13 and 24 (5193)
- 26 Valacyclovir/ (1255)
- 27 val?c#clovir.ab,ti,kf,rn,nm. (2120)
- 28 valtrex.ab,ti,kf,rn,nm. (41)
- 29 VALACV.ab,ti,kf,rn,nm. (2)
- 30 rapivir.ab,ti,kf,rn,nm. (0)
- 31 talavir.ab,ti,kf,rn,nm. (0)
- 32 vacv.ab,ti,kf,rn,nm. (1032)
- 33 valcyclor.ab,ti,kf,rn,nm. (0)
- 34 valdacir.ab,ti,kf,rn,nm. (0)
- 35 valherpes.ab,ti,kf,rn,nm. (0)
- 36 virval.ab,ti,kf,rn,nm. (0)
- 37 zelitrex.ab,ti,kf,rn,nm. (6)
- 38 (124832-26-4 or 124832-27-5 or 124832-28-6 or 125581-72-8 or 142963-59-5 or 142963-60-8 or 142963-71-1).ab,ti,kf,rf,nm. (3)



39 (256u87 or 8s9act5d06 or "acyclovir, l valyl ester" or bw256u87 or "d-valacyclovir" or g447s0t1vc or mz1iw7q79d).ab,ti,kf,rn,nm. (1253)

40 27 or 28 or 29 or 30 or 31 or 32 or 33 or 34 or 35 or 36 or 37 or 38 or 39 (3091)

41 26 or 40 (3091)

42 25 and 41 (89)

### 8.2.2 Embase

Embase session results (17 Feb 2025)

#51 #50 NOT ('conference abstract'/it OR 'conference paper'/it OR 'conference review'/it) (36)

#50 #49 NOT [medline]/lim (55)

#49 #13 AND #32 AND #48 (212)

#48 #33 OR #47 (11987)

#47 #34 OR #35 OR #36 OR #37 OR #38 OR #39 OR #40 OR #41 OR #42 OR #43 OR #44 OR #45 OR #46 (11974)

#46 256u87:ab,ti,tn,rn OR 8s9act5d06:ab,ti,tn,rn OR 'acyclovir, l valyl ester':ab,ti,tn,rn OR bw256u87:ab,ti,tn,rn OR 'd-valacyclovir':ab,ti,tn,rn OR

g447s0t1vc:ab,ti,tn,rn OR mz1iw7q79d:ab,ti,tn,rn (21)

#45 '124832-26-4':ab,ti,tn,rn OR '124832-27-5':ab,ti,tn,rn OR '124832-28-6':ab,ti,tn,rn OR '125581-72-8':ab,ti,tn,rn OR '142963-59-5':ab,ti,tn,rn OR

'142963-60-8':ab,ti,tn,rn OR '142963-71-1':ab,ti,tn,rn (10277)

#44 zelitrex:ab,ti,tn (176)

#43 virval:ab,ti,tn (2)

#42 valherpes:ab,ti,tn (2)

#41 valdacir:ab,ti,tn (0)

#40 valcyclor:ab,ti,tn (0)

#39 vacv:ab,ti,tn (1233)

#38 talavir:ab,ti,tn (1)

#37 rapivir:ab,ti,tn (0)

#36 valacv:ab,ti,tn (8)

#35 valtrex:ab,ti,tn (625)

#34 val\$c?clovir:ab,ti,tn (2805)

#33 'valaciclovir'/exp (10658)

#32 #19 OR #31 (1697866)

#31 #20 OR #21 OR #22 OR #23 OR #24 OR #25 OR #26 OR #27 OR #28 OR #29 OR #30 (1070028)

#30 'peri partum':ti,ab,kw (582)

#29 peripartum:ti,ab,kw (11333)

#28 'ante partum':ti,ab,kw (657)

#27 antepartum:ti,ab,kw (10698)

#26 pregnan\*:ti,ab,kw,jt (881710)  
 #25 'peri natal\*:ti,ab,kw (456)  
 #24 perinatal\*:ti,ab,kw (135095)  
 #23 'pre natal\*:ti,ab,kw (2140)  
 #22 prenatal\*:ti,ab,kw (172594)  
 #21 'ante natal\*:ti,ab,kw (1076)  
 #20 antenatal\*:ti,ab,kw (73787)  
 #19 #14 OR #15 OR #16 OR #17 OR #18 (1440515)  
 #18 'pregnancy complication'/exp (690697)  
 #17 'pregnant woman'/de (128163)  
 #16 'perinatal care'/exp (79800)  
 #15 'pregnancy'/exp (931524)  
 #14 'prenatal care'/exp (195966)  
 #13 #3 OR #12 (120596)  
 #12 #4 OR #5 OR #6 OR #7 OR #8 OR #9 OR #10 OR #11 (95301)  
 #11 (saliva\* NEAR/2 gland\* NEAR/2 disease\*):ti,ab,kw (957)  
 #10 (inclusion\* NEAR/2 disease\*):ti,ab,kw (2995)  
 #9 hcmv:ab,ti,kw (8555)  
 #8 cytomegal\*:ab,ti,kw (65883)  
 #7 'herpesvirus 5':ab,ti,kw OR 'herpes virus 5':ab,ti,kw (254)  
 #6 hhv5:ab,ti,kw OR 'hhv 5':ab,ti,kw(103)  
 #5 (saliva\* NEAR/2 gland\* NEAR/2 virus\*):ab,ti,kw (161)  
 #4 cmv:ab,ti,kw (55512)  
 #3 #1 OR #2 (88087)  
 #2 'cytomegalovirus'/exp (50709)  
 #1 'cytomegalovirus infection'/exp (51054)

### 8.2.3 Cochrane

Date Run: 17/02/2025 14:44:57

#1 [mh "Cytomegalovirus Infections"] (1063)  
 #2 [mh ^"Cytomegalovirus"] (462)  
 #3 #1 or #2 (1129)  
 #4 CMV:ab,ti (2323)  
 #5 (saliva\* NEAR/2 gland\* NEAR/2 virus\*):ti,ab (2)  
 #6 (hhv5 or hhv 5):ti,ab (49)  
 #7 (herpesvirus 5 or "herpes virus 5"):ti,ab 72  
 #8 cytomegal\*:ab,ti (2053)



- #9 hcmv:ab,ti (78)
- #10 (inclusion\* NEAR/2 disease\*):ti,ab (1247)
- #11 (saliva\* NEAR/2 gland\* NEAR/2 disease\*):ti,ab (24)
- #12 #4 or #5 or #6 or #7 or #8 or #9 or #10 or #11 (4426)
- #13 #3 or #12 (4520)
- #14 [mh ^"Prenatal Care"] (2418)
- #15 [mh "Pregnancy"] (34889)
- #16 [mh "Pregnancy complications"] (18375)
- #17 [mh ^"perinatal care"] (270)
- #18 [mh ^"pregnant women"] (1060)
- #19 #14 or #15 or #16 or #17 or #18 (39511)
- #20 (antenatal\* or ante-natal\* or prenatal\* or pre-natal\* or perinatal\* or peri-natal\* or pregnan\* or antepartum or ante-partum or peripartum or peri-partum):ti,ab (82641)
- #21 pregnant:ab,ti,so (31522)
- #22 pregnanc\*:ab,ti,so (62577)
- #23 #20 or #21 or #22 (82934)
- #24 #19 or #23 (97402)
- #25 #13 and #24 (285)
- #26 [mh "Valacyclovir"] (318)
- #27 val?c\*clovir:ab,ti (535)
- #28 valtrex:ab,ti (33)
- #29 VALACV:ab,ti (2)
- #30 rapivir:ab,ti (0)
- #31 talavir:ab,ti (0)
- #32 vacv:ab,ti (14)
- #33 valcyclor:ab,ti (0)
- #34 valdacir:ab,ti (0)
- #35 valherpes:ab,ti (0)
- #36 virval:ab,ti (0)
- #37 zelitrex:ab,ti (2)
- #38 ("124832-26-4" or "124832-27-5" or "124832-28-6" or "125581-72-8" or "142963-59-5" or "142963-60-8" or "142963-71-1"):ab,ti (7)
- #39 (256u87 or 8s9act5d06 or "acyclovir, l valyl ester" or bw256u87 or "d-valacyclovir" or g447s0t1vc or mz1iw7q79d):ab,ti (4)
- #40 #27 or #28 or #29 or #30 or #31 or #32 or #33 or #34 or #35 or #36 or #37 or #38 or #39 (546)
- #41 #26 or #40 (566)

- #42 #25 and #41 (20)
- #43 seroconver\*:ab,ti (4652)
- #44 [mh "Seroconversion"] (163)
- #45 #43 or #44 (4663)
- #46 [mh "Infections"] (110861)
- #47 infection:ab,ti (88822)
- #48 #46 or #47 (166676)
- #49 #45 or #48 (168420)
- #50 #42 and #49 (20)

### 8.3 Study selection

Figure 13 – Study flow selection Treatment with valaciclovir

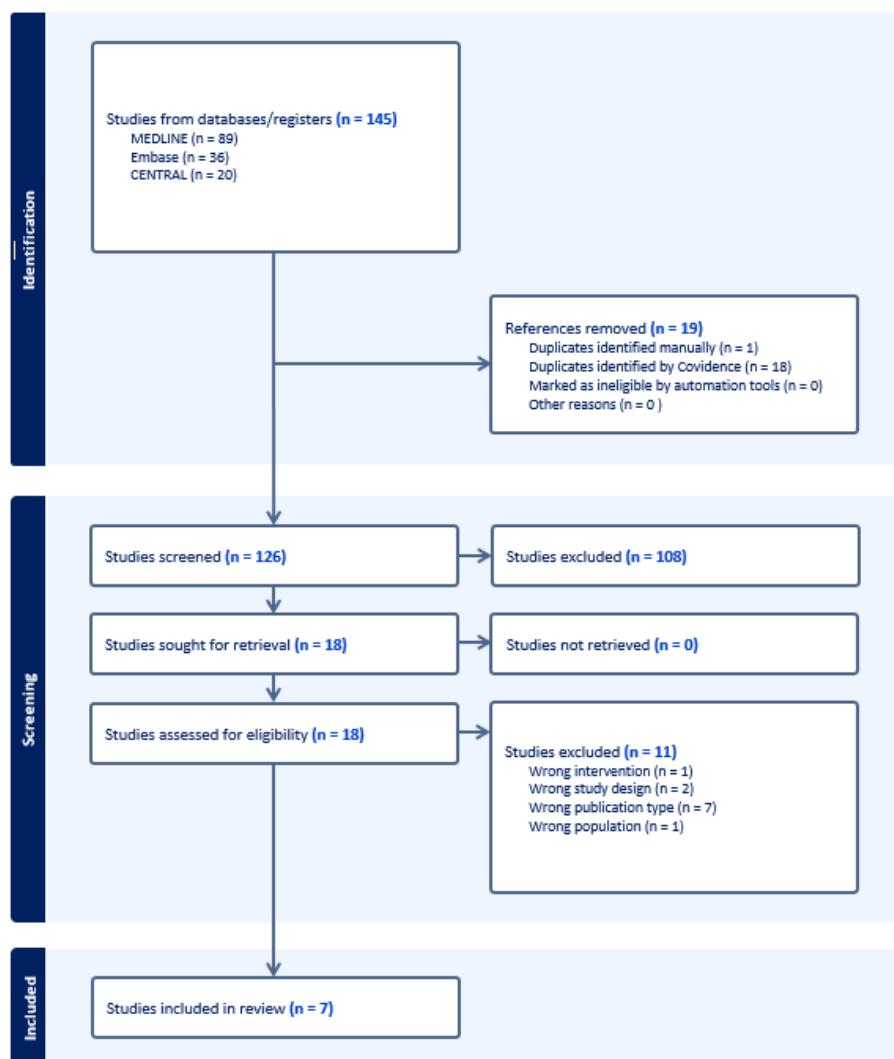


Figure provided by Covidence



### 8.3.1 Studies excluded with reason

**Table 13 – Excluded primary studies for treatment with valaciclovir**

| Study and year          | Exclusion reason                              |
|-------------------------|-----------------------------------------------|
| Chatzakis 2024          | Exclusion reason: wrong publication type (SR) |
| D'Antonio 2023          | Exclusion reason: wrong publication type (SR) |
| DeSantis 2020           | Exclusion reason: wrong publication type      |
| DelaCalle 2022          | Exclusion reason: wrong intervention          |
| Fitzpatrik 2022         | Exclusion reason: wrong publication type (SR) |
| Hamilton 2014           | Exclusion reason: wrong publication type (SR) |
| Nigro 2023              | Exclusion reason: wrong study design          |
| Roxby 2014              | Exclusion reason: wrong population            |
| Rybak-Krzyszkowska 2023 | Exclusion reason: wrong publication type (SR) |
| Schurder 2021           | Exclusion reason: wrong study design          |
| Ville 2021              | Exclusion reason: wrong publication type      |

SR systematic review: checked for references only.

## Appendix 1.1. Appraisal

The selected studies are one RCT (Shahar-Nissan)(24) that is evaluated with the ROB2(25) evaluation tool *and 6 observational studies (Egglof(26), Faure-Bardon(27), Zammarchi(28), Amir(29), Jacquemard(30), Leruez-Ville(31))* that are evaluated with the ROBINS-I(32) evaluation tool (reference).

**Table 14 – ROB2 evaluation for RCT on treatment with valaciclovir**

| <i>Risk of Bias</i>                                                                                                    | <i>Shahar-Nissan 2020(24)</i>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
|------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Domain 1: Risk of bias arising from the randomization process</b>                                                   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
| <b>Low</b>                                                                                                             | <p>Randomisation was done with computer-generated random number tables. Randomisation was done separately for participants infected periconceptionally or during the first trimester and was done in blocks of four; two of every four participants received drug, and the remaining two received placebo. Each recruited patient received a serial randomisation code. This code matched the randomisation code on the study medication bottle labels. The bottles were identical, and there was no way to know which contained valaciclovir and which placebo. The physicians of the study group enrolled the participants, with randomisation done by a separate designated pharmaceutical team outside the hospital, who provided participants with a randomisation number in a sequential matter. The designated randomization code remained blinded throughout study conduct.</p> <p>Participants in the treatment arm received valaciclovir (8 g per day); participants in the control group received the same number of identical-looking placebo tablets. Both valaciclovir and placebo were prepared by an outside pharmacy and delivered in boxes labelled with only the randomisation number in order to maintain masking. Patients and researchers were masked to participant allocation throughout the entire study period</p> |
| <b>Domain 2: Risk of bias due to deviations from the intended interventions (effect of assignment to intervention)</b> |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
| <b>High</b>                                                                                                            | <p>Per protocol analysis</p> <ul style="list-style-type: none"> <li>- exclusion of 2 patients assigned to the valaciclovir group because of complete non-adherence</li> <li>- exclusion of termination of pregnancy before amniocentesis, but after randomisation (1 in valaciclovir group and 1 in the placebo-group)</li> <li>- exclusion of 1 non-viable pregnancy after randomization in the valaciclovir group</li> </ul>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
| <b>Domain 3: Risk of bias due to missing outcome data</b>                                                              |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
| <b>High</b>                                                                                                            | <p>Results on amniocentesis were missing in 4 fetuses from the valaciclovir group and 3 from the placebo group. Amniocentesis results for the four neonates with positive urine PCR were presumed positive for the purpose of our data analysis (three of the neonates—twins and another fetus—were in the placebo group and one in the valaciclovir group). In a post-hoc analysis, after exclusion of these six participants (and seven fetuses), the primary</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |



|                                                                   |                                                                                                                                                                                                                                                                                                               |
|-------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                                                                   | endpoint results remained unchanged, with four (9%) of 43 amniocenteses positive in the valaciclovir group compared with 11 (26%) of 42 in the placebo group (p=0.038). No information on missing data for other outcomes.                                                                                    |
| <b>Domain 4: Risk of bias in measurement of the outcome</b>       |                                                                                                                                                                                                                                                                                                               |
| <b>Low for primary outcome: CMV PCR at amniocentesis</b>          | PCR CMV DNA amniocentesis is an objective measurement, performed in the same way for both groups                                                                                                                                                                                                              |
| <b>High for secondary outcomes</b>                                | Some of the follow-up data is collected by phone call.                                                                                                                                                                                                                                                        |
| <b>Domain 5: Risk of bias in selection of the reported result</b> |                                                                                                                                                                                                                                                                                                               |
| <b>Some concerns</b>                                              | Not sufficient details in the registered protocol on how symptoms after birth would be measured/collected. Accurate and detailed description of follow-up after birth in the publication, but are the outcomes systematically collected for the study? Selective reporting on secondary outcomes is possible. |
| <b>Overall risk of bias = High</b>                                |                                                                                                                                                                                                                                                                                                               |

Table 15 – ROBINS-I Treatment with valaciclovir

| <b>Amir 2023(29)</b>                                                                      | <b>Egloff 2023(26)</b>                                                                                                      | <b>Faure-Bardon 2021(27)</b>                                                                                                                 | <b>Jacquemard 2007(30)</b>                                                                                                 | <b>Leruez Ville 2016(31)</b>                                                       | <b>Zammarchi 2023(28)</b>                                                                                                                                                        |
|-------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Domain 1: bias due to confounding</b>                                                  |                                                                                                                             |                                                                                                                                              |                                                                                                                            |                                                                                    |                                                                                                                                                                                  |
| <b>Moderate</b><br>No adjustments for confounding                                         | <b>Moderate</b><br>Propensity score matching for timing of infection, residual confounding possible                         | <b>Moderate</b><br>Propensity score matching for timing of infection and gestational age at amniocentesis, but low number of events          | <b>Moderate</b><br>No attempt was made neither to match nor to compare outcomes of treated with untreated fetuses directly | <b>Moderate</b><br>No adjustments for confounding                                  | <b>Moderate</b><br>Inverse probability weighting, but low number of events to account for the confounding effect of the observed pretreatment variables, risk of over-adjustment |
| <b>Domain 2: bias due to selection of participants</b>                                    |                                                                                                                             |                                                                                                                                              |                                                                                                                            |                                                                                    |                                                                                                                                                                                  |
| <b>Serious</b><br>Historical controls, exclusions for non-adherence and early termination | <b>Serious</b><br>Controls include patients that declined treatment as well as patients from before treatment was available | <b>Serious</b><br>Controls include patients that declined treatment as well as patients from before treatment was available; exclusion if no | <b>Serious</b><br>Controls include patients that declined treatment as well as patients from before treatment was          | <b>Serious</b><br>Historical controls; exclusion of fetuses with normal ultrasound | <b>Serious</b><br>Controls include patients that declined treatment as well as patients from before treatment were available; 4 centers no                                       |



|                                                                    |                                                                                                    |                                                                                                                    |                                                                                           |                                                                                                                                                                      |                                                       |
|--------------------------------------------------------------------|----------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------|
|                                                                    |                                                                                                    | amniocentesis was performed                                                                                        | available. Only symptomatic fetuses.                                                      | and with severe brain abnormalities                                                                                                                                  | data for untreated patients; non-systematic screening |
| <b>Domain 3: bias in classification of interventions</b>           |                                                                                                    |                                                                                                                    |                                                                                           |                                                                                                                                                                      |                                                       |
| <b>Low</b><br>Clear dosing and monitoring                          | <b>Low</b><br>Clear dosing and monitoring                                                          | <b>Low</b><br>Clear dosing and monitoring                                                                          | <b>Low</b><br>Clear dosing and monitoring                                                 | <b>Low</b><br>Clear dosing and monitoring                                                                                                                            | <b>Low</b><br>Clear dosing and monitoring             |
| <b>Domain 4: bias due to deviation from intended interventions</b> |                                                                                                    |                                                                                                                    |                                                                                           |                                                                                                                                                                      |                                                       |
| <b>Low</b><br>Adherence >90%, minor temporary interruptions        | <b>Moderate</b><br>Possible variation in duration of treatment (after amniocentesis), ITT analysis | <b>Low</b><br>Minimal deviations: one patient temporarily stopped treatment                                        | <b>Low</b><br>1 mother stopped taking valaciclovir 3d before delivery at 38w of gestation | <b>Low</b><br>Adherence >90%, 2 patients stopped treatment, one of them temporarily                                                                                  | <b>Moderate</b><br>Minor deviations, ITT analysis     |
| <b>Domain 5: bias due to missing outcome data</b>                  |                                                                                                    |                                                                                                                    |                                                                                           |                                                                                                                                                                      |                                                       |
| <b>Low</b><br>Outcome largely complete, 2 cases imputed            | <b>Moderate</b><br>No amniocentesis in 8% of valaciclovir and 42% of control group.                | <b>Moderate</b><br>269/310 women underwent amniocentesis; missing data could bias results if not missing at random | <b>Moderate</b><br>No information on (handling of) missing data                           | <b>Moderate</b><br>No information on (handling of) missing data                                                                                                      | <b>Moderate</b><br>180/447 no amniocentesis           |
| <b>Domain 6: bias in measurement of outcomes</b>                   |                                                                                                    |                                                                                                                    |                                                                                           |                                                                                                                                                                      |                                                       |
| <b>Low</b><br>Objective lab result                                 | <b>Low</b><br>Primary outcome objective lab result.                                                | <b>Low</b><br>Objective lab result                                                                                 | <b>Moderate</b><br>Primary outcome is viral load and drug concentration;                  | <b>Moderate</b><br>Not sufficient detail on how they collected information for primary outcome in valaciclovir group and if they did the same in historical control. | <b>Low</b><br>Objective lab result                    |
| <b>Domain 7: bias in selection of reported result</b>              |                                                                                                    |                                                                                                                    |                                                                                           |                                                                                                                                                                      |                                                       |
| <b>Moderate</b>                                                    | <b>Low</b>                                                                                         | <b>Low</b>                                                                                                         | <b>Low</b>                                                                                | <b>Low</b>                                                                                                                                                           | <b>Low</b>                                            |



|                                                                                                |                                                  |                                                  |                                                                                                                               |                                                                 |                                                  |
|------------------------------------------------------------------------------------------------|--------------------------------------------------|--------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------|--------------------------------------------------|
| Only comparative data on primary outcome, potential selective reporting for secondary outcomes | All pre-specified outcomes appear to be reported | All pre-specified outcomes appear to be reported | The reported outcomes are consistent with the study objectives. The aim was not to demonstrate clinical efficacy of the drug. | The reported outcomes are consistent with the study objectives. | All pre-specified outcomes appear to be reported |
| <b>Overall risk of bias</b>                                                                    |                                                  |                                                  |                                                                                                                               |                                                                 |                                                  |
| <b>Serious</b>                                                                                 | Serious                                          | Serious                                          | Serious                                                                                                                       | Serious                                                         | Serious                                          |

## 8.4 Evidence tables

| <b>Shahar-Nissan 2020(24)</b>                   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
|-------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Design</b>                                   | RCT                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |
| <b>Source of funding and competing interest</b> | None                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
| <b>Setting</b>                                  | Single centre, Israel, 2015-2018                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
| <b>Sample size</b>                              | N=100                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
| <b>Statistical analysis</b>                     | <p>A sample size of 38 (corrected for continuity from 32) patients for each study group was originally calculated as sufficient to detect an assumed reduction in the incidence of cytomegalovirus transmission from 40% in the placebo group to 10% in the valaciclovir group, with 80% power by two-sided test at a 5% significance level</p> <p>Statistical analyses were done according to per protocol principles. We used Fisher's exact test to compare proportions and the independent <i>t</i> test to compare means between groups at baseline. Primary analyses included only participants who received at least one dose of study drug. To investigate correlation between twin foetuses in the study we used a generalised linear mixed model to evaluate the outcomes between the two study groups, adjusting for gestational age and period of therapy. <math>p &lt; 0.05</math> was considered the threshold of statistical significance.</p> |
| <b>Eligibility criteria</b>                     | Pregnant patients aged 18 years or older, with serological evidence of a primary cytomegalovirus infection acquired during the periconceptional period or the first trimester of pregnancy. Women were included before the 16th week of gestation so that a minimum of 6 weeks of treatment could be achieved.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
| <b>Exclusion criteria</b>                       | If unable to swallow capsules, suffering from severe vomiting or any pre-existing liver disease, renal dysfunction, or bone marrow suppression, receiving antiviral therapy or immunosuppressive therapy before the study, known hypersensitivity to aciclovir                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
| <b>Intervention</b>                             | Valaciclovir (VAVC) 2 x 4g/d (tablets 500 mg) until amniocentesis ( $\geq 7$ weeks after estimated maternal infection and $\geq 21$ weeks of gestation) (N=50).                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |

|                                             |                                                                                                                                                                                            |                                                                           |                                           |                                             |
|---------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------|-------------------------------------------|---------------------------------------------|
| <b>Comparator</b>                           | Placebo (capsules of lactose monohydrate (233 mg), carboxymethyl cellulose (233 mg), and calcium carbonate (233 mg) in identical gelatine capsules in same regimen as valaciclovir (N=50)) |                                                                           |                                           |                                             |
| <b>Patient characteristics</b>              |                                                                                                                                                                                            | <b>All patients</b>                                                       | <b>Valaciclovir</b>                       | <b>Placebo</b>                              |
| <b>Mean age</b>                             |                                                                                                                                                                                            | No info                                                                   | 32.7y (SD 4.1)                            | 31.1y (SD 4.0)                              |
| <b>Timing infection</b>                     |                                                                                                                                                                                            | Periconceptual 54%<br>First trimester 46%                                 | 58%<br>42%                                | 51%<br>49%                                  |
| <b>Time between infection and treatment</b> |                                                                                                                                                                                            | All patients<br>Periconceptual 63d (SD 19)<br>First trimester 42d (SD 13) | 54d (SD 19)<br>61d (SD 19)<br>44d (SD 14) | 54d (SD 20)<br>66.5d (SD 18)<br>41d (SD 13) |
| <b>GA at start treatment</b>                |                                                                                                                                                                                            | 10.9w (IQR 9.9-13.6)                                                      | 11.5w (SD 2.9)                            | 11.2w (SD 2.4)                              |
| <b>GA at amniocentesis</b>                  |                                                                                                                                                                                            | No info                                                                   | No info                                   | No info                                     |
| <b>Treatment duration</b>                   |                                                                                                                                                                                            | No info                                                                   | No info                                   | No info                                     |
| <b>Results</b>                              |                                                                                                                                                                                            |                                                                           |                                           |                                             |
|                                             | <b>Critical Outcomes</b>                                                                                                                                                                   | <b>Valaciclovir</b>                                                       | <b>Placebo</b>                            | <b>Reported effect size</b>                 |
|                                             | Termination of pregnancy                                                                                                                                                                   | 6% (3/49)                                                                 | 18% (9/50)                                |                                             |
|                                             | In case of positive amniocentesis                                                                                                                                                          | 25% (1/4)                                                                 | 45% (5/11)                                |                                             |
|                                             | with symptoms                                                                                                                                                                              | 25% (1/4)                                                                 | 18% (2/11)                                |                                             |
|                                             | without symptoms                                                                                                                                                                           | 0% (0/4)                                                                  | 27% (3/11)                                |                                             |
|                                             | Severely symptomatic newborns                                                                                                                                                              | 5% (2/43)                                                                 | 12% (5/41)                                |                                             |
|                                             | Severe adverse events mother                                                                                                                                                               | None                                                                      | None                                      |                                             |
|                                             | <b>Important Outcomes</b>                                                                                                                                                                  |                                                                           |                                           |                                             |
|                                             | cCMV at amniocentesis                                                                                                                                                                      | 11% (5/45)                                                                | 30% (14/47)                               | OR 0.29 (95%CI: 0.09-0.9) p=0.027           |
|                                             | Periconceptual infection                                                                                                                                                                   | 12% (3/26)                                                                | 13% (3/24)                                |                                             |
|                                             | First trimester infection                                                                                                                                                                  | 11% (2/19)                                                                | 48% (11/23)                               |                                             |
|                                             | cCMV at birth                                                                                                                                                                              | 21% (9/43)                                                                | 39% (16/41)                               |                                             |
|                                             | Moderate symptoms at birth                                                                                                                                                                 | None                                                                      | None                                      |                                             |
|                                             | Hearing loss                                                                                                                                                                               | 2% (1/43)                                                                 | 12% (5/41)                                |                                             |
|                                             | Miscarriage                                                                                                                                                                                | 2% (1/50)                                                                 |                                           | Excluded from analysis                      |
|                                             | <b>Adverse events</b>                                                                                                                                                                      |                                                                           |                                           | p=0.49                                      |



|                                       |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |             |             |  |
|---------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------|-------------|--|
|                                       | Headache                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    | 20% (9/45)  | 13% (6/45)  |  |
|                                       | Non-specific rash                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           | 2% (1/45)   | 7% (3/45)   |  |
|                                       | Nausea                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      | 29% (13/45) | 22% (10/45) |  |
|                                       | Abdominal pain                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | 9% (4/45)   | 4% (2/45)   |  |
|                                       | Thrombocytopenia                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            | 2% (1/45)   | 0% (0/45)   |  |
| <b>Limitations and other comments</b> | <p>All cases of severely symptomatic newborns were hearing loss, except for 1 treated patient that had bilateral subependymal cysts on brain imaging.</p> <p>Description of postnatal follow-up after birth, but insufficient details on systematic collection of the outcomes.</p> <p>Postnatal evaluation of infected infants included a full physical examination, blood count, liver function tests, brain ultrasound, hearing assessment by brainstem evoked audiometry, and an ophthalmological examination. Follow-up of neonate growth, development, and hearing status was done in the cytomegalovirus follow-up clinic or by a maternal telephone interview.</p> <p>Definition of periconceptional = 4 weeks before and 3 weeks after recorded date of the last menstruation<br/>first trimester = 3-14 weeks</p> <p>Definition of maternal primary infection = seroconversion of IgG from negative to positive during pregnancy, or an IgG with low avidity in the presence of specific IgM and a subsequent, substantial rise in IgG titre.</p> |             |             |  |

### Amir 2023(29)

|                                 |                                                                                                                                                                                                                                                                                                                                                                                                                   |
|---------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Design</b>                   | Retrospective cohort                                                                                                                                                                                                                                                                                                                                                                                              |
| <b>Source of funding (COI?)</b> | Source of funding not reported; No reported conflict of interest                                                                                                                                                                                                                                                                                                                                                  |
| <b>Setting</b>                  | Single centre, Israel, 2012-2022                                                                                                                                                                                                                                                                                                                                                                                  |
| <b>Sample size</b>              | N=195                                                                                                                                                                                                                                                                                                                                                                                                             |
| <b>Statistical analysis</b>     | Baseline characteristics were summarized by mean and standard deviation. Findings between women who started treatment in the periconception period or during the first trimester were compared using Fisher exact test and independent <i>t</i> test. Odds ratios and 95% confidence intervals were calculated for the primary outcome for each subgroup and compared with the placebo arm in our previous study. |
| <b>Eligibility criteria</b>     | Pregnant patients with (serological evidence of) primary early CMV infection acquired during the periconception period or the first trimester of pregnancy, treated with Valaciclovir and with normal renal function; max 9 (for periconceptional) or 8w (for first trimester infection) after infection                                                                                                          |

|                                |                                                                                                                                                                                           |                                           |                     |                             |
|--------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------|---------------------|-----------------------------|
| <b>Exclusion criteria</b>      | Arrival out of time frame of the revised protocol; refusal to take the medication; start R/ but used <50% of prescribed meds; termination of pregnancy early after the first clinic visit |                                           |                     |                             |
| <b>Intervention</b>            | Valaciclovir 2 x 4g/d or 4 x 2g/d until amniocentesis (20-22 weeks of gestation) (N=195 offered treatment, N=184 in ITT analysis, N=178 in PP analysis).                                  |                                           |                     |                             |
| <b>Comparator</b>              | Historical control = placebo group from RCT Shahar Nissan et al. 2020 (N=47)                                                                                                              |                                           |                     |                             |
| <b>Patient characteristics</b> |                                                                                                                                                                                           | <b>All patients</b>                       | <b>Valaciclovir</b> | <b>Placebo</b>              |
|                                | <b>Mean age</b>                                                                                                                                                                           |                                           | No info             | No info                     |
|                                | <b>Periconceptual infection</b>                                                                                                                                                           | 30.47y (SD 3.73)                          |                     |                             |
|                                | <b>First trimester infection</b>                                                                                                                                                          | 31.42y (SD 4.38)                          |                     |                             |
|                                | <b>Timing infection</b>                                                                                                                                                                   | Periconceptual 33%<br>First trimester 67% | No info             | No info                     |
|                                | <b>Time between infection and treatment</b>                                                                                                                                               |                                           |                     |                             |
|                                | <b>Periconceptual infection</b>                                                                                                                                                           |                                           | 47d (SD 12)         |                             |
|                                | <b>First trimester infection</b>                                                                                                                                                          |                                           | 39d (SD 10)         |                             |
| <b>Results</b>                 | <b>GA at start treatment</b>                                                                                                                                                              |                                           |                     |                             |
|                                | <b>Periconceptual infection</b>                                                                                                                                                           |                                           | 55d (SD 12)         |                             |
|                                | <b>First trimester infection</b>                                                                                                                                                          |                                           | 93d (SD 19)         |                             |
|                                | <b>GA at amniocentesis</b>                                                                                                                                                                |                                           |                     |                             |
|                                | <b>Periconceptual infection</b>                                                                                                                                                           |                                           | 21.28w (SD 0.59)    |                             |
|                                | <b>First trimester infection</b>                                                                                                                                                          |                                           | 21.44w (SD 0.55)    |                             |
|                                | <b>Treatment duration</b>                                                                                                                                                                 |                                           |                     |                             |
|                                | <b>Periconceptual infection</b>                                                                                                                                                           |                                           | 13w (calculated)    |                             |
|                                | <b>First trimester infection</b>                                                                                                                                                          |                                           | 8w (calculated)     |                             |
| <b>Critical outcomes</b>       |                                                                                                                                                                                           | <b>Valaciclovir</b>                       | <b>Placebo</b>      | <b>Reported effect size</b> |
|                                | Termination of pregnancy                                                                                                                                                                  | 5% (9/195), excluded from analysis        |                     |                             |

|                                       |                                                                                                                                                                                                             |                                         |                                       |                                                                                                                         |
|---------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------|---------------------------------------|-------------------------------------------------------------------------------------------------------------------------|
|                                       | Severe adverse events in mother                                                                                                                                                                             | None                                    | No info                               |                                                                                                                         |
|                                       | <b>Important outcomes</b>                                                                                                                                                                                   |                                         |                                       |                                                                                                                         |
|                                       | cCMV at amniocentesis                                                                                                                                                                                       | 9% (17/184) (ITT)<br>8% (14/178) (PP)   | 30% (14/47) (ITT)<br>30% (14/47) (PP) |                                                                                                                         |
|                                       | Periconceptual infection                                                                                                                                                                                    | 2% (1/61) (ITT)<br>0% (0/59) (PP)       | 13% (3/24) (ITT)<br>13% (3/24) (PP)   | OR 0.12 (95%CI: 0.0-1.59)<br>p=0.66                                                                                     |
|                                       | First trimester infection                                                                                                                                                                                   | 13% (16/123) (ITT)<br>12% (14/119) (PP) | 48% (11/23)(ITT)<br>48% (11/23) (PP)  | OR 0.16 (95%CI: 0.06-0.49)<br>p<0.001<br>OR 0.15 (95%CI: 0.05-0.45)<br>p<0.001                                          |
|                                       | Miscarriage                                                                                                                                                                                                 | 1% excluded from analysis               |                                       |                                                                                                                         |
|                                       | <b>Adverse events</b>                                                                                                                                                                                       |                                         |                                       |                                                                                                                         |
|                                       | Nausea or vomiting                                                                                                                                                                                          | 1% (2/185)                              | No info                               |                                                                                                                         |
|                                       | Acute renal failure *                                                                                                                                                                                       | 0.5% (1/184)                            | No info                               | 1 transient renal failure in women with single kidney; treatment stop 4 days and resumed after creatinine normalisation |
|                                       |                                                                                                                                                                                                             |                                         |                                       |                                                                                                                         |
| <b>Limitations and other comments</b> | Use of the placebo group of RCT by Shahar-Nissan et al(24). as a control group for this observational study.<br>No comparative results on critical outcomes.                                                |                                         |                                       |                                                                                                                         |
|                                       | Definition of periconceptual = 4 weeks before and 3 weeks after recorded date of the last menstruation<br>first trimester = 3 – 14 weeks of gestation                                                       |                                         |                                       |                                                                                                                         |
|                                       | Definition of maternal primary infection = specific IgM only with IgG that become positive on repeated assay performed later, or specific IgM with low IgG avidity that increased on a second repeat assay. |                                         |                                       |                                                                                                                         |

**Egloff 2023(26)**

|                                                   |                                                                                                                                                                                                                                                                                                                                                       |                                                                                           |                         |                             |
|---------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------|-------------------------|-----------------------------|
| <b>Design</b>                                     | Retrospective cohort                                                                                                                                                                                                                                                                                                                                  |                                                                                           |                         |                             |
| <b>Source of funding and conflict of interest</b> | Not reported                                                                                                                                                                                                                                                                                                                                          |                                                                                           |                         |                             |
| <b>Setting</b>                                    | Multicentre (3), France, 2014-2021                                                                                                                                                                                                                                                                                                                    |                                                                                           |                         |                             |
| <b>Sample size</b>                                | N=143                                                                                                                                                                                                                                                                                                                                                 |                                                                                           |                         |                             |
| <b>Statistical analysis</b>                       | A logistic regression analysis adjusted for the propensity score was then used to estimate the treatment effect. Continuous data were presented as median (interquartile range (IQR)) and categorical data as <i>n</i> (%). Student's <i>t</i> -test was used to compare amniotic and neonatal viral load, after log transformation of the variables. |                                                                                           |                         |                             |
| <b>Eligibility criteria</b>                       | Women with primary CMV infection (confirmed on virological testing) during pregnancy or periconceptional period                                                                                                                                                                                                                                       |                                                                                           |                         |                             |
| <b>Exclusion criteria</b>                         | Primary infection occurred > 1m before date of conception; referral due to US abnormality related to congenital CMV infection; VACV treatment initiated after positive result following amniocentesis; infection not confirmed on virological testing                                                                                                 |                                                                                           |                         |                             |
| <b>Intervention</b>                               | Valaciclovir 8g until amniocentesis (≥ 21 weeks of gestation and ≥ 6 weeks after onset of the infection, according to the usual management in the participating center) or birth (discussed with patients if PCR positive at amniocentesis) (N=29).                                                                                                   |                                                                                           |                         |                             |
| <b>Comparator</b>                                 | No treatment (before treatment or declined treatment) (N=84)                                                                                                                                                                                                                                                                                          |                                                                                           |                         |                             |
| <b>Patient characteristics</b>                    |                                                                                                                                                                                                                                                                                                                                                       | <b>All patients</b>                                                                       | <b>Valaciclovir</b>     | <b>No treatment</b>         |
|                                                   | <b>Mean age</b>                                                                                                                                                                                                                                                                                                                                       | 32.1y (IQR 29-34)                                                                         | 32.4y (IQR 29-34)       | 32.4y (IQR 29-34)           |
|                                                   | <b>Timing infection</b>                                                                                                                                                                                                                                                                                                                               | Periconceptional 17%<br>First trimester 57%<br>Second trimester 24%<br>Third trimester 1% | 24%<br>61%<br>14%<br>2% | 12%<br>55%<br>32%<br>1%     |
|                                                   | <b>Time between infection and treatment</b>                                                                                                                                                                                                                                                                                                           |                                                                                           | 49d (IQR 36-56)         |                             |
|                                                   | <b>GA at start treatment</b>                                                                                                                                                                                                                                                                                                                          |                                                                                           | 13.6w (IQR 11.2-17.3)   |                             |
|                                                   | <b>GA at amniocentesis</b>                                                                                                                                                                                                                                                                                                                            | 21.5w (IQR 20.6-23.1)                                                                     | 21.6w (IQR 20.3-23.6)   | 22.2w (IQR 21.1-28.4)       |
|                                                   | <b>Treatment duration</b>                                                                                                                                                                                                                                                                                                                             |                                                                                           | 72d (IQR 58-105)        | No info                     |
|                                                   |                                                                                                                                                                                                                                                                                                                                                       |                                                                                           |                         |                             |
| <b>Results</b>                                    |                                                                                                                                                                                                                                                                                                                                                       |                                                                                           |                         |                             |
|                                                   | <b>Critical outcomes</b>                                                                                                                                                                                                                                                                                                                              | <b>Valaciclovir</b>                                                                       | <b>No treatment</b>     | <b>Reported effect size</b> |
|                                                   | Severe adverse events in mother                                                                                                                                                                                                                                                                                                                       | None                                                                                      | No info                 |                             |



|  |                                            |             |             |                                       |
|--|--------------------------------------------|-------------|-------------|---------------------------------------|
|  | <b>Important outcomes</b>                  |             |             |                                       |
|  | cCMV at amniocentesis                      | 19% (10/54) | 33% (16/49) |                                       |
|  | Periconceptional infection                 | 8% (1/13)   | 0% (0/3)    |                                       |
|  | First trimester infection                  | 23% (8/35)  | 32% (10/31) |                                       |
|  | Second trimester infection                 | 17% (1/6)   | 46% (6/13)  |                                       |
|  | cCMV at birth                              | 19% (11/59) | 40% (34/84) | OR 0.4 (95%CI: 0.18-0.90),<br>p=0.029 |
|  | Periconceptional infection                 | 7% (1/14)   | 10% (1/10)  | p=1.0                                 |
|  | First trimester infection                  | 22% (8/36)  | 41% (19/46) | p=0.068                               |
|  | Second trimester infection                 | 25% (2/8)   | 52% (14/27) | p=0.244                               |
|  | <b>Adverse events</b>                      |             |             |                                       |
|  | Back pain                                  | 7% (4/59)   | No info     |                                       |
|  | Dizziness                                  | 2% (1/59)   | No info     |                                       |
|  | Nausea or vomiting                         | 5% (3/59)   | No info     |                                       |
|  | Acute renal failure*                       | 2% (1/59)   | No info     | Resolved after sTOP medication        |
|  | Macrocytosis                               | 2% (1/59)   | No info     |                                       |
|  | <b>Other outcomes</b>                      |             |             |                                       |
|  | Microcephaly                               | 7% (2/29)   | 1% (1/84)   |                                       |
|  | Abnormal brain US at birth                 | 9% (5/58)   | 11% (9/83)  |                                       |
|  | Periconceptional infection                 | 7% (1/14)   | 0% (0/10)   |                                       |
|  | First trimester infection                  | 11% (4/36)  | 15% (7/46)  |                                       |
|  | Second trimester infection                 | 0% (0/8)    | 7% (2/27)   |                                       |
|  | Abnormal brain MRI at birth (from all MRI) | 57% (4/7)   | 30% (6/20)  |                                       |
|  | Periconceptional infection                 | 100% (1/1)  | 0% (0/2)    |                                       |
|  | First trimester infection                  | 50% (3/6)   | 42% (5/12)  |                                       |
|  | Second trimester infection                 | 0% (0/0)    | 17% (1/6)   |                                       |
|  | Abnormal brain MRI at birth (all patients) |             |             |                                       |
|  | Periconceptional infection                 | 7% (4/58)   | 7% (6/83)   |                                       |



|                                       |                                                                                                                                                                                                                                                                                                                  |           |            |  |
|---------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------|------------|--|
|                                       | First trimester infection                                                                                                                                                                                                                                                                                        | 7% (1/14) | 0% (0/10)  |  |
|                                       | Second trimester infection                                                                                                                                                                                                                                                                                       | 8% (3/36) | 11% (5/46) |  |
|                                       |                                                                                                                                                                                                                                                                                                                  | 0% (0/8)  | 4% (1/27)  |  |
|                                       | *One patient with no relevant medical history developed acute renal failure (creatinine level, 291 µmol/L) associated with back pain and pruritus 14 days after initiation of VCV treatment at 9.4 weeks' gestation. Renal function resolved spontaneously within a few days after discontinuation of treatment. |           |            |  |
| <b>Limitations and other comments</b> | No comparative results on critical outcomes available. No information per patient on (severe) symptoms at birth. No information on hearing loss.                                                                                                                                                                 |           |            |  |
|                                       | Definition of periconceptual infection = 4 weeks before and 2 weeks after the date of conception<br>first trimester infection = 2-14 weeks of gestation<br>second trimester infection = 14-28 weeks of gestation<br>third trimester infection >28 weeks                                                          |           |            |  |
|                                       | Definition of maternal primary infection = seroconversion identified on two sequential serum samples, with negative CMV-specific IgG in the first sample and positive CMV-IgG in the second sample in the same laboratory, or positive CMV-specific IgG and CMV-IgM, and low IgG avidity.                        |           |            |  |

**Faure Bardon 2023(27)**

|                                                   |                                                                                                                                                                                                                                                                                                                 |
|---------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Design</b>                                     | Retrospective cohort                                                                                                                                                                                                                                                                                            |
| <b>Source of funding and conflict of interest</b> | Not reported                                                                                                                                                                                                                                                                                                    |
| <b>Setting</b>                                    | Single center, France, 2009-2020                                                                                                                                                                                                                                                                                |
| <b>Sample size</b>                                | N=130                                                                                                                                                                                                                                                                                                           |
| <b>Statistical analysis</b>                       | Propensity score matching for the timing of MPI (periconceptual or first trimester) and the gestational age at amniocentesis. The effect of VACV was assessed using odds ratios (OR) obtained by standard logistic regression, with periconceptual infection and gestational age at amniocentesis as covariates |
| <b>Eligibility criteria</b>                       | Maternal primary CMV infection (CMV-MPI) diagnosed by maternal serological screening before 14 weeks gestation                                                                                                                                                                                                  |
| <b>Exclusion criteria</b>                         | No amniocentesis performed                                                                                                                                                                                                                                                                                      |



|                                       |                                                                                                                                                               |                                           |                       |                                                           |
|---------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------|-----------------------|-----------------------------------------------------------|
| <b>Intervention</b>                   | Valaciclovir 2 x 4g/d until amniocentesis (≥8 weeks after maternal infection and ≥ 17 weeks of gestation) or birth (if PCR positive at amniocentesis) (N=65). |                                           |                       |                                                           |
| <b>Comparator</b>                     | No treatment (before treatment or declined treatment) (N=65)                                                                                                  |                                           |                       |                                                           |
| <b>Patient characteristics</b>        |                                                                                                                                                               | <b>All patients</b>                       | <b>Valaciclovir</b>   | <b>No treatment</b>                                       |
|                                       | <b>Mean age</b>                                                                                                                                               | No info                                   | No info               | No info                                                   |
|                                       | <b>Timing infection</b>                                                                                                                                       | Periconceptual 42%<br>First trimester 58% | 43%<br>57%            | 40%<br>60%                                                |
|                                       | <b>Time between infection and treatment</b>                                                                                                                   | No info                                   | No info               | No info                                                   |
|                                       | <b>GA at start treatment</b>                                                                                                                                  |                                           | 12.71w (IQR 10-13.86) |                                                           |
|                                       | <b>GA at amniocentesis</b>                                                                                                                                    | No info                                   | 17.6w (IQR 17.1-18.1) | 18.3w (IQR 17.6-19.3)                                     |
|                                       | <b>Treatment duration</b>                                                                                                                                     |                                           | 7w (IQR 4-8w)         |                                                           |
| <b>Results</b>                        |                                                                                                                                                               |                                           |                       |                                                           |
|                                       | <b>Critical outcomes</b>                                                                                                                                      | <b>Valaciclovir</b>                       | <b>No treatment</b>   | <b>Reported effect size</b>                               |
|                                       | Severe adverse events in mother                                                                                                                               | None                                      | No info               |                                                           |
|                                       | <b>Important outcomes</b>                                                                                                                                     |                                           |                       |                                                           |
|                                       | cCMV at amniocentesis                                                                                                                                         | 12% (8/65)                                | 29% (19/65)           | OR 0.318 (95%CI: 0.12-0.841), p=0.021<br>p=0.6<br>p=0.027 |
|                                       | Periconceptual infection                                                                                                                                      | 4% (1/28)                                 | 8% (2/26)             |                                                           |
|                                       | First trimester infection                                                                                                                                     | 19% (7/37)                                | 44% (17/39)           |                                                           |
|                                       | <b>Adverse events</b>                                                                                                                                         |                                           |                       |                                                           |
|                                       | Acute renal failure                                                                                                                                           | 1.5% (1/65)                               | No info               | resolved after stop medication                            |
| <b>Other</b>                          |                                                                                                                                                               |                                           |                       |                                                           |
| <b>Limitations and other comments</b> | No comparative results on critical outcomes.                                                                                                                  |                                           |                       |                                                           |
|                                       | Definition of periconceptual < 0 weeks of gestation<br>first trimester = 0-14 weeks of gestation                                                              |                                           |                       |                                                           |



Definition of primary maternal infection = The diagnosis of CMV-MPI was made by maternal serology before 14 weeks of gestation, based on IgG and IgM measurement, followed by IgG avidity tests in cases with positive IgM and positive IgG. Cases with high IgG avidity < 14 weeks' gestation were not considered MPI and were excluded from the study.

**Zammarchi 2023(28)**

|                                |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |                                                                         |                                     |                     |
|--------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------|-------------------------------------|---------------------|
| <b>Design</b>                  | Retrospective cohort                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |                                                                         |                                     |                     |
| <b>Source of funding</b>       | This study has been supported by the Department of Experimental and Clinical Medicine, University of, Florence, Italy (Fondi ex 60%) assigned to Prof Lorenzo Zammarchi. Authors from Pavia were supported by Ministero della Salute, Ricerca Corrente (grant number: 053618). The authors report no conflict of interest.                                                                                                                                                                       |                                                                         |                                     |                     |
| <b>Setting</b>                 | Multicentre (11), Italy, 2010-2022                                                                                                                                                                                                                                                                                                                                                                                                                                                               |                                                                         |                                     |                     |
| <b>Sample size</b>             | N=447                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |                                                                         |                                     |                     |
| <b>Statistical analysis</b>    | Descriptive statistics were provided: proportions for categorical variables and means and SD for quantitative variables; Crude odds ratios and mean differences were calculated when appropriate. We adopted a marginal structural model approach using inverse probability weighting to account for the confounding effect of the observed pretreatment variables, selection because of outcome missingness and censoring by TOP (the newborn's outcomes were not observed in the case of TOP). |                                                                         |                                     |                     |
| <b>Eligibility criteria</b>    | Pregnant patients presenting with a primary CMV infection acquired in the periconception period and up to 24 weeks of gestation                                                                                                                                                                                                                                                                                                                                                                  |                                                                         |                                     |                     |
| <b>Exclusion criteria</b>      | Creatinine level of >1.1 mg/dL and glomerular filtration of <90 mL/min                                                                                                                                                                                                                                                                                                                                                                                                                           |                                                                         |                                     |                     |
| <b>Intervention</b>            | Valaciclovir 4 x 2g/d until amniocentesis (≥8 weeks after estimated timing of primary infection and ≥ 20 1/7 weeks of gestation) or birth (if PCR positive at amniocentesis and abnormal US) (N=205). If amniocentesis is refused, treatment until 26 weeks of gestation.                                                                                                                                                                                                                        |                                                                         |                                     |                     |
| <b>Comparator</b>              | No treatment (before treatment or declined treatment) (N=242)                                                                                                                                                                                                                                                                                                                                                                                                                                    |                                                                         |                                     |                     |
| <b>Patient characteristics</b> |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | <b>All patients</b>                                                     | <b>Valaciclovir</b>                 | <b>No treatment</b> |
|                                | <b>Mean age</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | 31.4y (SD5.4)                                                           | No info                             | No info             |
|                                | <b>Timing infection</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                          | Periconceptional 21%<br>First trimester 65.5%<br>Second trimester 13.5% | 17%<br>69%<br>13%                   | 24%<br>62%<br>14%   |
|                                | <b>Time between infection and treatment</b><br><b>Periconceptional infection</b><br><b>First trimester infection</b>                                                                                                                                                                                                                                                                                                                                                                             |                                                                         | 7.9w (SD 4.6)<br><br>15.6w (SD 5.5) |                     |



|  |                                   |                |                                             |                |
|--|-----------------------------------|----------------|---------------------------------------------|----------------|
|  | <b>Second trimester infection</b> |                | 8w (SD 3.9)<br>4.6w (SD 3.1)                |                |
|  | <b>GA at start treatment</b>      |                | No info                                     |                |
|  | <b>Timing treatment start</b>     |                | 25% first trimester<br>75% second trimester |                |
|  | <b>GA at amniocentesis</b>        | 20.8w (SD 1.6) | 20.7w (SD 1.3)                              | 20.9w (SD 1.9) |
|  | <b>Treatment duration</b>         |                | 58d (SD 32)                                 |                |

### Results

|  | <b>Critical outcomes</b>          | <b>Valaciclovir</b> | <b>No treatment</b> | <b>Reported effect size</b>                                                 |
|--|-----------------------------------|---------------------|---------------------|-----------------------------------------------------------------------------|
|  | Termination of pregnancy          | 3% (7/206)          | 10% (24/244)        | OR 0.36, p=0.0021 (95%CI: 0.17-0.75)                                        |
|  | In case of positive amniocentesis | 25% (5/20)          | 49% (18/37)         |                                                                             |
|  | Periconceptual infection          | 10% (3/30)          | 10% (6/58)          | OR 0.74 (90%CI 0.22-2.51),<br>p=0.688 OR 0.25<br>(90%CI 0.08-0.75), p=0.039 |
|  | First trimester infection         | 3% (3/93)           | 12% (18/152)        |                                                                             |
|  | Second trimester infection        | 1% (1/83)           | 0% (0/32)           |                                                                             |
|  | Severely symptomatic newborns     | 1% (2/185)          | 8% (18/213)         |                                                                             |
|  | Periconceptual infection          | 0% (0/22)           | 8% (4/49)           |                                                                             |
|  | First trimester infection         | 2% (2/85)           | 9% (12/132)         |                                                                             |
|  | Second trimester infection        | 0% (0/78)           | 6% (2/32)           |                                                                             |
|  | Severe adverse events in mother   | None                | No info             |                                                                             |
|  | <b>Important outcomes</b>         |                     |                     |                                                                             |

|  |                            |              |              |                                                 |
|--|----------------------------|--------------|--------------|-------------------------------------------------|
|  | cCMV at amniocentesis      | 15% (20/136) | 28% (37/134) | OR 0.39 (90%CI: 0.22-0.68)<br>p=0.005           |
|  | Periconceptual infection   | 12% (3/25)   | 31% (10/32)  | OR 0.33 (90%CI: 0.09-1.23)<br>p=0.164           |
|  | First trimester infection  | 14% (10/73)  | 25% (25/100) | OR 0.57 (90%CI: 0.28-1.15)<br>p=0.191           |
|  | Second trimester infection | 18% (7/38)   | 100% (2/2)   |                                                 |
|  | cCMV at birth              | 23%          | 25%          | OR 0.85 (90%CI: 0.57-1.26)<br>p=0.5             |
|  | Periconceptual infection   | 14% (3/22)   | 18% (9/49)   | OR 1.06 (90%CI: 0.23-4.92)<br>p=0.947           |
|  | First trimester infection  | 26% (22/85)  | 20% (27/132) | OR 0.96 (90%CI: 0.48-1.95)<br>p=0.934           |
|  | Second trimester infection | 22% (17/78)  | 56% (18/32)  |                                                 |
|  | Moderate symptoms at birth | 0.5% (1/185) | 0% (0/213)   |                                                 |
|  | Hearing loss               | 0.5% (1/185) | 5% (10/213)  |                                                 |
|  | Miscarriage                | 0.5% (1/206) | 0.8% (4/244) |                                                 |
|  | <b>Adverse events</b>      |              |              |                                                 |
|  | Headache                   | 2.5% (5/205) | No info      |                                                 |
|  | Fatigue                    | 1.5% (3/205) | No info      |                                                 |
|  | Dizziness                  | 1% (2/205)   | No info      |                                                 |
|  | Gastro-intestinal problems | 2.5% (5/205) | No info      |                                                 |
|  | Acute renal failure *      | 2% (4/205)   | No info      | Resolved after suspension or<br>stop medication |
|  | <b>Other outcomes</b>      |              |              |                                                 |
|  | Abnormal US at birth       | 0.5% (1/206) | 3% (8/244)   |                                                 |
|  | Periconceptual infection   | 0% (0/36)    | 2% (1/58)    |                                                 |
|  | First trimester infection  | 0% (0/143)   | 5% (7/152)   |                                                 |



|                                       |                                                                                                                                                                                                                                                                                                                                                                                                                                                       |              |              |  |
|---------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------|--------------|--|
|                                       | Second trimester infection                                                                                                                                                                                                                                                                                                                                                                                                                            | 4% (1/27)    | 0% (0/34)    |  |
|                                       | Abnormal MRI at birth                                                                                                                                                                                                                                                                                                                                                                                                                                 | 0.5% (1/206) | 2% (4/244)   |  |
|                                       | Periconceptual infection                                                                                                                                                                                                                                                                                                                                                                                                                              | 0% (0/36)    | 3% (2/58)    |  |
|                                       | First trimester infection                                                                                                                                                                                                                                                                                                                                                                                                                             | 0.7% (1/143) | 0.7% (1/152) |  |
|                                       | Second trimester infection                                                                                                                                                                                                                                                                                                                                                                                                                            | 0% (0/27)    | 3% (1/34)    |  |
|                                       | No information of the number of patients that had MRI taken, so denominator is all patients                                                                                                                                                                                                                                                                                                                                                           |              |              |  |
| <b>Limitations and other comments</b> | Severe symptoms include hearing loss, abnormal brain imaging and/or retinal haemorrhages or chorioretinal atrophy<br>Symptomatic cCMV infection at birth is defined according to the ESPID definition. Retrieval of clinical data from electronic or paper medical records according to the availability in the different participating centers, which may be incomplete.                                                                             |              |              |  |
|                                       | Definition of periconceptual = 4 weeks before and 3 weeks after recorded date of the last menstruation<br>First trimester = 3-14 weeks of gestation<br>Second trimester = 14- 24 weeks of gestation                                                                                                                                                                                                                                                   |              |              |  |
|                                       | Definition of maternal primary infection = CMV immunoglobulin G (IgG) seroconversion defined as a negative IgG test during pregnancy followed by a positive IgG test later in the gestation; (2) CMV IgG and immunoglobulin M (IgM) positive with low IgG avidity index during pregnancy; and (3) CMV IgG and IgM positive, intermediate IgG avidity index, and detected CMV DNA in at least 1 body fluid (blood, saliva, or urine) during pregnancy. |              |              |  |
|                                       | *The 4 women (1.9%) with renal toxicity (3 women treated with 2 g 4 times a day and 1 treated with the 4 g 2 times a day) presented a slight increase of creatinine level (maximum creatinine level of 1.5 mg/dL in 1 patient), which was reversible after treatment suspension. In 2 women with an increase in creatinine level, the treatment was discontinued before the planned.                                                                  |              |              |  |

**Jacquemard 2007(30)**

|                                                   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |                     |                     |
|---------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------|---------------------|
| <b>Design</b>                                     | Pro- and retrospective cohort                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |                     |                     |
| <b>Source of funding and conflict of interest</b> | Not reported                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |                     |                     |
| <b>Setting</b>                                    | Monocentre, France, 2003-2005                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |                     |                     |
| <b>Sample size</b>                                | N = 44                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |                     |                     |
| <b>Statistical analysis</b>                       | Although no attempt was made either to match nor to compare outcomes of treated with untreated fetuses directly, outcomes were also collected from all cases of symptomatic CMV infection presenting for 12 months prior to the start of this study and from those who declined treatment during the study period.                                                                                                                                                                                                                                                                                                                                                         |                     |                     |
| <b>Eligibility criteria</b>                       | <p>We offered VCV therapy in cases of confirmed fetal CMV infection after primary maternal infection when the foetus showed biological and/or ultrasound markers of infection and when the parents had elected to continue with the pregnancy.</p> <p>Presence of one or more of the following ultrasound features: grade 3 hyperechogenic bowels, ascites, growth restriction with an estimated fetal weight below the 10th percentile, ventriculomegaly with ventricular width of at least 10 mm at the level of the atrium, or any other brain anomaly. Fetal thrombocytopenia below 100 000/dL and/or elevated GGT were features of a symptomatic fetal infection.</p> |                     |                     |
| <b>Exclusion criteria</b>                         | Women who had a history of hepatic disease, renal dysfunction, bone marrow suppression, or known VCV intolerance were not offered therapy.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |                     |                     |
| <b>Intervention</b>                               | Valaciclovir 4 x 2g per day from day of enrolment (after amniocentesis) up to delivery or TOP (N=20 women, 21 fetuses)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |                     |                     |
| <b>Comparator (control group)</b>                 | 24 singleton pregnancies that presented at 19–38 weeks of pregnancy with a symptomatic intrauterine infection, but either presented 12 months before treatment was offered or declined intrauterine treatment with valaciclovir (N=24)                                                                                                                                                                                                                                                                                                                                                                                                                                     |                     |                     |
| <b>Patient characteristics</b>                    | <b>All patients</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        | <b>Valaciclovir</b> | <b>No treatment</b> |
|                                                   | Mean age                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | No info             | No info             |
|                                                   | Timing infection                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           | No info             | No info             |
|                                                   | Time between infection and treatment                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       | No info             | No info             |
|                                                   | GA at start treatment                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      | 28w (range 22-34)   |                     |
|                                                   | GA at amniocentesis                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        | 28w (range 22-34)   |                     |
|                                                   | Treatment duration                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         | 7w (range 1-12w)    |                     |
| <b>Results</b>                                    |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |                     |                     |
|                                                   | <b>Critical outcomes</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | <b>Valaciclovir</b> | <b>No treatment</b> |



|                                       |                                                                                                                                                                                                                                                                                                                                                                                                                                                        |            |             |        |
|---------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------|-------------|--------|
|                                       | Termination of pregnancy                                                                                                                                                                                                                                                                                                                                                                                                                               | 33% (7/21) | 50% (12/24) |        |
|                                       | Severely symptomatic newborns                                                                                                                                                                                                                                                                                                                                                                                                                          | 14% (3/21) | 4% (1/24)   |        |
|                                       | Severe adverse events in mother                                                                                                                                                                                                                                                                                                                                                                                                                        | None       | No info     |        |
|                                       | <b>Important outcomes</b>                                                                                                                                                                                                                                                                                                                                                                                                                              |            |             |        |
|                                       | Moderate symptoms at birth                                                                                                                                                                                                                                                                                                                                                                                                                             | None       | None        |        |
|                                       | Hearing loss                                                                                                                                                                                                                                                                                                                                                                                                                                           | 14% (3/21) | 0% (0/24)   |        |
|                                       | Miscarriage                                                                                                                                                                                                                                                                                                                                                                                                                                            | 5% (1/21)  | 4% (1/24)   |        |
|                                       | Severe CID including microcephaly                                                                                                                                                                                                                                                                                                                                                                                                                      | 0% (0/21)  | 4% (1/24)   |        |
|                                       | TOP + IUFD or severe CID (Poor outcome)                                                                                                                                                                                                                                                                                                                                                                                                                | 38% (8/21) | 58% (14/24) | p=0.42 |
|                                       | <b>Adverse events</b>                                                                                                                                                                                                                                                                                                                                                                                                                                  | None       |             |        |
|                                       | <b>Other outcomes</b>                                                                                                                                                                                                                                                                                                                                                                                                                                  |            |             |        |
|                                       | 1 treated patient with X-linked dominant neurocutaneous syndrome with microcephaly and bilateral deafness (unrelated to CMV)                                                                                                                                                                                                                                                                                                                           |            |             |        |
|                                       | Hepatitis at birth (+ hearing loss)                                                                                                                                                                                                                                                                                                                                                                                                                    | 5% (1/21)  | 0% (0/24)   |        |
| <b>Limitations and other comments</b> | Severely symptomatic newborns include hearing loss and severe CID. Cytomegalic inclusion disease (CID) is defined by the association of growth restriction, microcephaly, jaundice, hepatosplenomegaly, and thrombocytopenia.<br>No other patients with severe CNS involvement at birth, because pregnancy was terminated. Hearing loss more often in untreated children, but these foetuses were already infected with CMV at the start of treatment! |            |             |        |
|                                       | Definition of maternal primary infection = not specified                                                                                                                                                                                                                                                                                                                                                                                               |            |             |        |
|                                       |                                                                                                                                                                                                                                                                                                                                                                                                                                                        |            |             |        |

**Leruez Ville 2016(31)**

|                                                   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |                      |                     |                     |
|---------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------|---------------------|---------------------|
| <b>Design</b>                                     | Open label Phase II study with one arm + historical control                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |                      |                     |                     |
| <b>Source of funding and conflict of interest</b> | Not reported                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |                      |                     |                     |
| <b>Setting</b>                                    | Multicentre (6), France 2012-2014                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |                      |                     |                     |
| <b>Sample size</b>                                | N = 41 (phase II study) and N=47 for historical controls                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |                      |                     |                     |
| <b>Statistical analysis</b>                       | To estimate the sample size according to Simon optimal 2-stage design, we assumed that a proportion of asymptomatic neonates of <60% was not clinically relevant in relation to valaciclovir efficacy, while a proportion of >80% was deemed acceptable. With a type I error fixed at 0.05 and a power of 80%, we needed 11 infected fetuses for the first stage: if at least 8 cases in stage 1 had a good outcome (were asymptomatic), then 32 additional infected fetuses would be included in stage 2 for a total of 43. If at least 31 of the 43 cases were asymptomatic at birth, valaciclovir would be judged to have a positive effect. Analyses of efficacy data were performed for all included fetuses (intention to treat). The proportion of asymptomatic neonates and its 95% confidence interval were calculated with an unbiased estimator. In the historical comparator group, we used a random effects model to calculate the overall proportion of asymptomatic neonates and a 95% confidence interval for all 47 cases from the 3 studies. We used nonparametric tests for all secondary analyses. |                      |                     |                     |
| <b>Eligibility criteria</b>                       | Eligible women were pregnant with an infected fetus identified by a positive CMV polymerase chain reaction assay in amniotic fluid, sampled by amniocentesis >21 weeks, together with the presence of 1 extracerebral ultrasound features compatible with CMV infection and/or 1 isolated cerebral abnormality and/or 1 of the following laboratory findings in fetal blood: fetal platelet count <100,000/mm <sup>3</sup> or CMV DNA viral load >3000 copies/mL.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |                      |                     |                     |
| <b>Exclusion criteria</b>                         | The presence of severe ultrasound brain abnormalities (n=25) at least one: ventriculomegaly ≥ 15 mm, periventricular hyper-echogenicity, hydrocephaly, microcephaly <3 SD, mega-cisterna magna >10 mm, vermian hypoplasia, porencephaly, lissencephaly, periventricular cysts, abnormal corpus callosum) and the absence of any ultrasound feature of infection or laboratory abnormality (n=22 asymptomatic) in fetal blood were exclusion criteria.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |                      |                     |                     |
| <b>Intervention</b>                               | Valaciclovir 4 x 2g from enrolment (amniocentesis > 21 weeks) until delivery or TOP or 24 treatment weeks (whichever was first) (N=41, 2 sets of twins)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |                      |                     |                     |
| <b>Comparator (control group)</b>                 | Historical controls: 47 cases reported in 3 suitable published studies with ultrasound abnormalities matching inclusion criteria (Jacquemard 2007, Picone 2008, Guerra 2008)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |                      |                     |                     |
| <b>Patient characteristics</b>                    |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        | <b>All patients</b>  | <b>Valaciclovir</b> | <b>No treatment</b> |
|                                                   | <b>Mean age</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        | 31.2 (IQR 28.6-33.9) | No info             | No info             |
|                                                   | <b>Timing primary infection</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        | median 10 weeks      | No info             | No info             |
|                                                   | <b>Time between infection and treatment</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            | No info              | 16w (IQR 12.3-18.6) | No info             |
|                                                   | <b>GA at treatment</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |                      | 26w (range 22-35w)  |                     |
|                                                   | <b>GA at amniocentesis</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |                      | No info             |                     |

|                                       |                                                                                                                                                                                                                        |                                                                                         |                     |  |
|---------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------|---------------------|--|
|                                       | Treatment duration                                                                                                                                                                                                     |                                                                                         | 89d (range 41-102)  |  |
| <b>Results</b>                        |                                                                                                                                                                                                                        |                                                                                         |                     |  |
|                                       | <b>Critical outcomes</b>                                                                                                                                                                                               | <b>Valaciclovir</b>                                                                     | <b>No treatment</b> |  |
|                                       | Termination of pregnancy                                                                                                                                                                                               | 5% (2/43)                                                                               | No info             |  |
|                                       | Severe adverse events in mother                                                                                                                                                                                        | None                                                                                    | No info             |  |
|                                       | <b>Important outcomes</b>                                                                                                                                                                                              |                                                                                         |                     |  |
|                                       | Hearing loss                                                                                                                                                                                                           | 12% (5/43)                                                                              | No info             |  |
|                                       | <b>Adverse events</b>                                                                                                                                                                                                  | No changes in creatinine<br>AST-ALT increases not<br>clinically relevant and<br><40IU/L |                     |  |
|                                       | <b>Other outcomes</b>                                                                                                                                                                                                  |                                                                                         |                     |  |
|                                       | Proportion of asymptomatic neonates                                                                                                                                                                                    | 79% (34/43)                                                                             | 43% (20/47)         |  |
|                                       | Growth restriction at birth                                                                                                                                                                                            | 2% (1/43)                                                                               | No info             |  |
|                                       | Thrombocytopaenia at birth                                                                                                                                                                                             | 2% (1/43)                                                                               | No info             |  |
| <b>Limitations and other comments</b> | Only comparative data on 'asymptomatic' neonates. No comparative data on separate symptoms. Not possible to distinguish between severe symptoms AND severe ultrasound brain abnormalities are excluded from the study. |                                                                                         |                     |  |
|                                       | Definition of maternal primary infection = seroconversion or the concomitant presence of CMV-specific IgM antibodies and low IgG avidity                                                                               |                                                                                         |                     |  |

## 8.5 Meta-analysis

### 8.5.1 cCMV at amniocentesis (vertical transmission at amniocentesis/ foetal infection)

Figure 14 – Metanalysis for cCMV at amniocentesis for patients treated and not treated with valaciclovir

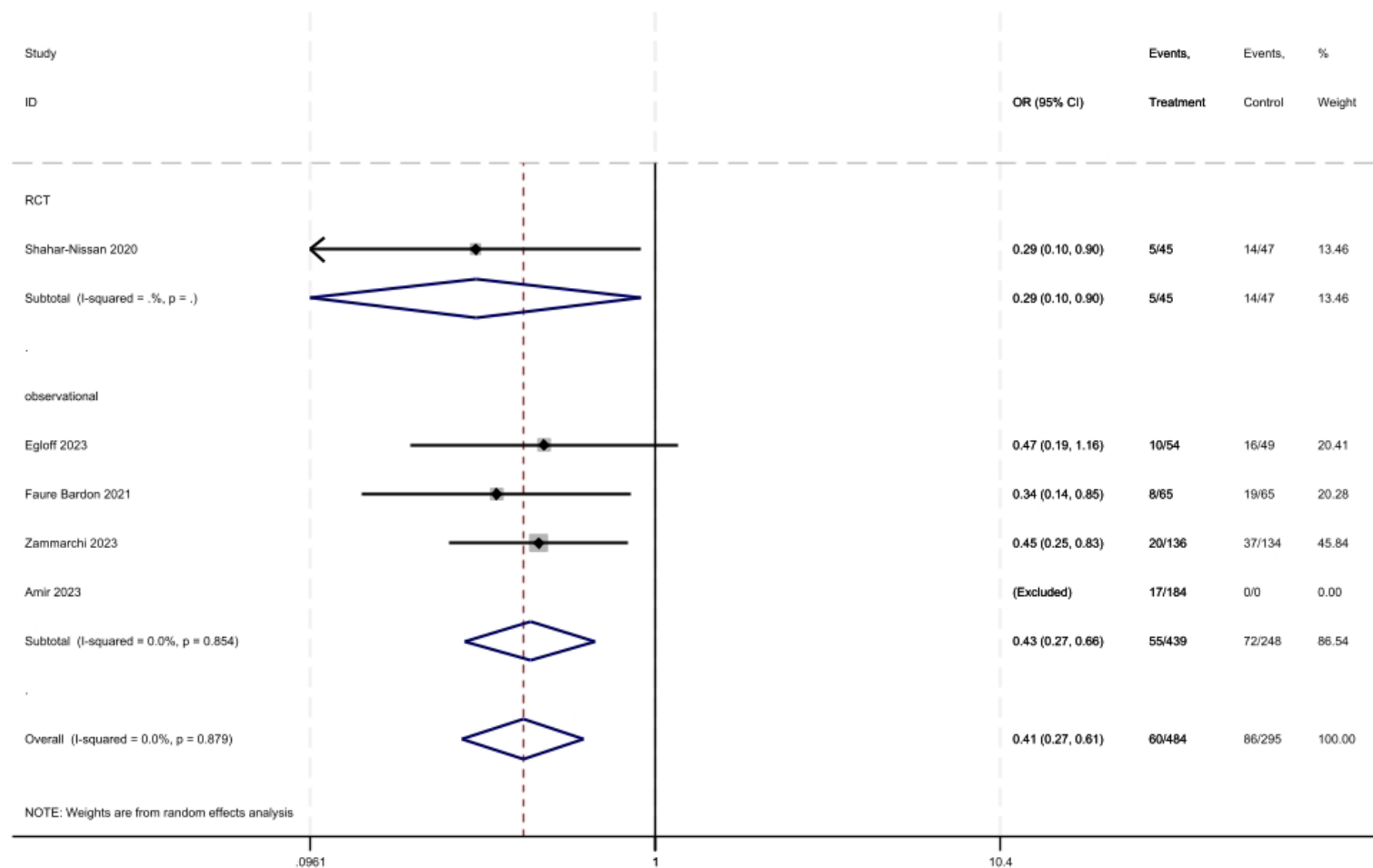
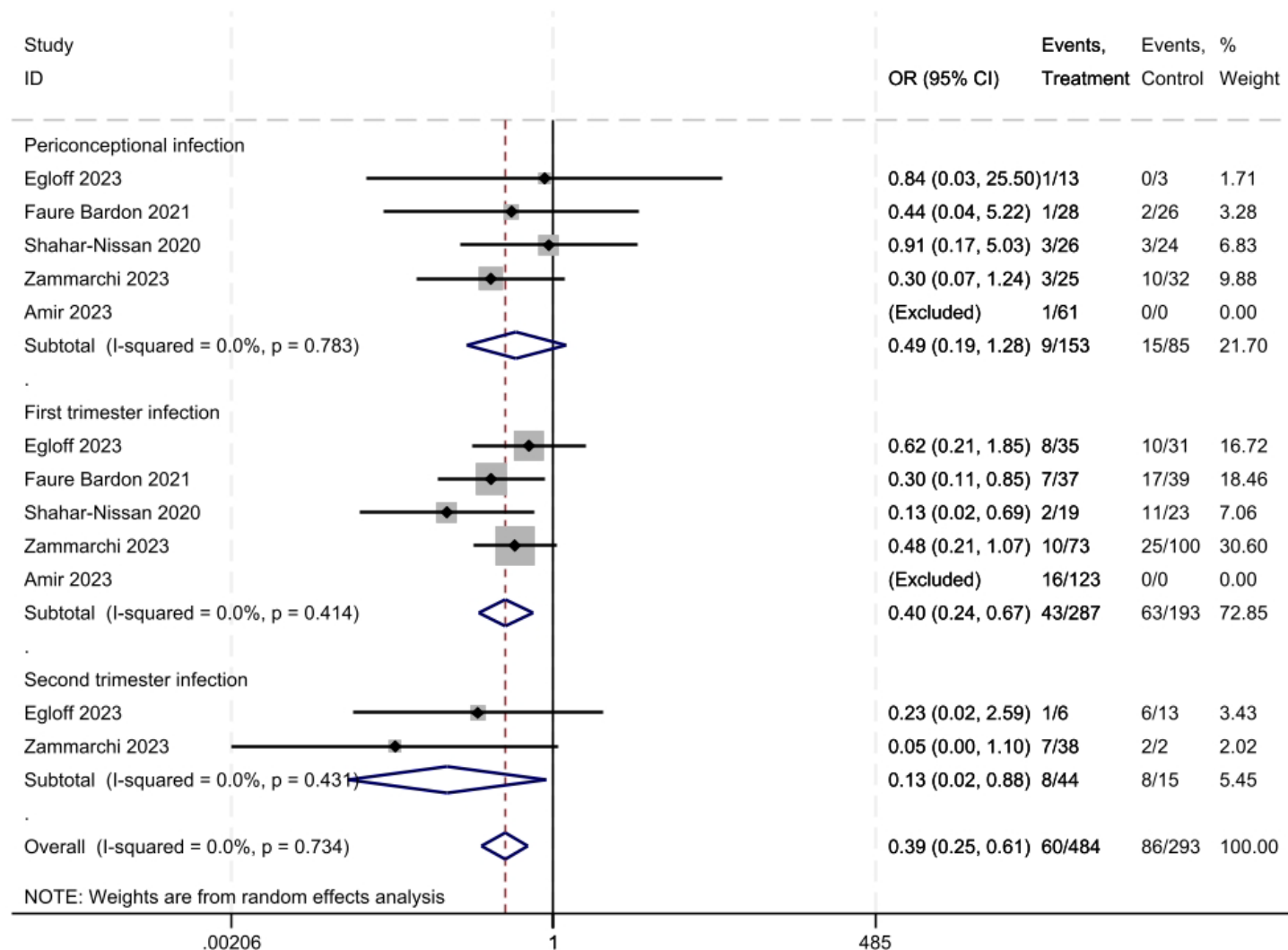


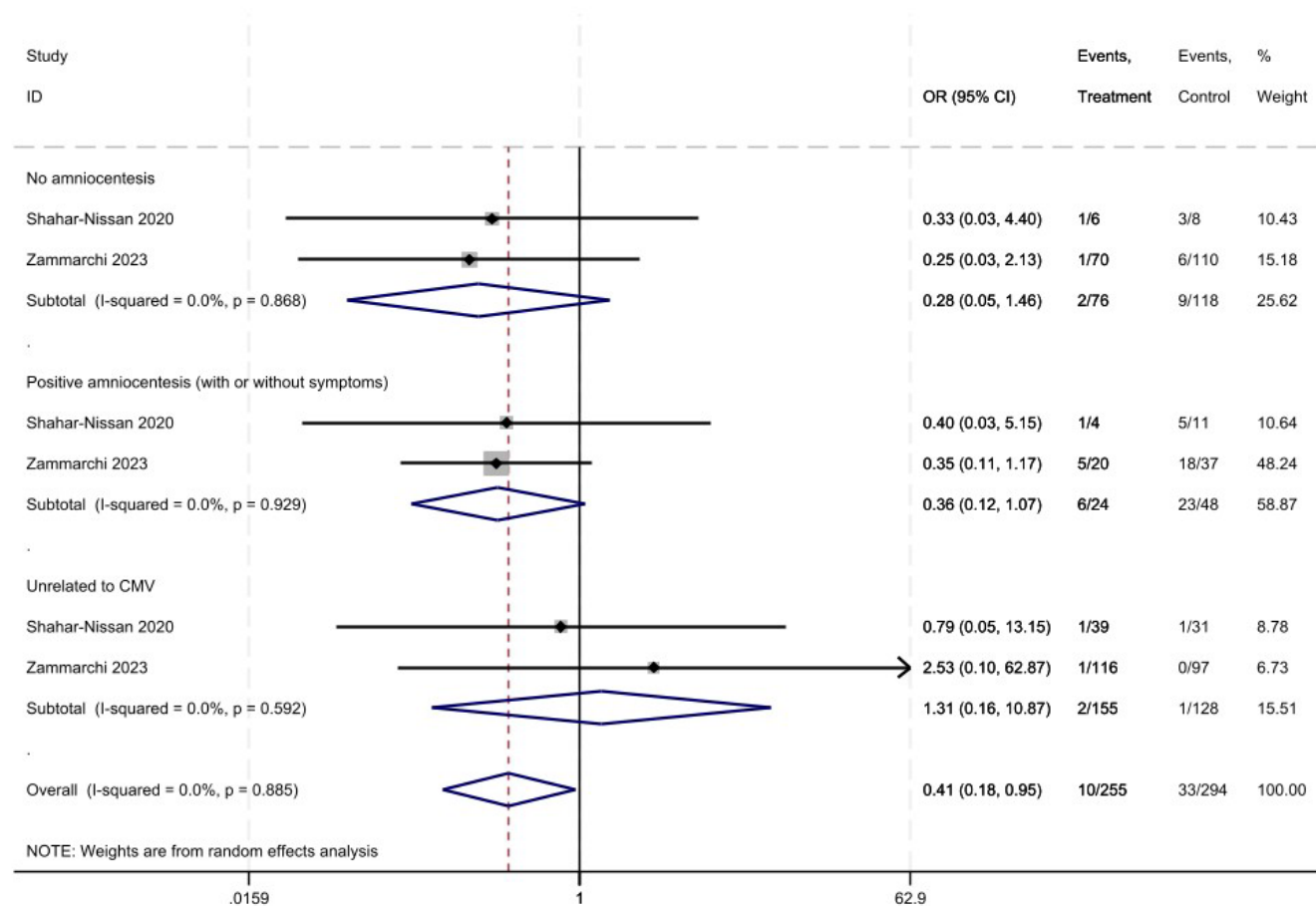


**Figure 15 – Metanalysis for cCMV at amniocentesis for patients treated and not treated with valaciclovir, stratified by trimester of seroconversion**



### 8.5.2 Termination of pregnancy

Figure 16 – Metanalysis for TOP for patients treated and not treated with valaciclovir





### 8.5.3 cCMV at birth

Figure 17 – Metanalysis for cCMV at birth for patients treated and not treated with valaciclovir

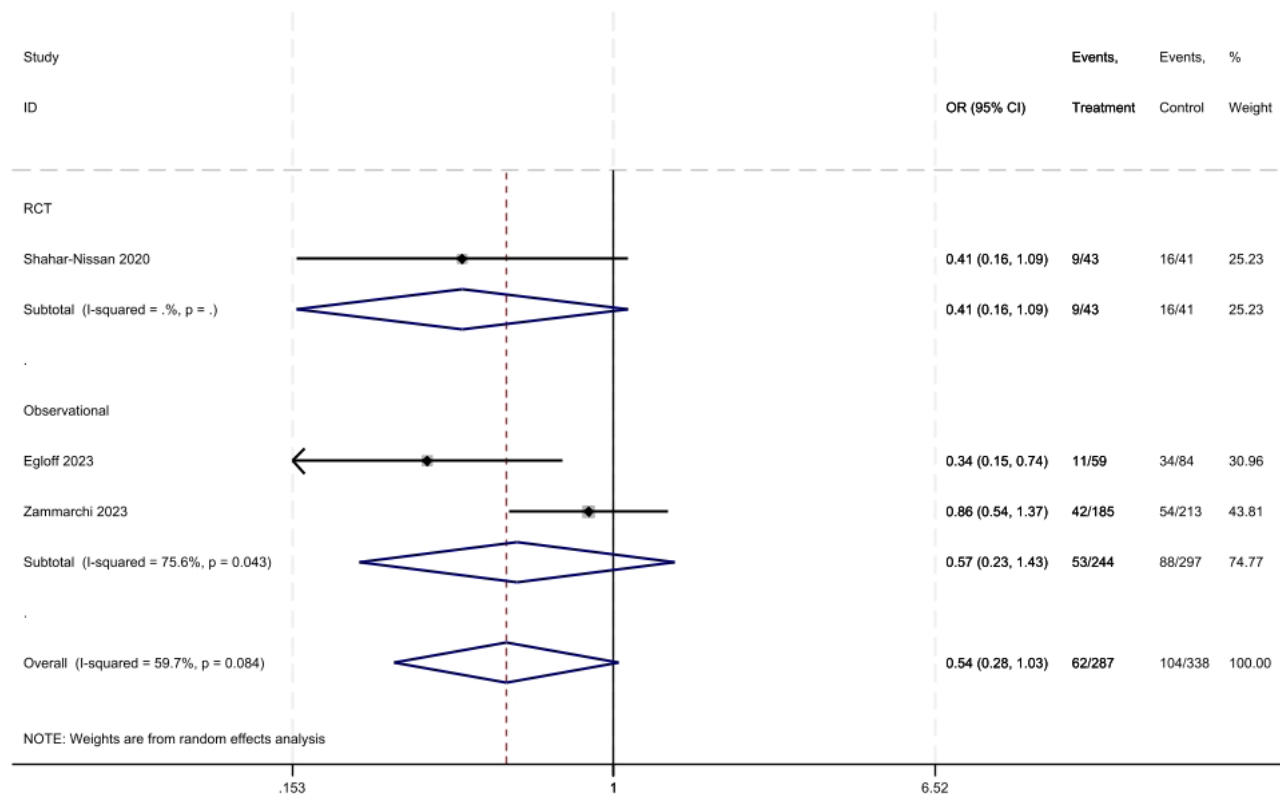
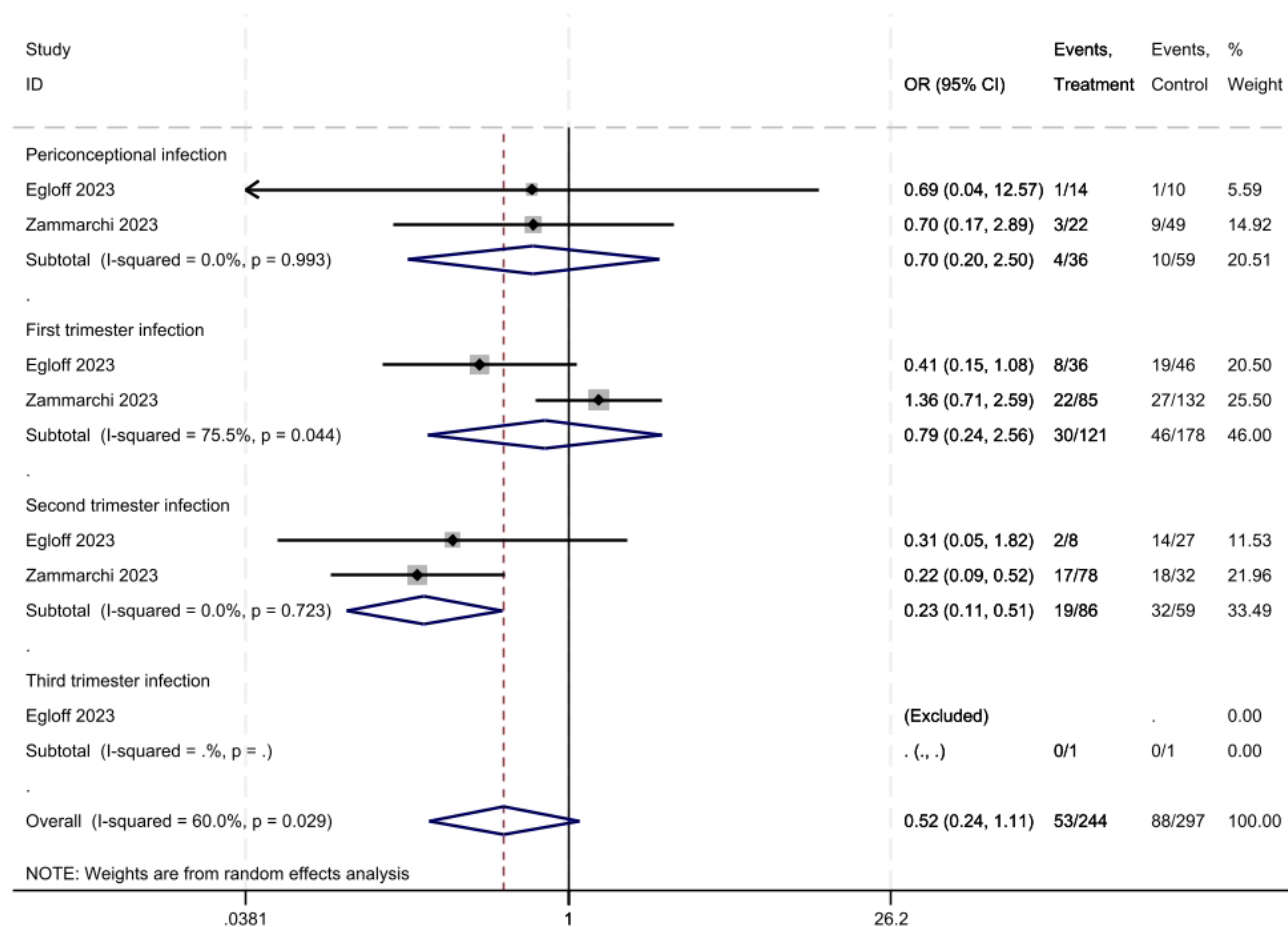


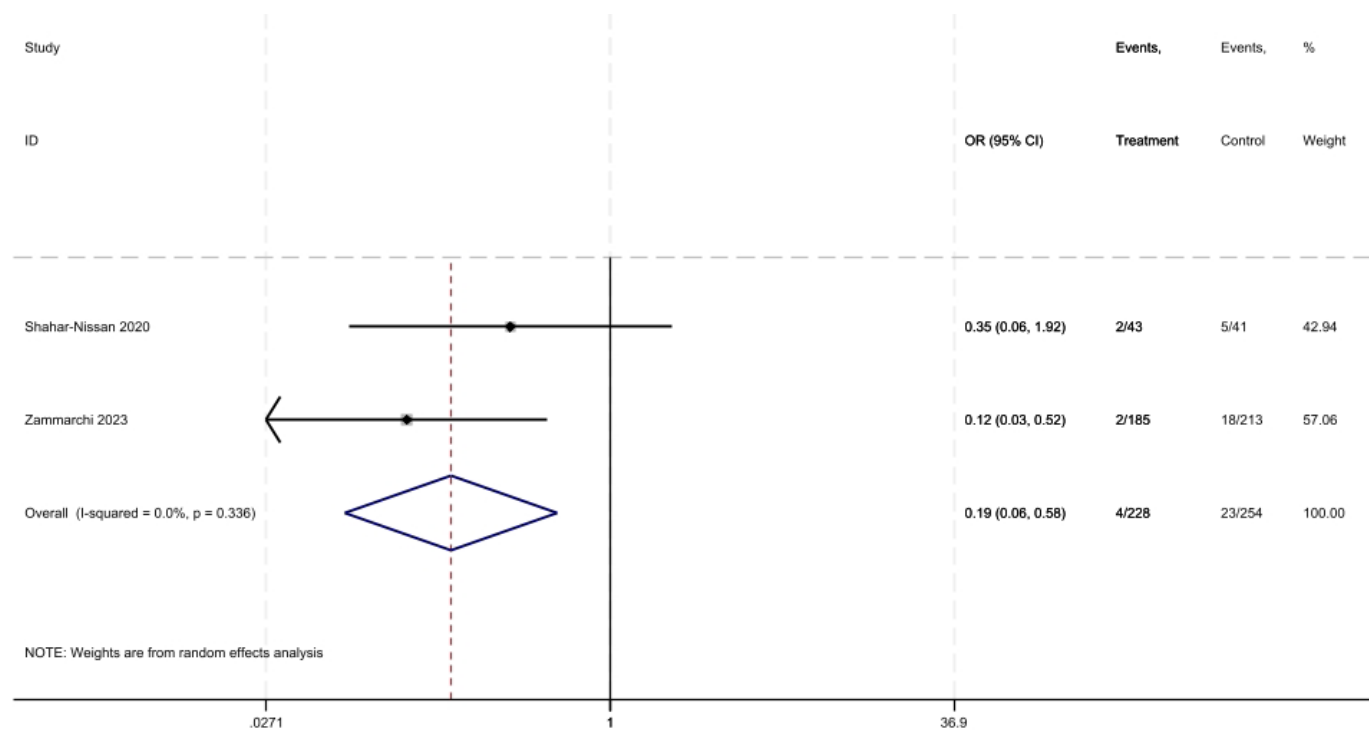
Figure 18 – Metanalysis for cCMV at birth for patients treated and not treated with valaciclovir, stratified by trimester of seroconversion





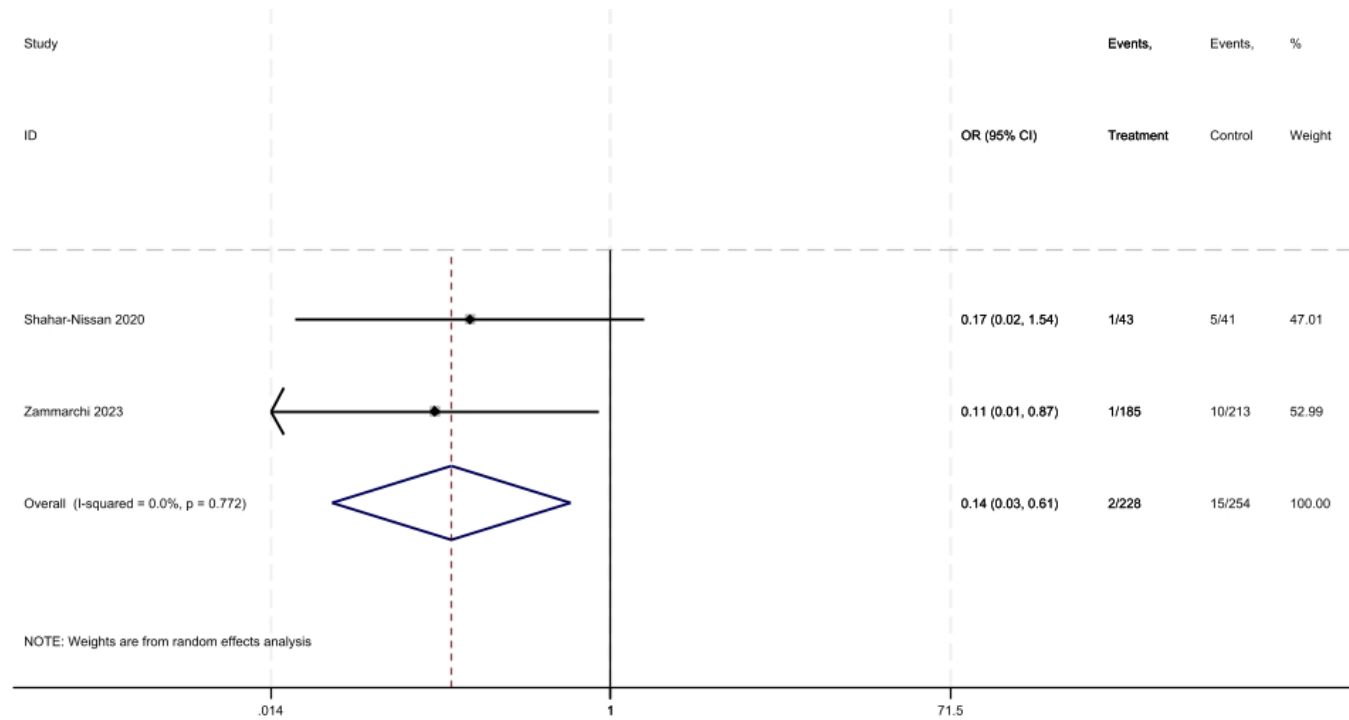
### Appendix 1.1.1. Severely symptomatic newborns

Figure 19 – Metanalysis for severely symptomatic newborns for patients treated and not treated with valaciclovir





### Appendix 1.1.2. Hearing loss

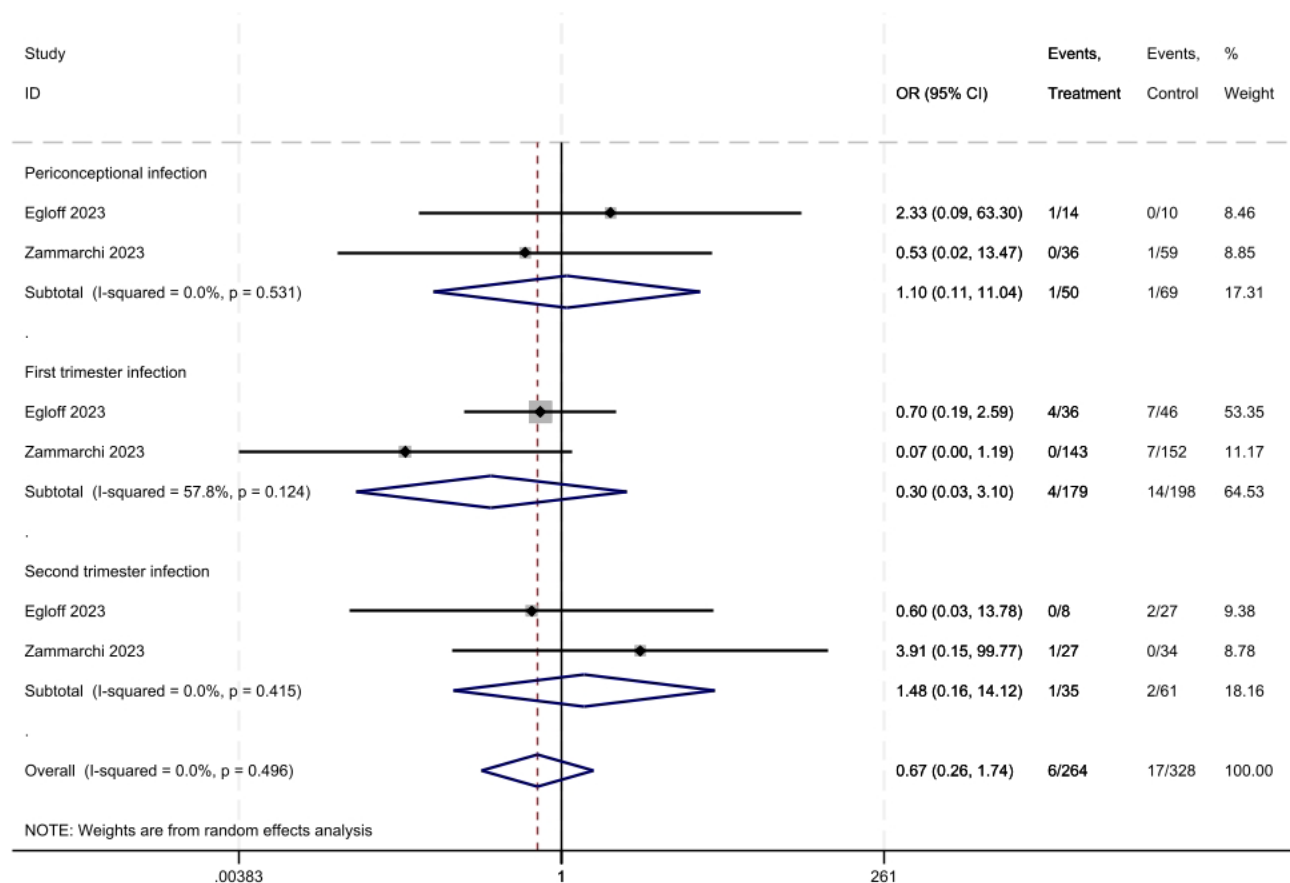




### 8.5.4 Brain anomalies at birth

#### ON ULTRASOUND

Figure 20 – Metanalysis for brain abnormalities at birth on ultrasound for patients treated and not treated with valaciclovir

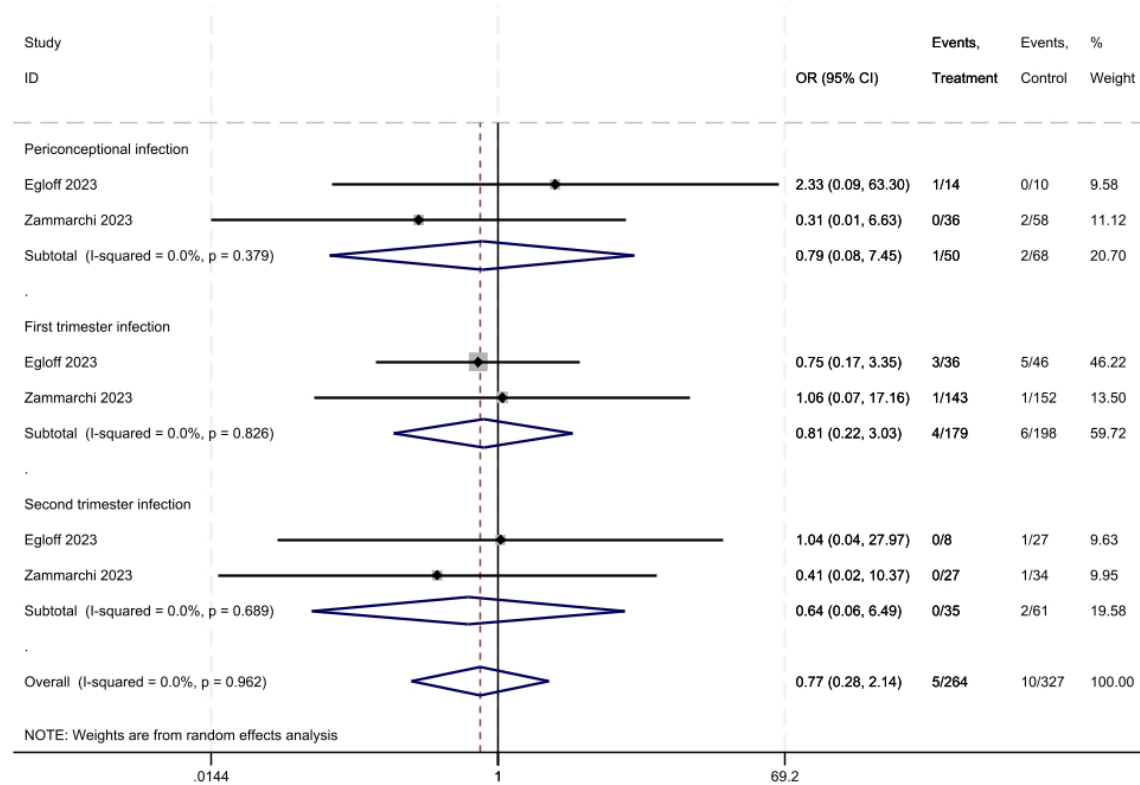




## ON MRI

To be consistent in the two studies, denominator is all patients, not patients with MRI performed.

**Figure 21 – Metanalysis for brain abnormalities at birth on MRI for patients treated and not treated with valaciclovir**



| Certainty assessment                             |                                |                        |               |              |                        |                      | No of patients      |                         | Effect                 |                                                  | Certainty of the evidence        | Importance |
|--------------------------------------------------|--------------------------------|------------------------|---------------|--------------|------------------------|----------------------|---------------------|-------------------------|------------------------|--------------------------------------------------|----------------------------------|------------|
| No of studies                                    | Study design                   | Risk of bias           | Inconsistency | Indirectness | Imprecision            | Other considerations | Valaciclovir 8g/day | Placebo or no treatment | Relative (95% CI)      | Absolute (95% CI)                                |                                  |            |
| cCMV at amniocentesis                            |                                |                        |               |              |                        |                      |                     |                         |                        |                                                  |                                  |            |
| 4                                                | non-randomised studies and RCT | serious <sup>a,b</sup> | not serious   | not serious  | not serious            | none                 | 43/300 (14.3%)      | 86/295 (29.2%)          | OR 0.41 (0.27 to 0.61) | 147 fewer per 1,000 (from 192 fewer to 91 fewer) | ⊕○○○ Very low <sup>a,b</sup>     | IMPORTANT  |
| cCMV at amniocentesis periconceptual infection   |                                |                        |               |              |                        |                      |                     |                         |                        |                                                  |                                  |            |
| 4                                                | non-randomised studies and RCT | serious <sup>a,b</sup> | not serious   | not serious  | serious <sup>c,d</sup> | none                 | 8/92 (8.7%)         | 15/85 (17.6%)           | OR 0.49 (0.19 to 1.28) | 81 fewer per 1,000 (from 137 fewer to 29 more)   | ⊕○○○ Very low <sup>a,b,c,d</sup> | IMPORTANT  |
| cCMV at amniocentesis first trimester infection  |                                |                        |               |              |                        |                      |                     |                         |                        |                                                  |                                  |            |
| 4                                                | non-randomised studies and RCT | serious <sup>a,b</sup> | not serious   | not serious  | not serious            | none                 | 27/164 (16.5%)      | 63/193 (32.6%)          | OR 0.40 (0.24 to 0.67) | 164 fewer per 1,000 (from 226 fewer to 87 fewer) | ⊕○○○ Very low <sup>a,b</sup>     | IMPORTANT  |
| cCMV at amniocentesis second trimester infection |                                |                        |               |              |                        |                      |                     |                         |                        |                                                  |                                  |            |



|                                                                         |                                |                        |                      |             |                        |      |                   |                    |                           |                                                     |                                     |           |
|-------------------------------------------------------------------------|--------------------------------|------------------------|----------------------|-------------|------------------------|------|-------------------|--------------------|---------------------------|-----------------------------------------------------|-------------------------------------|-----------|
| 2                                                                       | non-randomised studies and RCT | serious <sup>a</sup>   | not serious          | not serious | serious <sup>c</sup>   | none | 8/44<br>(18.2%)   | 8/15<br>(53.3%)    | OR 0.13<br>(0.02 to 0.88) | 404 fewer per 1,000<br>(from 511 fewer to 35 fewer) | ⊕○○○<br>Very low <sup>a,c</sup>     | IMPORTANT |
| <b>termination of pregnancy</b>                                         |                                |                        |                      |             |                        |      |                   |                    |                           |                                                     |                                     |           |
| 2                                                                       | non-randomised study and RCT   | serious <sup>b,e</sup> | not serious          | not serious | serious <sup>c</sup>   | none | 10/255<br>(3.9%)  | 33/294<br>(11.2%)  | OR 0.41<br>(0.18 to 0.95) | 63 fewer per 1,000<br>(from 89 fewer to 8 fewer)    | ⊕○○○<br>Very low <sup>b,c,e</sup>   | CRITICAL  |
| <b>termination of pregnancy in patients with positive amniocentesis</b> |                                |                        |                      |             |                        |      |                   |                    |                           |                                                     |                                     |           |
| 2                                                                       | non-randomised study and RCT   | serious <sup>b,e</sup> | not serious          | not serious | serious <sup>c,d</sup> | none | 6/24<br>(25.0%)   | 23/48<br>(47.9%)   | OR 0.36<br>(0.12 to 1.07) | 230 fewer per 1,000<br>(from 380 fewer to 17 more)  | ⊕○○○<br>Very low <sup>b,c,d,e</sup> | CRITICAL  |
| <b>cCMV at birth</b>                                                    |                                |                        |                      |             |                        |      |                   |                    |                           |                                                     |                                     |           |
| 3                                                                       | non-randomised studies and RCT | serious <sup>a,b</sup> | serious <sup>f</sup> | not serious | not serious            | none | 62/287<br>(21.6%) | 104/338<br>(30.8%) | OR 0.54<br>(0.28 to 1.03) | 114 fewer per 1,000<br>(from 147 fewer to 22 fewer) | ⊕○○○<br>Very low <sup>a,b,f</sup>   | IMPORTANT |
| <b>cCMV at birth periconceptional infection</b>                         |                                |                        |                      |             |                        |      |                   |                    |                           |                                                     |                                     |           |
| 2                                                                       | non-randomised studies         | serious <sup>a</sup>   | not serious          | not serious | serious <sup>c,d</sup> | none | 4/36<br>(11.1%)   | 10/59<br>(16.9%)   | OR 0.7<br>(0.2 to 2.5)    | 44 fewer per 1,000<br>(from 130 fewer to            | ⊕○○○<br>Very low <sup>a,c,d</sup>   | IMPORTANT |



|                                                 |                              |                        |                           |             |                        |      |                |                |                        |                                                          |                                |           |
|-------------------------------------------------|------------------------------|------------------------|---------------------------|-------------|------------------------|------|----------------|----------------|------------------------|----------------------------------------------------------|--------------------------------|-----------|
|                                                 |                              |                        |                           |             |                        |      |                |                |                        | 168 more)                                                |                                |           |
| <b>cCMV at birth first trimester infection</b>  |                              |                        |                           |             |                        |      |                |                |                        |                                                          |                                |           |
| <b>2</b>                                        | non-randomised studies       | serious <sup>a</sup>   | very serious <sup>g</sup> | not serious | serious <sup>d</sup>   | none | 30/121 (24.8%) | 46/178 (25.8%) | OR 0.79 (0.24 to 2.56) | <b>43 fewer per 1,000</b> (from 98 fewer to 95 more)     | ⊕○○○ Very low <sup>a,d,g</sup> | IMPORTANT |
| <b>cCMV at birth second trimester infection</b> |                              |                        |                           |             |                        |      |                |                |                        |                                                          |                                |           |
| <b>2</b>                                        | non-randomised studies       | serious <sup>a</sup>   | not serious               | not serious | serious <sup>c</sup>   | none | 19/86 (22.1%)  | 32/59 (54.2%)  | OR 0.23 (0.11 to 0.51) | <b>328 fewer per 1,000</b> (from 427 fewer to 161 fewer) | ⊕○○○ Very low <sup>a,c</sup>   | IMPORTANT |
| <b>cCMV at birth third trimester infection</b>  |                              |                        |                           |             |                        |      |                |                |                        |                                                          |                                |           |
| <b>1</b>                                        | non-randomised study         | serious <sup>a</sup>   | not serious               | not serious | not serious            | none | 0/1 (0.0%)     | 0/1 (0.0%)     | not estimable          |                                                          | ⊕○○○ Very low <sup>a</sup>     | IMPORTANT |
| <b>miscarriage or stillbirth</b>                |                              |                        |                           |             |                        |      |                |                |                        |                                                          |                                |           |
| <b>1</b>                                        | non-randomised study         | serious <sup>e</sup>   | not serious               | not serious | serious <sup>c,d</sup> | none | 1/206 (0.5%)   | 4/244 (1.6%)   | OR 0.29 (0.03 to 2.64) | <b>12 fewer per 1,000</b> (from 16 fewer to 26 more)     | ⊕○○○ Very low <sup>c,d,e</sup> | IMPORTANT |
| <b>severely symptomatic newborns</b>            |                              |                        |                           |             |                        |      |                |                |                        |                                                          |                                |           |
| <b>2</b>                                        | non-randomised study and RCT | serious <sup>b,e</sup> | not serious               | not serious | serious <sup>c</sup>   | none | 4/228 (1.8%)   | 23/254 (9.1%)  | OR 0.19 (0.06 to 0.58) | <b>72 fewer per 1,000</b> (from 85 fewer to 42 fewer)    | ⊕○○○ Very low <sup>b,c,e</sup> | CRITICAL  |

| hearing loss at follow-up |                              |                        |             |             |                      |      |              |               |                        |                                                |                                   |           |
|---------------------------|------------------------------|------------------------|-------------|-------------|----------------------|------|--------------|---------------|------------------------|------------------------------------------------|-----------------------------------|-----------|
| 2                         | non-randomised study and RCT | serious <sup>b,e</sup> | not serious | not serious | serious <sup>c</sup> | none | 2/228 (0.9%) | 15/254 (5.9%) | OR 0.14 (0.03 to 0.61) | 50 fewer per 1,000 (from 57 fewer to 23 fewer) | ⊕○○○<br>Very low <sup>b,c,e</sup> | IMPORTANT |
| Severe adverse events     |                              |                        |             |             |                      |      |              |               |                        |                                                |                                   |           |
| 1                         | RCT                          | serious <sup>b</sup>   | not serious | not serious | not serious          | none | 0/45 (0%)    | 0/45 (0%)     | not estimable          | 0 events                                       | ⊕○○○<br>Very low <sup>b,c</sup>   | CRITICAL  |

**CI:** confidence interval; **OR:** odds ratio

*Explanations*

a. observational studies: serious selection bias because of a retrospective cohort an patient who declined treatment as control and residual confounding or lack of events to correct for confounding, with moderate concerns from treatment deviations and missing outcome data

b. RCT: deviations from intended interventions and missing outcome data from per-protocol exclusions and imputation, with some concern for selective reporting of secondary outcomes

c. The 95% confidence interval for this outcome is wide due to small sample size and/or low number of events

d. The 95% confidence interval includes both the possibility of benefit and the possibility of harm or no effect at all

e. observational study: serious selection bias and lack of events to correct for confounding despite inverse probability weighting, with some concern from minor treatment deviations

f. point estimate of effect is different in the two included studies

g. point estimate of effect is opposite (benefit versus harm) in the two included studies



### 8.6.2 Tertiary prevention

**Table 17 – Summary table for GRADE for therapy with valaciclovir for tertiary prevention**

| Certainty assessment                          |                        |                      |               |                      |                                   |                      | № of patients       |                         | Effect               |                                                  | Certainty of the evidence  | Importance |
|-----------------------------------------------|------------------------|----------------------|---------------|----------------------|-----------------------------------|----------------------|---------------------|-------------------------|----------------------|--------------------------------------------------|----------------------------|------------|
| № of studies                                  | Study design           | Risk of bias         | Inconsistency | Indirectness         | Imprecision                       | Other considerations | Valaciclovir 8g/day | Placebo or no treatment | Relative (95% CI)    | Absolute (95% CI)                                |                            |            |
| termination of pregnancy                      |                        |                      |               |                      |                                   |                      |                     |                         |                      |                                                  |                            |            |
| 1                                             | non-randomised studies | serious <sup>a</sup> | not serious   | serious <sup>b</sup> | Very serious <sup>c,d</sup>       | none                 | 7/21 (33.3%)        | 12/24 (50.0%)           | OR 0.50 (0.15-1.68)  | 167 fewer per 1,000 (from 370 fewer to 127 more) | ⊕○○○ Very low <sup>a</sup> | CRITICAL   |
| severe symptoms = hearing loss and severe CID |                        |                      |               |                      |                                   |                      |                     |                         |                      |                                                  |                            |            |
| 1                                             | non-randomised studies | serious <sup>a</sup> | not serious   | serious <sup>b</sup> | extremelly serious <sup>c,d</sup> | none                 | 3/21 (14.3%)        | 1/24 (0.0%)             | OR 3.38 (0.37-40.02) | 101 more per 1,000 (from 26 fewer to 593 more)   | ⊕○○○ Very low <sup>a</sup> | CRITICAL   |

CI: confidence interval; OR: odds ratio

**Explanations**

- observational study: serious selection bias and concerns regarding confounding, missing data and measurement of outcomes
- only infected fetuses with symptoms were included
- The 95% confidence interval for this outcome is wide due to small sample size and/or low number of events
- The 95% confidence interval includes both the possibility of benefit and the possibility of harm or no effect at all

## 9 NEGATIVE AMNIOCENTESIS: FREQUENCY OF CMV-ASSOCIATED SEQUELAE AND FOLLOW-UP

This chapter addresses two research questions using a single search strategy, that was based on the patient population and the performing of amniocentesis.

The first question “Results of amniocentesis to predict the risk of CMV-related sequelae when cCMV is detected at birth” compares the risk of CMV-related sequelae in the foetus or child between children born to mothers who had a primary infection and a positive amniocentesis with cCMV detected at birth, and children born to mothers who had a primary infection and a negative amniocentesis and still had cCMV detected at birth.

The second question “Follow up in case of negative amniocentesis” examines whether, in pregnancies with a negative amniocentesis, a more intensive antenatal post-amniocentesis follow-up was linked with better detection of CMV-related sequelae in the foetus or child and better pregnancy outcome.

The results are presented together because both are needed in practice to decide on the follow-up of a negative amniocentesis.

### 9.1 PECO

**Table 18 – PECO for results of amniocentesis to predict the risk of congenital CMV-related sequelae when cCMV is detected at birth**

| Category        | Definition                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
|-----------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Objective       | To evaluate whether amniocentesis results can serve as a reliable prognostic tool to predict CMV-related sequelae when cCMV is detected at birth                                                                                                                                                                                                                                                                                                                                   |
| Review Question | In pregnant patients with confirmed primary CMV infection, what is the risk of CMV-related sequelae in the foetus or child in case of negative amniocentesis compared to positive amniocentesis, when both result in positive cCMV at birth?                                                                                                                                                                                                                                       |
| Population      | Pregnant patients with a primary CMV infection                                                                                                                                                                                                                                                                                                                                                                                                                                     |
| Exposure        | Positive CMV DNA PCR at amniocentesis (index test) and positive CMV DNA PCR at birth or within the first three weeks of life (reference standard)                                                                                                                                                                                                                                                                                                                                  |
| Comparator      | Negative CMV DNA PCR at amniocentesis (index test) and positive CMV DNA PCR at birth within the first three weeks of life (reference standard)                                                                                                                                                                                                                                                                                                                                     |
| Outcomes        | Critical: Severe antenatal imaging anomalies, termination of pregnancy, severely symptomatic newborns, cerebral palsy, motor developmental delay, cognitive developmental delay.<br>Other: see patient important outcomes. Accuracy measurements for amniocentesis for the presence of cCMV at birth                                                                                                                                                                               |
| Study design    | Good quality systematic reviews, single arms of RCTs, prospective and retrospective cohort studies, case-control studies<br>Excluded: 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 foetuses or children at birth according to their amniocentesis result. Studies where index and/or reference standard testing not include CMV DNA PCR. |
| Analysis        | By trimester of seroconversion, by treatment status                                                                                                                                                                                                                                                                                                                                                                                                                                |



Table 19 – PICO for follow-up of negative amniocentesis

| Category               | Definition                                                                                                                                                                                                                                                        |
|------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Objective</b>       | To assess whether adding enhanced imaging after a negative amniocentesis meaningfully improves the detection of CMV-related sequelae and leads to better patient-important outcomes compared with routine imaging.                                                |
| <b>Review Question</b> | In pregnant patients with a negative amniocentesis for CMV, does enhanced imaging (e.g., serial ultrasound, fetal MRI) compared with routine imaging improve detection of late CMV-related sequelae and lead to better clinical outcomes for the foetus or child? |
| <b>Population</b>      | Pregnant patients with confirmed primary CMV infection and a negative amniocentesis result                                                                                                                                                                        |
| <b>Intervention</b>    | Enhanced imaging: regular/serial targeted ultrasound and/or fetal MRI beyond standard obstetric care.                                                                                                                                                             |
| <b>Comparator</b>      | No enhanced imaging, i.e., only routine standard-of-care imaging.                                                                                                                                                                                                 |
| <b>Outcomes</b>        | Critical: Severe antenatal imaging anomalies, termination of pregnancy, severely symptomatic newborns, cerebral palsy, motor developmental delay, cognitive developmental delay.<br>Other: see patient important outcomes.                                        |
| <b>Study design</b>    | Good quality systematic reviews, randomised controlled trials, prospective and retrospective cohort studies. Excluded: narrative reviews, case reports, case series, congress proceedings.                                                                        |
| <b>Analysis</b>        | Stratification by trimester of seroconversion (periconceptional, first, second, third); by treatment status                                                                                                                                                       |

## 9.2 Search strategies

All review questions related to amniocentesis were addressed using a single search strategy, as these questions shared the same population and index test criteria.

The search was performed on October 21st 2025

### 9.2.1 Medline through Pubmed

1. "Amniocentesis"[MeSH] OR amniocentesis[tiab] OR "amniotic fluid sampl\*" [tiab] OR "amniotic fluid test\*" [tiab] OR "amniotic fluid analys\*" [tiab] OR amnio\*[tiab] OR "prenatal diagnos\*" [tiab] OR "antenatal diagnos\*" [tiab] OR "antenatal screening\*" [tiab] OR "prenatal screening\*" [tiab] OR "fetal diagnos\*" [tiab] OR "foetal diagnos\*" [tiab] OR "intrauterine diagnos\*" [tiab]

2. "Cytomegalovirus Infections"[MeSH] OR "Cytomegalovirus"[MeSH] OR cytomegalovirus[tiab] OR CMV[tiab] OR hhv5[tiab] OR "hhv 5"[tiab] OR "HHV-5"[tiab] OR "herpesvirus 5"[tiab] OR "herpes virus 5"[tiab] OR hcmv[tiab] OR "salivary gland virus"[tiab] OR "salivary gland disease"[tiab] OR "inclusion disease"[tiab] OR "cytomegalic inclusion disease"[tiab] OR "congenital cytomegalovirus infection"[tiab]

3. #1 AND #2

### 9.2.2 Embase

1. 'amniocentesis'/exp OR amniocentesis:ti,ab,kw OR 'amniotic fluid sampl\*':ti,ab,kw OR 'amniotic fluid test\*':ti,ab,kw OR 'amniotic fluid analys\*':ti,ab,kw OR amnio\*:ti,ab,kw OR 'prenatal diagnos\*':ti,ab,kw OR 'antenatal diagnos\*':ti,ab,kw OR 'antenatal screening\*':ti,ab,kw OR 'prenatal screening\*':ti,ab,kw OR 'fetal diagnos\*':ti,ab,kw OR 'foetal diagnos\*':ti,ab,kw OR 'intrauterine diagnos\*':ti,ab,kw

2. 'congenital cytomegalovirus infection'/exp OR 'Human cytomegalovirus'/exp OR cytomegalovirus:ti,ab,kw OR CMV:ti,ab,kw OR hhv5:ti,ab,kw OR 'hhv 5':ti,ab,kw OR 'HHV-5':ti,ab,kw OR 'herpesvirus 5':ti,ab,kw OR 'herpes virus 5':ti,ab,kw OR hcmv:ti,ab,kw OR 'salivary gland virus':ti,ab,kw OR 'salivary gland disease':ti,ab,kw OR 'inclusion disease':ti,ab,kw OR 'cytomegalic inclusion disease':ti,ab,kw OR 'congenital cytomegalovirus infection':ti,ab,kw

Following methodology described in GRADE Guidance 42, and after discussion with the guideline development group, minimal important difference thresholds corresponding to a utility value of 0 were used for critical outcomes.

3. #1 AND #2

### 9.2.3 Cochrane

1. [mh "Amniocentesis"] OR amniocentesis:ti,ab,kw OR ("amniotic fluid sample" OR "amniotic fluid samples"):ti,ab,kw OR ("amniotic fluid test" OR "amniotic fluid tests"):ti,ab,kw OR ("amniotic fluid analysis" OR "amniotic fluid analyses"):ti,ab,kw OR amnio\*:ti,ab,kw OR ("prenatal diagnosis" OR "prenatal diagnoses"):ti,ab,kw OR ("antenatal diagnosis" OR "antenatal diagnoses"):ti,ab,kw OR ("antenatal screening" OR "antenatal screenings"):ti,ab,kw OR ("prenatal screening" OR "prenatal screenings"):ti,ab,kw OR ("fetal diagnosis" OR "fetal diagnoses"):ti,ab,kw OR ("foetal diagnosis" OR "foetal diagnoses"):ti,ab,kw OR ("intrauterine diagnosis" OR "intrauterine diagnoses"):ti,ab,kw

2. [mh "Cytomegalovirus Infections, Congenital"] OR [mh "Cytomegalovirus Infections"] OR [mh "Cytomegalovirus"] OR cytomegalovirus:ti,ab,kw OR CMV:ti,ab,kw OR hhv5:ti,ab,kw OR "hhv 5":ti,ab,kw OR "HHV-5":ti,ab,kw OR "herpesvirus 5":ti,ab,kw OR "herpes virus 5":ti,ab,kw OR hcmv:ti,ab,kw OR "salivary gland virus":ti,ab,kw OR "salivary gland disease":ti,ab,kw OR "inclusion disease":ti,ab,kw OR "cytomegalic inclusion disease":ti,ab,kw OR "congenital cytomegalovirus infection":ti,ab,kw

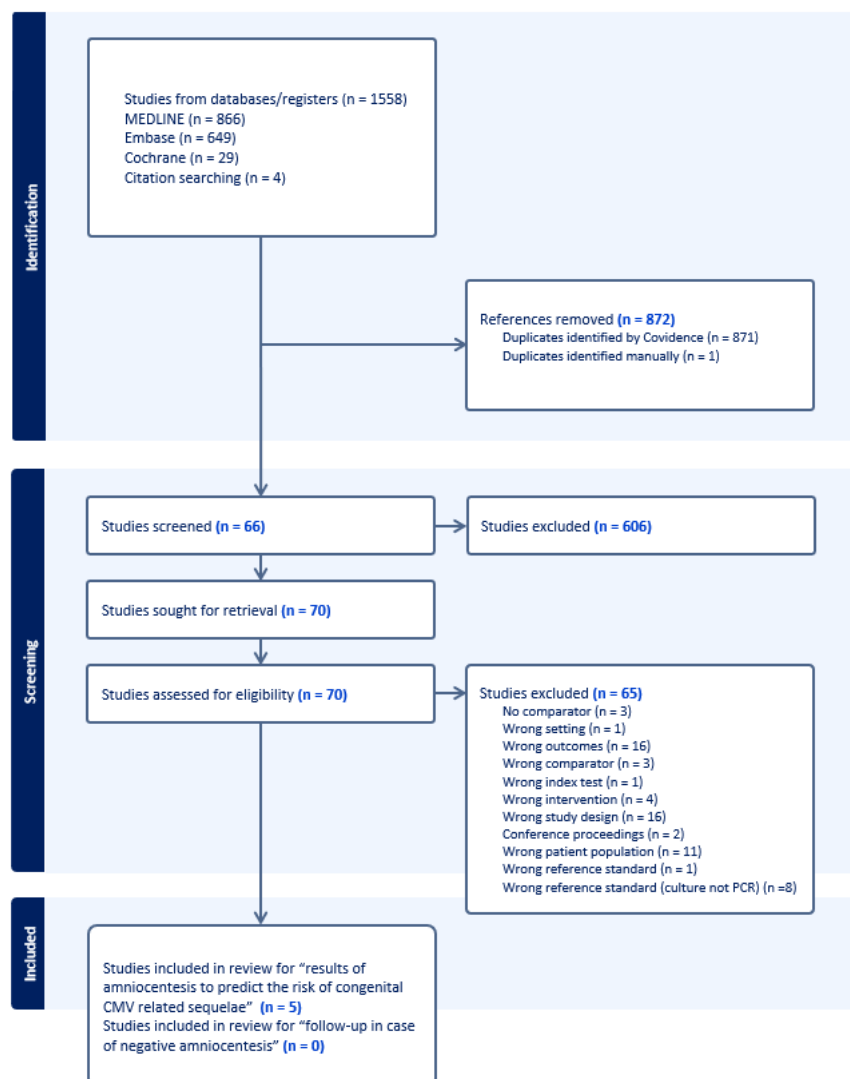
3. #1 AND #2

4. #3 NOT ("case report" OR "case reports"):ti,ab,kw



### 9.3 Study selection

**Figure 22 – Study flow selection for amniocentesis to predict the risk of CMV-related sequelae when cCMV is detected at birth**



Modified from figure provided by Covidence

### 9.3.1 Studies excluded with reason

**Table 20 – Excluded primary studies for amniocentesis to predict the risk of CMV-related sequelae when cCMV is detected at birth**

| Study and year         | Reason for exclusion                                        |
|------------------------|-------------------------------------------------------------|
| Antsaklis 2000         | Exclusion reason: Wrong reference standard                  |
| Avidor 2004            | Exclusion reason: Wrong study design                        |
| Azam 2001              | Exclusion reason: Wrong reference standard                  |
| Bartek 2025            | Exclusion reason: Wrong patient population                  |
| Bodeus 1999            | Exclusion reason: Wrong outcomes                            |
| Buca 2021              | Exclusion reason: Wrong patient population                  |
| Catanzarite 1993       | Exclusion reason: Wrong study design                        |
| Chatzakis 2023*        | Exclusion reason: Wrong study design                        |
| D'Alberti 2024         | Exclusion reason: Wrong study design                        |
| Daiminger 2005         | Exclusion reason: Wrong intervention                        |
| DeConinck 2024         | Exclusion reason: No comparator                             |
| Denef 2022             | Exclusion reason: Wrong outcomes                            |
| DeSantis 2020          | Exclusion reason: Wrong study design                        |
| Dinsmoor 2022          | Exclusion reason: Wrong outcomes                            |
| Donner 1993            | Exclusion reason: Wrong reference standard (culture no PCR) |
| Donner 1994            | Exclusion reason: Wrong outcomes                            |
| Enders 2001            | Exclusion reason: Wrong index test                          |
| Enders 2011            | Exclusion reason: Wrong intervention                        |
| Enders 2017            | Exclusion reason: Wrong study design                        |
| Enders 2017            | Exclusion reason: Wrong outcomes                            |
| Fattuoni 2016          | Exclusion reason: Wrong study design                        |
| Feldman 2011           | Exclusion reason: Wrong reference standard                  |
| Fernicola 2024         | Exclusion reason: Wrong study design                        |
| Fourgeaud 2025         | Exclusion reason: Wrong outcomes                            |
| Gabbay-Benziv 2012     | Exclusion reason: No comparator                             |
| Goegebuer 2009         | Exclusion reason: Wrong reference standard (culture no PCR) |
| Gouarin 2001           | Exclusion reason: Wrong outcomes                            |
| Guerra 2000            | Exclusion reason: No comparator                             |
| Hughes 2019            | Exclusion reason: Conference proceedings                    |
| Hui 2022               | Exclusion reason: Wrong outcomes                            |
| Kagan 2017             | Exclusion reason: Wrong study design                        |
| Kanengisser-Pines 2009 | Exclusion reason: Wrong intervention                        |
| Lazzarotto 1998        | Exclusion reason: Wrong patient population                  |
| Lazzarotto 2014        | Exclusion reason: Wrong study design                        |
| Leruez-Ville 2016      | Exclusion reason: Wrong patient population                  |
| Liesnard 2000          | Exclusion reason: Wrong reference standard (culture no PCR) |



|                                                                                                                        |                                                             |
|------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------|
| Lipitz 1997                                                                                                            | Exclusion reason: Wrong outcomes                            |
| Lipitz 2002                                                                                                            | Exclusion reason: Wrong reference standard (culture no PCR) |
| Lipitz 2020                                                                                                            | Exclusion reason: Wrong patient population                  |
| Mappa 2023                                                                                                             | Exclusion reason: Wrong patient population                  |
| Margioulas-Siarkou 2015                                                                                                | Exclusion reason: Wrong outcomes                            |
| MedinaGarcía 2024                                                                                                      | Exclusion reason: Wrong intervention                        |
| Miller 2009                                                                                                            | Exclusion reason: Wrong patient population                  |
| Minsart 2018                                                                                                           | Exclusion reason: Wrong comparator                          |
| Moaven 1995                                                                                                            | Exclusion reason: Wrong study design                        |
| Naing 2016                                                                                                             | Exclusion reason: Wrong study design                        |
| NCT02351102 2015                                                                                                       | Exclusion reason: Wrong study design                        |
| Nigro 2005                                                                                                             | Exclusion reason: Wrong study design                        |
| Patel 2024                                                                                                             | Exclusion reason: Wrong study design                        |
| Picone 2013                                                                                                            | Exclusion reason: Wrong comparator                          |
| Revello 1995                                                                                                           | Exclusion reason: Wrong comparator                          |
| Revello 2008                                                                                                           | Exclusion reason: Wrong outcomes                            |
| Revello 2014                                                                                                           | Exclusion reason: Wrong outcomes                            |
| Satilmis 2007                                                                                                          | Exclusion reason: Wrong setting                             |
| Seidel 2020                                                                                                            | Exclusion reason: Wrong patient population                  |
| Seidenari 2025                                                                                                         | Exclusion reason: Wrong patient population                  |
| Shahar-Nissan 2019                                                                                                     | Exclusion reason: Conference proceedings                    |
| Simonazzi 2017                                                                                                         | Exclusion reason: Wrong reference standard                  |
| Toti 2023                                                                                                              | Exclusion reason: Wrong study design                        |
| TugrulErsak 2023                                                                                                       | Exclusion reason: Wrong outcomes                            |
| VandenEede 2025                                                                                                        | Exclusion reason: Wrong patient population                  |
| Visentin 2012                                                                                                          | Exclusion reason: Wrong reference standard (culture no PCR) |
| Zavattoni 2014                                                                                                         | Exclusion reason: Wrong outcomes                            |
| Zavattoni 2016                                                                                                         | Exclusion reason: Wrong outcomes                            |
| Zhang 2025                                                                                                             | Exclusion reason: Wrong patient population                  |
| * Impossible to directly compare outcomes of infected fetuses or children at birth according to amniocentesis results. |                                                             |

## 9.4 Appraisal for amniocentesis to predict the risk of congenital CMV-related sequelae

Quality appraisal for the question related to amniocentesis to predict the risk of CMV-related sequelae when cCMV is detected at birth, was done using the QUAPAS(33) (Quality Assessment of Prognostic Accuracy Studies) instrument.

**Table 21 – QUAPAS appraisal, adapted for amniocentesis to predict the risk of congenital CMV-related sequelae**

|                                                                                                  | Bilavsky 2016(34)                                                                                                                                                                                                                                       | Shahar Nissan 2020(24) | Keymeulen 2021(17)                                                                                                                                                                                                                                                                                | Zammarchi 2023(28)                            | De Keersmaecker 2024(35)                                                                                                        |
|--------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------|
| <b>DOMAIN 1: PARTICIPANTS</b>                                                                    |                                                                                                                                                                                                                                                         |                        |                                                                                                                                                                                                                                                                                                   |                                               |                                                                                                                                 |
| Signalling questions                                                                             |                                                                                                                                                                                                                                                         |                        |                                                                                                                                                                                                                                                                                                   |                                               |                                                                                                                                 |
| Was a consecutive or random sample of participants enrolled?                                     | no                                                                                                                                                                                                                                                      | yes                    | no                                                                                                                                                                                                                                                                                                | unclear                                       | yes                                                                                                                             |
| Was a case-control design avoided?                                                               | no                                                                                                                                                                                                                                                      | yes                    | no                                                                                                                                                                                                                                                                                                | yes                                           | yes                                                                                                                             |
| Did the study avoid inappropriate selection criteria?                                            | yes                                                                                                                                                                                                                                                     | yes                    | yes                                                                                                                                                                                                                                                                                               | yes                                           | unclear                                                                                                                         |
| Summary                                                                                          |                                                                                                                                                                                                                                                         |                        |                                                                                                                                                                                                                                                                                                   |                                               |                                                                                                                                 |
| <i>Risk of Bias: Could selection of participants have introduced bias?</i>                       | <b>high risk</b>                                                                                                                                                                                                                                        | <b>low risk</b>        | <b>high risk</b>                                                                                                                                                                                                                                                                                  | <b>unclear risk</b>                           | <b>unclear risk</b>                                                                                                             |
| <i>Applicability: Are there concerns that the participants do not match the review question?</i> | <b>unclear concern</b>                                                                                                                                                                                                                                  | <b>low concern</b>     | <b>high concern</b>                                                                                                                                                                                                                                                                               | <b>low concern</b>                            | <b>low concern</b>                                                                                                              |
| Remarks:                                                                                         | The selection of patients are only live children with cCMV, made in a case-control manner. , we have no information of the proportion in cases vs controls of the reason for performing CMV related amniocentesis (screening vs referral for ultrasound |                        | The patients included in the registry are only live children with cCMV, Foetuses that were terminated were not included into the registry, therefore in the true positive amniocentesis group results may appear milder. Results of amniocentesis is available of only 18% of children.. The vast | Many patients included refused amniocentesis. | It is not clear what the meaning is of exclusion criteria "if data collection was incomplete". Selection bias may be introduced |



|                                                                                                                                             |                                                                                                                    |                                                              |                                                                                                                                                                  |                                                              |                                                              |
|---------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------|--------------------------------------------------------------|
|                                                                                                                                             | anomalies/maternal symptoms)                                                                                       |                                                              | majority of "reason for testing" was maternal seroconversion" but we cannot isolate this group in the analysis, so some non-primary infections may be included.t |                                                              |                                                              |
| <b>DOMAIN 2: INDEX TEST</b>                                                                                                                 |                                                                                                                    |                                                              |                                                                                                                                                                  |                                                              |                                                              |
| Signaling questions                                                                                                                         |                                                                                                                    |                                                              |                                                                                                                                                                  |                                                              |                                                              |
| Index test(s): name + method used                                                                                                           | Amniotic Fluid PCR CMV DNA<br>At birth: Positive urine culture or PCR in one center, CMV PCR in the second center. | Amniotic Fluid PCR CMV DNA<br>At birth: PCR CMV DNA in urine | Amniotic Fluid PCR CMV DNA<br>At birth: viral isolation and/or CMV DNA PCR on urine/saliva (if diagnosis after 21 weeks, PCR CMV DNA on dried blood spot)        | Amniotic Fluid PCR CMV DNA<br>At birth: PCR CMV DNA in urine | Amniotic Fluid PCR CMV DNA<br>At birth: PCR CMV DNA in urine |
| Was the method used to perform the index test valid and reliable?                                                                           | yes                                                                                                                | yes                                                          | yes                                                                                                                                                              | yes                                                          | yes                                                          |
| Was the method for performing the index test the same for all participants?                                                                 | unclear                                                                                                            | yes                                                          | unclear                                                                                                                                                          | yes                                                          | yes                                                          |
| Were the index test results interpreted without knowledge of the outcome?                                                                   | yes                                                                                                                | yes                                                          | yes                                                                                                                                                              | yes                                                          | yes                                                          |
| Summary                                                                                                                                     |                                                                                                                    |                                                              |                                                                                                                                                                  |                                                              |                                                              |
| <i>Risk of Bias: Could the conduct or interpretation of the index tests have introduced bias?</i>                                           | unclear risk                                                                                                       | low risk                                                     | unclear risk                                                                                                                                                     | low risk                                                     | low risk                                                     |
| <i>Applicability: Are there concerns that the index test or its conduct, interpretation, or threshold differs from the review question?</i> | low concern                                                                                                        | low concern                                                  | low concern                                                                                                                                                      | low concern                                                  | low concern                                                  |

|          |                                                                                                                                                                                                                                        |  |                                                                                   |  |  |
|----------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--|-----------------------------------------------------------------------------------|--|--|
| Remarks: | In centre 1 (Israel), for amniocentesis both rapid shell vial and CMV DNA PCR are performed and only if both negative it was considered negative.<br>cCMV at birth was detected with viral culture in a unknown proportion of patients |  | cCMV at birth was detected with viral culture in a unknown proportion of patients |  |  |
|----------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--|-----------------------------------------------------------------------------------|--|--|

**DOMAIN 3: OUTCOME**

## Signaling questions

|                                                   |                                                                                     |                                                   |                                                          |                                                                                                                                                             |                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
|---------------------------------------------------|-------------------------------------------------------------------------------------|---------------------------------------------------|----------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Which outcome(s) was/were assessed by this study? | Symptomatic cCMV infection according to Kimberlin et al, abnormal postnatal imaging | TOP, hearing loss, postnatal sonographic findings | Mildly, moderately and severely symptomatology at birth, | Miscarriage or TOP, presence of cCMV symptoms according to ESPID (European Society of Paediatric Infectious Diseases), use of valganciclovir in the newborn | Preterm birth, Small for Gestational Age, Microcephaly Symptomatic CMV disease (at least one petechiae, hepatomegaly, splenomegaly, IUGR, microcephaly or chorioretinitis). Motor deficit (need for physiotherapy later in life) early psychomotor development (global and fine motor abilities, language skills and social skills) hearing loss, visual impairment, learning disability (repeating a class) behavioral problems (analysed by child psychiatrists) |
|---------------------------------------------------|-------------------------------------------------------------------------------------|---------------------------------------------------|----------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|



|                                                                                 |                                                                                                                                                                                                                 |                                                                                                                                                                                                             |                                                                                                                                                                                                                                                                                            |                                                                                                                                                            |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
|---------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Which method was used to measure the outcome(s)?                                | Complete physical examination, blood test, fundoscopy, brain ultrasound (blinded to the amniocentesis results), hearing assessment (air and if need bone conduction BERA). Reports of radiological examination. | Full physical examination, blood count, liver function tests, brain ultrasound, audiometry, ophthalmological examination. Follow up in clinic or by interview (but no results really described for this FU) | There is no specific description on how symptomatology at birth or antenatal imaging results were measured, with the exception of a clear description of audiological evaluation (OAE, ABR or BRA) and eye exam, but there is a clear definition of mild/moderate or severe symptomatology | Clinical and laboratory data were retrieved from electronic or paper medical records according to the availability in the different participating centers. | Outcomes at birth: Standardised CMV follow up: examination by pediatrician, cranial ultrasound, eye fundus, ear examination (standard and BERA), this is for babies that had a positive amniocentesis and for all babies with a positive NU PCR even if amniocentesis negative Outcomes long term: Purpose made questionnaire distributed to patients (parents). Patient reported information on neonatal health, neurological outcomes, hearing outcomes and educational progress. |
| Was the method used to measure the outcome valid and reliable?                  | yes                                                                                                                                                                                                             | yes                                                                                                                                                                                                         | unclear                                                                                                                                                                                                                                                                                    | yes                                                                                                                                                        | unclear                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
| Was the same method used for all participants (per outcome)?                    | unclear                                                                                                                                                                                                         | yes                                                                                                                                                                                                         | unclear                                                                                                                                                                                                                                                                                    | unclear                                                                                                                                                    | yes                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
| Was the outcome measured without knowledge of the index test results?           | Brain imaging was blinded, otherwise unclear                                                                                                                                                                    | unclear                                                                                                                                                                                                     | unclear                                                                                                                                                                                                                                                                                    | unclear                                                                                                                                                    | unclear                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
| Summary:                                                                        |                                                                                                                                                                                                                 |                                                                                                                                                                                                             |                                                                                                                                                                                                                                                                                            |                                                                                                                                                            |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
| <i>Risk of Bias: Could the measurement of the outcome have introduced bias?</i> | <b>unclear risk</b>                                                                                                                                                                                             | <b>unclear risk</b>                                                                                                                                                                                         | <b>unclear risk</b>                                                                                                                                                                                                                                                                        | <b>unclear risk</b>                                                                                                                                        | <b>unclear risk</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |

|                                                                                               |                                                            |                 |                 |                                                                       |                                                                                                                                                                                                                                                                                                        |
|-----------------------------------------------------------------------------------------------|------------------------------------------------------------|-----------------|-----------------|-----------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <i>Applicability: Are there concerns that the outcome does not match the review question?</i> | unclear concern                                            | unclear concern | unclear concern | unclear concern                                                       | unclear concern                                                                                                                                                                                                                                                                                        |
| Remarks:                                                                                      | Unclear how outcomes are collected, especially in center 1 |                 |                 | Unclear how symptoms at birth were evaluated in the different centres | At birth there is a dedicated CMV examination only for children with a positive CMV PCR in NU. Evaluating long term FU with a questionnaire may introduce bias, since positive questionnaires are confirmed by documentation, but negative questionnaires mean that "the parents consider them normal" |
| <b>DOMAIN 4: FLOW AND TIMING</b>                                                              |                                                            |                 |                 |                                                                       |                                                                                                                                                                                                                                                                                                        |
| Signalling questions                                                                          |                                                            |                 |                 |                                                                       |                                                                                                                                                                                                                                                                                                        |
| Did all participants receive the index test?                                                  | no                                                         | no              | no              | no                                                                    | no                                                                                                                                                                                                                                                                                                     |
| Was treatment avoided after the index test was performed?                                     | yes                                                        | yes             | unclear         | unclear                                                               | yes                                                                                                                                                                                                                                                                                                    |
| Was the time horizon sufficient for capturing the outcome?                                    | yes                                                        | unclear         | no              | yes                                                                   | unclear                                                                                                                                                                                                                                                                                                |
| Was information on the outcome available for all participants?                                | yes                                                        | yes             | unclear         | yes                                                                   | yes                                                                                                                                                                                                                                                                                                    |
| Summary:                                                                                      |                                                            |                 |                 |                                                                       |                                                                                                                                                                                                                                                                                                        |
| <i>Risk of Bias: Could the study flow have introduced bias?</i>                               | high risk                                                  | high risk       | high risk       | high risk                                                             | high risk                                                                                                                                                                                                                                                                                              |



| <i>Applicability: Are there concerns that the time horizon does not match the review question?</i> | highconcern                                                                                                                                                                   | high concern                                                                                                                                                                                                        | high concern                                                                                                                                                                                                                                                                                                                                                                              | high concern                                                                                                                                                                                   | highconcern                                                                                                                                                                                                                                                                                                                                                                                                                     |
|----------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Remarks:                                                                                           | 756 infants were considered of which 301 had amniocentesis. Newborns were treated with valganciclovir, with different schemes according to centre. The follow-up time varies. | In women who refused amniocentesis (seven fetuses), amnio was presumed being negative in a CMV negative foetus and was presumed being positive in a CMV positive foetus. There are no long-term outcomes described. | The information on amniocentesis is available for only 18.2% of CMV + patients in the registry. Outcome measures of symptomatology at birth are available for all. Outcome measures of antenatal ultrasound are available for 139/233 in the amnio positive and 41/81 for amnio negative. Fetal MRI results were available only for 56/133 for amnio positive and 1/81 for amnio negative | 180/447 (40.2%) did not undergo amniocentesis, 12/116 (10.3%) newborn treated with Valaciclovir and a negative amniocentesis are lost to follow up. There are no long-term outcomes described. | The entry point is amniocentesis for seroconversion, it is unknown the proportion of seroconversions undergoing amniocentesis. The follow-up time varies, the range is between 1 to 8 years, so we do not have all the information at 8 years of age for all the children. We have long time data on 67/70 amniocentesis positives, 6/7 amniocentesis negatives and 160/220 amniocentesis negatives with negative cCMV at birth |
| Funding and author COI                                                                             | None declared                                                                                                                                                                 | None declared                                                                                                                                                                                                       | The research did not receive any specific grant from funding agencies in the public, commercial or non-profit sector                                                                                                                                                                                                                                                                      | None declared. Financially supported by the Italian ministry of health, University of Florence.                                                                                                | None declared                                                                                                                                                                                                                                                                                                                                                                                                                   |

## 9.5 Evidence tables for amniocentesis to predict the risk of congenital CMV-related sequelae

**Bilavsky E, Pardo J, Attias J, Levy I, Magny JF, Ville Y, Leruez-Ville M, Amir J. Clinical Implications for Children Born With Congenital Cytomegalovirus Infection Following a Negative Amniocentesis. Clin Infect Dis. 2016 Jul 1;63(1):33-8(34)**

|                                 |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |                                          |                                          |                         |
|---------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------|------------------------------------------|-------------------------|
| Country, data collection period | France and Israel,<br>01-2005 to 04-2015                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |                                          |                                          |                         |
| Population, sample size         | Infants with cCMV born from mothers with MPI and a negative amniocentesis (cases) (n=45) and children born with cCMV and positive amniocentesis (controls)(n=93) in two separate centres, centre 1 in Israel, centre 2 in France. 67.4% in both group infected following either periconceptional or first trimester MPI.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |                                          |                                          |                         |
| Key methodological features     | Case control study<br>Inclusion criteria: primary seroconversion Exclusion criteria: none listed for cases, controls are matched with same GA, in the same centre as cases 1:2                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |                                          |                                          |                         |
| Timing of amniocentesis         | At least 21 GW and 7 weeks after assumed date of maternal infection in center 1<br>20-21 GW and 6-7 weeks after maternal infection in center 2                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |                                          |                                          |                         |
| Outcome(s) assessed             | Symptomatic according to Kymberlin et al*, CNS symptoms, non-CNS symptoms, broader definition of symptomatic infants (any abnormal finding on brain US including periventricular hyper echogenicity, ventricular dilatation, pseudocyst of the germinal matrix and LSV), hearing loss, neurodevelopmental delay                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |                                          |                                          |                         |
| Outcome measurements            | <p>Outcomes at birth:</p> <p>in France: PCR DNA in saliva/urine within 10 days after birth;</p> <p>in Israel: culture or PCR in urine within the first two weeks of life.</p> <p>Complete physical examination, blood test, fundoscopy, brain ultrasound (blinded to the amniocentesis results), hearing assessment (air and if needed bone conduction BERA)</p> <p>Outcomes long term:</p> <p>No explanation of how long-term follow-up is performed.</p> <p>In center 1 all infants with any sign of CNS involvement (including imaging only) are given valganciclovir. In centre 2 infants with clinical symptoms of infection are given valganciclovir.</p> <p>Median FU 41.3 months for the cases and 36.8 for the controls. No description of lost to follow-up (although the occurrence is quoted in the discussion)</p> |                                          |                                          |                         |
| Results                         |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |                                          |                                          |                         |
|                                 | Outcome                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         | CMV at birth with positive amniocentesis | CMV at birth with negative amniocentesis | OR (95%CI) <sup>a</sup> |



|                                |                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |               |               |                       |
|--------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------|---------------|-----------------------|
|                                | Symptomatic at birth according to Kymberlin et al(36)*                                                                                                                                                                                                                                                                                                                                                                                                                    | 23/92 (25%)   | 2/46 (4.3%)   | 5.7 (1.29 to 25.45)   |
|                                | CNS symptoms                                                                                                                                                                                                                                                                                                                                                                                                                                                              | 24**          | 1**           |                       |
|                                | Non CNS symptoms                                                                                                                                                                                                                                                                                                                                                                                                                                                          | 10**          | 1**           |                       |
|                                | Symptomatic at birth including any ultrasound CNS anomaly                                                                                                                                                                                                                                                                                                                                                                                                                 | 53/92 (57.6%) | 11/46 (23.9%) | 2.4 (1.15 to 5)       |
|                                | Hearing loss at birth                                                                                                                                                                                                                                                                                                                                                                                                                                                     | 16/92 (17.3%) | 1/46 (2.1%)   | 8 (1.02 to 62.2)      |
|                                | Hearing loss long term                                                                                                                                                                                                                                                                                                                                                                                                                                                    | 12/92 (13%)   | 0/46 (0%)     | 12.56 (0.72 to 216.9) |
|                                | Neurodevelopmental delay                                                                                                                                                                                                                                                                                                                                                                                                                                                  | 2/92 (2.17%)  | 0/46 (0%)     | 2.51 (0.12 to 53.43)  |
|                                | <p><sup>a</sup> <b>Calculated with</b> <a href="https://www.medcalc.org/en/calc/odds_ratio.php">https://www.medcalc.org/en/calc/odds_ratio.php</a></p> <p>*CNS involvement such as microcephaly, intracranial calcifications, SNHL, and/or chorioretinitis and the presence of 1 or more non-CNS manifestations such as thrombocytopenia, hepatomegaly, splenomegaly, intra uterine growth description, hepatitis.</p> <p>** One child may have more than one symptom</p> |               |               |                       |
| Other                          |                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |               |               |                       |
| Limitations and other comments | <p>In centre 1 (Israel), for amniocentesis both rapid shell vial and CMV DNA PCR are performed and only if both negative it was considered negative.</p> <p>cCMV at birth was detected with viral culture in an unknown proportion of patients</p> <p>There are no details on how long term follow up is being performed.</p>                                                                                                                                             |               |               |                       |



**Shahar-Nissan K, Pardo J, Peled O, Krause I, Bilavsky E, Wiznitzer A, Hadar E, Amir J. Valaciclovir to prevent vertical transmission of cytomegalovirus after maternal primary infection during pregnancy: a randomised, double-blind, placebo-controlled trial. Lancet. 2020 Sep 12;396(10253):779-785(24)**

|                                 |                                                                                                                                                                                                                                                                                                                               |                                          |                                          |                         |
|---------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------|------------------------------------------|-------------------------|
| Country, data collection period | Israel,<br>11-2015 to 10-2018                                                                                                                                                                                                                                                                                                 |                                          |                                          |                         |
| Population, sample size         | Pregnant patients with primary infection in periconceptional or first trimester of pregnancy, included before the 16thGW. 47 treated with valaciclovir and 45 untreated                                                                                                                                                       |                                          |                                          |                         |
| Key methodological features     | Prospective RCT (the two arms were investigated separately), treatment of seroconversion with valaciclovir vs placebo.<br>Inclusion criteria: primary infection in periconceptional or first trimester of pregnancy, included before the 16thGW<br>Exclusion criteria: 18 GW, non-primary infection, inclusion after 16 weeks |                                          |                                          |                         |
| Timing of amniocentesis         | At least 21 weeks and 7 weeks after seroconversion                                                                                                                                                                                                                                                                            |                                          |                                          |                         |
| Outcome(s) assessed             | cCMV at amniocentesis cCMV at birth, termination of pregnancy, hearing loss, postnatal sonographic findings<br>All assessed in untreated and treated with valacyclovir                                                                                                                                                        |                                          |                                          |                         |
| Outcome measurements            | Outcomes at birth:<br>urinary PCR, full physical examination, blood count, liver function tests, brain ultrasound, audiometry, ophthalmological examination.                                                                                                                                                                  |                                          |                                          |                         |
| Results                         |                                                                                                                                                                                                                                                                                                                               |                                          |                                          |                         |
|                                 | Outcome                                                                                                                                                                                                                                                                                                                       | CMV at birth with positive amniocentesis | CMV at birth with negative amniocentesis | OR (95%CI) <sup>a</sup> |
|                                 | Termination of pregnancy (untreated)                                                                                                                                                                                                                                                                                          | 5/14 (35%)                               | 0/2 (0%)                                 | 1.89 (0.07 to 46.11)    |
|                                 | Termination of pregnancy (treated)                                                                                                                                                                                                                                                                                            | 1/5 (20%)                                | 0/4 (0%)                                 | 2.45 (0.079 to 76.13)   |
|                                 | Hearing loss at birth (untreated)*                                                                                                                                                                                                                                                                                            | 5/9 (55%)                                | 0/2 (0%)                                 | 2.89 (0.11 to 71.93)    |
|                                 | Hearing loss at birth (treated)*                                                                                                                                                                                                                                                                                              | 1/4 (25%)                                | 0/4 (0%)                                 | 3 (0.094 to 95.17)      |
|                                 | Postnatal sonographic findings (untreated)*                                                                                                                                                                                                                                                                                   | 0/9 (0%)                                 | 0/2 (0%)                                 | /                       |
|                                 | Postnatal sonographic findings (treated)*                                                                                                                                                                                                                                                                                     | 0/4 (0%)                                 | 1/4 *(25%)                               | 0.33 (0.01 to 10.57)    |



<sup>a</sup> Calculated with [https://www.medcalc.org/en/calc/odds\\_ratio.php](https://www.medcalc.org/en/calc/odds_ratio.php)

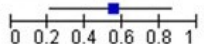
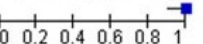
\*Foetuses undergoing TOP were taken out of the denominator

\*\* Focal bilateral subependymal cysts

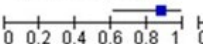
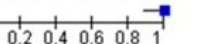
Other results: Diagnostic accuracy statistics of amniocentesis for CMV at birth according to treatment status

Diagnostic accuracy statistics of amniocentesis for CMV at birth according to treatment status

**Amniocentesis >=21GW and >=7 weeks after seroconversion (treated)**

| Study              | TP | FP | FN | TN | Sensitivity (95% CI) | Specificity (95% CI) | Sensitivity (95% CI)                                                                | Specificity (95% CI)                                                                |
|--------------------|----|----|----|----|----------------------|----------------------|-------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|
| Shahar-Nissan 2020 | 5  | 0  | 4  | 36 | 0.56 [0.21, 0.86]    | 1.00 [0.90, 1.00]    |  |  |

**Amniocentesis >=21GW and >=7 weeks after seroconversion (untreated)**

| Study              | TP | FP | FN | TN | Sensitivity (95% CI) | Specificity (95% CI) | Sensitivity (95% CI)                                                                | Specificity (95% CI)                                                                |
|--------------------|----|----|----|----|----------------------|----------------------|-------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|
| Shahar-Nissan 2020 | 14 | 0  | 2  | 31 | 0.88 [0.62, 0.98]    | 1.00 [0.89, 1.00]    |  |  |

Other results: Prognostic accuracy of amniocentesis for outcomes

| Outcome                              | True Positive | False Positive | False Negative | True Negative | Sensitivity <sup>b</sup><br>(95%CI) | Specificity <sup>b</sup><br>(95%CI) |
|--------------------------------------|---------------|----------------|----------------|---------------|-------------------------------------|-------------------------------------|
| Termination of pregnancy (untreated) | 5             | 9              | 1              | 32            | 83.33%<br>(35.88% to 99.58%)        | 78.05%<br>(62.39% to 89.44%)        |
| Termination of pregnancy (treated)   | 1             | 4              | 1              | 39            | 50.00%<br>(1.26% to 98.74%)         | 90.70%<br>(77.86% to 97.41%)        |
| Hearing loss at birth (untreated)*   | 5             | 4              | 0              | 32            | 100.00%<br>(47.82% to 100.00%)      | 88.89%<br>(73.94% to 96.89%)        |
| Hearing loss at birth (treated)*     | 1             | 3              | 0              | 39            | 100.00%<br>(2.50% to 100.00%)       | 92.86%<br>(80.52% to 98.50%)        |
| Postnatal sonographic                | 0             | 0              | 0              | 43            | /                                   | /                                   |



|                                |                                                                                                                                                                                                                                                                            |   |   |   |    |                                  |                                    |
|--------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---|---|---|----|----------------------------------|------------------------------------|
|                                | findings<br>(untreated)*                                                                                                                                                                                                                                                   |   |   |   |    |                                  |                                    |
|                                | Postnatal<br>sonographic<br>findings<br>(treated)*                                                                                                                                                                                                                         | 0 | 4 | 1 | 38 | 0.00%<br>(0.00%<br>to<br>97.50%) | 90.48%<br>(77.38%<br>to<br>97.34%) |
|                                | <sup>b</sup> Calculated with <a href="https://www.medcalc.org/en/calc/diagnostic_test.php">https://www.medcalc.org/en/calc/diagnostic_test.php</a>                                                                                                                         |   |   |   |    |                                  |                                    |
| Limitations and other comments | For more details on this study see 8.4 Six participants refused amniocentesis (one twin pregnancy). Three neonates were negative for CMV therefore their amniocentesis was presumed to be negative. Four neonates with positive CMV at birth were presumed to be positive. |   |   |   |    |                                  |                                    |

**Keymeulen, A, Leenheer D, Alexandra C, Veerle C, Sabine L, Ludo M, Christine VM, Ingeborg D, Koenraad S. Results of a multicenter registry for congenital cytomegalovirus infection in Flanders, Belgium: From prenatal diagnosis over neonatal management to therapy. Early Hum Dev. 2021 Dec;163:105499.(17)**

|                                 |                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
|---------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Country, data collection period | Belgium,<br>01-2007 to 12-2020                                                                                                                                                                                                                                                                                                                                                                                                                                              |
| Population, sample size         | Children with cCMV registered the multicentre registry for CMV in Flanders.<br>889 diagnosed with cCMV in the neonatal period, amniocentesis performed in 18.2% of patients<br>n=162                                                                                                                                                                                                                                                                                        |
| Key methodological features     | Registry study, retrospective analysis<br>Inclusion criteria: cCMV at birth, detected by viral isolation and/or CMV DNA PCR on urine/saliva (if diagnosis after 21 weeks, PCR CMV DNA on dried blood spot)<br>Exclusion criteria:for the analysis: no amniocentesis performed                                                                                                                                                                                               |
| Timing of amniocentesis         | > 21 weeks of gestation                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
| Outcome(s) assessed             | Abnormal foetal ultrasound,<br>Abnormal antenatal MRI<br>Mild symptoms at birth (isolated (max2) clinical non-significant or transient findings: IUGR, petechiae, mild hepatosplenomegaly, mild thrombopenia, anaemia leukopenia, mild elevates AST/ALT, cholestasis.).<br>Moderate symptoms at birth (more than 2 mild symptoms, or with persistent -more than 2 weeks- biological/haematological abnormalities or with mild lesions on CNS imaging -LSV, isolated cysts). |



|                                                                  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |                                          |                                          |                         |
|------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------|------------------------------------------|-------------------------|
|                                                                  | Severely symptomatic newborns (CNS involvement: neurological signs, - convulsions, microcephaly- or chorioretinitis or lesions on CNS -calcifications, moderate to severe ventriculomegaly, multiple cysts, extensive white matter changes, cerebellar/cerebral hypoplasia, hippocampus dysplasia, migration disorders, polymicrogyria-. Children with severe single organ disease, severe multi organ disease or with life threatening disease. Children with isolated hearing loss.                                                                              |                                          |                                          |                         |
| Outcome measurements                                             | Data are collected at the moment of enrolment (timing of seroconversion, foetal ultrasound, amniocentesis, prenatal MRI, reason for testing at birth, timing of diagnosis, diagnostic tools, demographic features, parity, clinical features at birth, laboratory results, results of CNS imaging -US, MRI- audiological testing, ophthalmological investigation and antiviral therapy. Testing is done preferably within 1 month after birth. Audiological evaluation (OAE, ABR or BRA) and eye exam. Clear definition of mild/moderate or severe symptomatology. |                                          |                                          |                         |
| Results (limited to the included children who had amniocentesis) |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |                                          |                                          |                         |
|                                                                  | Outcome                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            | CMV at birth with positive amniocentesis | CMV at birth with negative amniocentesis | OR (95%CI) <sup>a</sup> |
|                                                                  | Mild symptomatic at birth                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          | 13/ 233 (5.6%)                           | 3/81 (3.7%)                              | 1.5 (0.41 to 5.42)      |
|                                                                  | Moderately symptomatic at birth                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    | 17/233 (7.3%)                            | 12/81 (14.8%)                            | 0.49 (0.22 to 1.07)     |
|                                                                  | Severe symptomatic at birth                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        | 55/233 (23.6%)                           | 17/81 (21%)                              | 1.12 (0.61 to 2.04)     |
|                                                                  | Abnormal fetal ultrasound                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          | 22/139 (15.8%)                           | 2/41 (4.9%)                              | 3.24 (0.73 to 14.38)    |
|                                                                  | Abnormal fetal MRI                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | 16/56 (28.6%)                            | 0/1 (0%)*                                | 0.87 (0.03 to 22.53)    |
|                                                                  | <sup>a</sup> <b>Calculated with</b> <a href="https://www.medcalc.org/en/calc/odds_ratio.php">https://www.medcalc.org/en/calc/odds_ratio.php</a>                                                                                                                                                                                                                                                                                                                                                                                                                    |                                          |                                          |                         |
| Other                                                            |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |                                          |                                          |                         |
| Limitations and other comments                                   | Children born after 2018 were retrospectively re classified as mild/moderate/severe symptomatic. 83.9% of children were investigated after known maternal seroconversion during pregnancy<br>*MRI is not usually performed if amniocentesis is negative.                                                                                                                                                                                                                                                                                                           |                                          |                                          |                         |



Zammarchi L, Tomasoni LR, Liuzzi G, Simonazzi G, Dionisi C, Mazzarelli LL, Seidenari A, Maruotti GM, Ornaghi S, Castelli F, Abbate I, Bordi L, Mazzotta S, Fusco P, Torti C, Calò Carducci FI, Baccini M, Modi G, Galli L, Lilleri D, Furione M, Zavattoni M, Ricciardi A, Arossa A, Vimercati A, Lovatti S, Salomè S, Raimondi F, Sarno L, Sforza A, Fichera A, Caforio L, Trotta M, Lazzarotto T; MEGAL-ITALI Working Group. Treatment with valacyclovir during pregnancy for prevention of congenital cytomegalovirus infection: a real-life multicenter Italian observational study. *Am J Obstet Gynecol MFM*. 2023 Oct;5(10):101101(28)

|                                                                  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |                                          |                                           |                         |
|------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------|-------------------------------------------|-------------------------|
| Country, data collection period                                  | Italy,<br>01-2010 to 06-2022                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |                                          |                                           |                         |
| Population, sample size                                          | Pregnant patients satisfying AIFA criteria for valaciclovir treatment. 447 patients included. 270 had amniocentesis performed. (136 treated with valacyclovir, 134 untreated with valaciclovir)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |                                          |                                           |                         |
| Key methodological features                                      | Multicentre retrospective cohort study, including women both treated and not treated with valaciclovir<br>inclusion criteria: MPI infection before 24GW<br>Exclusion criteria: for the analysis: no amniocentesis performed                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |                                          |                                           |                         |
| Timing of amniocentesis                                          | At least 8 weeks after primary infection, not before 20+1/7GW                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |                                          |                                           |                         |
| Outcome(s) assessed                                              | CMV DNA in saliva or urine within 2 weeks from birth; Miscarriage or stillbirth, termination of pregnancy, Symptomatology at birth according to ESPID (“Symptomatic” cCMV should be considered as “severe,” “moderate” or “mild” disease. a. “Mild” disease includes those with isolated (1 or 2 at most), otherwise, clinically insignificant or transient findings, such as petechiae, mild hepatomegaly or splenomegaly or biochemical/hematologic abnormalities (such as thrombocytopenia, anaemia, leukopenia, borderline raised liver enzyme abnormalities or conjugated hyperbilirubinemia) or SGA (defined as weight for gestational age <−2 standard deviations) without microcephaly. b. “Severe” disease includes those with central nervous system (CNS) involvement (abnormal neurologic or ophthalmologic examination, microcephaly or neuroimaging consistent with cCMV disease [such as calcifications, moderate to severe ventriculomegaly, cysts, white matter changes, cerebral or cerebellar hypoplasia, hippocampal dysplasia, neuronal migration abnormalities]) or with life-threatening disease.) SNHL is considered by the majority as “severe cCMV”.use of valganciclovir in the newborn |                                          |                                           |                         |
| Outcome measurements                                             | Clinical and laboratory data were retrieved from electronic or paper medical records according to the availability in the different participating centers.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |                                          |                                           |                         |
| Results (limited to the included children who had amniocentesis) |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |                                          |                                           |                         |
|                                                                  | Outcome                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            | CMV at birth with positive amniocentesis | CMV at birth with negative amniocentesis* | OR (95%CI) <sup>a</sup> |
|                                                                  | Miscarriage (untreated)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            | 2/37 (5.4%)                              | 0/6 (0%)                                  | 0.86 (0.03 to 20.19)    |
|                                                                  | Miscarriage (treated)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | 0/19 (0%)                                | 0/16 (0%)                                 | /                       |



|                                                                                                               | Termination of pregnancy (untreated)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | 18/37 (48.6%) | 0/6 (0%)       | 6.41 (0.34 to 120.09) |                      |                                  |                                  |                      |    |    |    |    |                      |                      |                      |                      |                |    |   |   |    |                   |                   |  |  |       |    |    |    |    |                      |                      |                      |                      |                |    |   |    |    |                   |                   |  |
|---------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------|----------------|-----------------------|----------------------|----------------------------------|----------------------------------|----------------------|----|----|----|----|----------------------|----------------------|----------------------|----------------------|----------------|----|---|---|----|-------------------|-------------------|--|--|-------|----|----|----|----|----------------------|----------------------|----------------------|----------------------|----------------|----|---|----|----|-------------------|-------------------|--|
|                                                                                                               | Termination of pregnancy (treated)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     | 5/19 (26.31%) | 0/16 (0%)      | 9.3 (0.47 to 181.13)  |                      |                                  |                                  |                      |    |    |    |    |                      |                      |                      |                      |                |    |   |   |    |                   |                   |  |  |       |    |    |    |    |                      |                      |                      |                      |                |    |   |    |    |                   |                   |  |
|                                                                                                               | Symptomatic cCMV neonates (untreated)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | 5/15 (33.33%) | 0/6 (0%)       | 4.61 (0.22 to 96.1)   |                      |                                  |                                  |                      |    |    |    |    |                      |                      |                      |                      |                |    |   |   |    |                   |                   |  |  |       |    |    |    |    |                      |                      |                      |                      |                |    |   |    |    |                   |                   |  |
|                                                                                                               | Symptomatic cCMV neonates (treated)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    | 3/14 (21.4%)  | 0/16 (0%)      | 7.96 (0.37 to 167.53) |                      |                                  |                                  |                      |    |    |    |    |                      |                      |                      |                      |                |    |   |   |    |                   |                   |  |  |       |    |    |    |    |                      |                      |                      |                      |                |    |   |    |    |                   |                   |  |
|                                                                                                               | <sup>a</sup> <b>Calculated with</b> <a href="https://www.medcalc.org/en/calc/odds_ratio.php">https://www.medcalc.org/en/calc/odds_ratio.php</a>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |               |                |                       |                      |                                  |                                  |                      |    |    |    |    |                      |                      |                      |                      |                |    |   |   |    |                   |                   |  |  |       |    |    |    |    |                      |                      |                      |                      |                |    |   |    |    |                   |                   |  |
| Other results: Diagnostic accuracy statistics of amniocentesis for CMV at birth according to treatment status | Diagnostic accuracy statistics of amniocentesis for CMV at birth according to treatment status                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |               |                |                       |                      |                                  |                                  |                      |    |    |    |    |                      |                      |                      |                      |                |    |   |   |    |                   |                   |  |  |       |    |    |    |    |                      |                      |                      |                      |                |    |   |    |    |                   |                   |  |
|                                                                                                               | <div><div><div><b>Amniocentesis &gt;20GW and &gt;=8 weeks after primary infection (untreated)</b></div><table><tr><th>Study</th><th>TP</th><th>FP</th><th>FN</th><th>TN</th><th>Sensitivity (95% CI)</th><th>Specificity (95% CI)</th><th>Sensitivity (95% CI)</th><th>Specificity (95% CI)</th></tr><tr><td>Zammarchi 2023</td><td>35</td><td>0</td><td>6</td><td>90</td><td>0.85 [0.71, 0.94]</td><td>1.00 [0.96, 1.00]</td><td></td><td></td></tr></table></div><div><div><b>Amniocentesis &gt;20GW and &gt;=8 weeks after primary infection (treated)</b></div><table><tr><th>Study</th><th>TP</th><th>FP</th><th>FN</th><th>TN</th><th>Sensitivity (95% CI)</th><th>Specificity (95% CI)</th><th>Sensitivity (95% CI)</th><th>Specificity (95% CI)</th></tr><tr><td>Zammarchi 2023</td><td>19</td><td>0</td><td>16</td><td>87</td><td>0.54 [0.37, 0.71]</td><td>1.00 [0.96, 1.00]</td><td></td><td></td></tr></table></div></div> |               |                |                       |                      |                                  |                                  | Study                | TP | FP | FN | TN | Sensitivity (95% CI) | Specificity (95% CI) | Sensitivity (95% CI) | Specificity (95% CI) | Zammarchi 2023 | 35 | 0 | 6 | 90 | 0.85 [0.71, 0.94] | 1.00 [0.96, 1.00] |  |  | Study | TP | FP | FN | TN | Sensitivity (95% CI) | Specificity (95% CI) | Sensitivity (95% CI) | Specificity (95% CI) | Zammarchi 2023 | 19 | 0 | 16 | 87 | 0.54 [0.37, 0.71] | 1.00 [0.96, 1.00] |  |
| Study                                                                                                         | TP                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     | FP            | FN             | TN                    | Sensitivity (95% CI) | Specificity (95% CI)             | Sensitivity (95% CI)             | Specificity (95% CI) |    |    |    |    |                      |                      |                      |                      |                |    |   |   |    |                   |                   |  |  |       |    |    |    |    |                      |                      |                      |                      |                |    |   |    |    |                   |                   |  |
| Zammarchi 2023                                                                                                | 35                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     | 0             | 6              | 90                    | 0.85 [0.71, 0.94]    | 1.00 [0.96, 1.00]                |                                  |                      |    |    |    |    |                      |                      |                      |                      |                |    |   |   |    |                   |                   |  |  |       |    |    |    |    |                      |                      |                      |                      |                |    |   |    |    |                   |                   |  |
| Study                                                                                                         | TP                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     | FP            | FN             | TN                    | Sensitivity (95% CI) | Specificity (95% CI)             | Sensitivity (95% CI)             | Specificity (95% CI) |    |    |    |    |                      |                      |                      |                      |                |    |   |   |    |                   |                   |  |  |       |    |    |    |    |                      |                      |                      |                      |                |    |   |    |    |                   |                   |  |
| Zammarchi 2023                                                                                                | 19                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     | 0             | 16             | 87                    | 0.54 [0.37, 0.71]    | 1.00 [0.96, 1.00]                |                                  |                      |    |    |    |    |                      |                      |                      |                      |                |    |   |   |    |                   |                   |  |  |       |    |    |    |    |                      |                      |                      |                      |                |    |   |    |    |                   |                   |  |
| Other results: Prognostic accuracy of amniocentesis for outcomes                                              | Outcome                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | True Positive | False Positive | False Negative        | True Negative        | Sensitivity <sup>b</sup> (95%CI) | Specificity <sup>b</sup> (95%CI) |                      |    |    |    |    |                      |                      |                      |                      |                |    |   |   |    |                   |                   |  |  |       |    |    |    |    |                      |                      |                      |                      |                |    |   |    |    |                   |                   |  |
|                                                                                                               | Miscarriage (untreated)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | 2             | 33             | 0                     | 96                   | 100% (15.81% to 100%)            | 74.42% (65.99% to 81.69%)        |                      |    |    |    |    |                      |                      |                      |                      |                |    |   |   |    |                   |                   |  |  |       |    |    |    |    |                      |                      |                      |                      |                |    |   |    |    |                   |                   |  |
|                                                                                                               | Miscarriage (treated)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | 0             | 0              | 0                     | 136                  | /                                | /                                |                      |    |    |    |    |                      |                      |                      |                      |                |    |   |   |    |                   |                   |  |  |       |    |    |    |    |                      |                      |                      |                      |                |    |   |    |    |                   |                   |  |
|                                                                                                               | Termination of pregnancy (untreated)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | 18            | 19             | 0                     | 96                   | 100.00% (81.47% to 100.00%)      | 83.48% (75.41% to 89.75%)        |                      |    |    |    |    |                      |                      |                      |                      |                |    |   |   |    |                   |                   |  |  |       |    |    |    |    |                      |                      |                      |                      |                |    |   |    |    |                   |                   |  |
|                                                                                                               | Termination of pregnancy (treated)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     | 5             | 15             | 1                     | 103                  | 83.33% (35.88% to 99.58%)        | 87.29% (79.90% to 92.71%)        |                      |    |    |    |    |                      |                      |                      |                      |                |    |   |   |    |                   |                   |  |  |       |    |    |    |    |                      |                      |                      |                      |                |    |   |    |    |                   |                   |  |



|                                |                                                                                                                                                                                           |   |    |   |     |                             |    |                           |    |
|--------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---|----|---|-----|-----------------------------|----|---------------------------|----|
|                                | Symptomatic cCMV neonates (untreated)                                                                                                                                                     | 5 | 10 | 0 | 96  | 100.00% (47.82% to 100.00%) | to | 90.57% (83.33% to 95.38%) | to |
|                                | Symptomatic cCMV neonates (treated)                                                                                                                                                       | 3 | 11 | 0 | 103 | 100.00% (29.24% to 100.00%) | to | 90.35% (83.39% to 95.08%) | to |
|                                | <sup>b</sup> Calculated with <a href="https://www.medcalc.org/en/calc/diagnostic_test.php">https://www.medcalc.org/en/calc/diagnostic_test.php</a>                                        |   |    |   |     |                             |    |                           |    |
| Limitations and other comments | For more details on this study see 8.4180/447 (40.2%) did not undergo amniocentesis, *12/116 (10.3%) newborn treated with Valaciclovir and a negative amniocentesis are lost to follow up |   |    |   |     |                             |    |                           |    |

|                                 |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
|---------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                                 | <b>De Keersmaecker B, Vanwinkel S, Lagrou K, Jansen K, Naulaers G, De Catte L. Neonatal and short-term outcome after late vertical transmission in congenital CMV-infected fetuses following primary first-trimester maternal seroconversion. BMJ Paediatr Open. 2024 Sep 10;8(1):e002773(35)</b>                                                                                                                                                                                                                                                                                                                                                  |
| Country, data collection period | Belgium, 11-2006 to 03-2019                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
| Population, sample size         | Pregnant patients with MPI before 14 GW; n=343, 45 excluded because of prenatal administration of immunoglobulins -n=13- fetal demise -n=2-, absence of results of CMV at birth -n=30)                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
| Key methodological features     | Retrospective cohort;<br>Inclusion criteria: <14 weeks, primary seroconversion, for whom amniocentesis was performed, investigated with CMV PCR<br>Exclusion criteria: no amniocentesis performed.<br>Exclusion criteria: no PCR CMV at birth on neonatal urine, antenatal treatment, data collection incomplete<br>Outcomes are collected in children with positive amniocentesis and positive CMV DNA PCR at birth (median follow up 4 years (1-8)), negative amniocentesis and positive CMV DNA PCR at birth (Median follow up 4.4 years (1 - 8)), negative amniocentesis and negative CMV DNA PCR at birth (median follow up 3.4 years (1-8)). |
| Timing of amniocentesis         | Around 21 GW                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |
| Outcome(s) assessed             | Preterm birth, Small for Gestational Age, Microcephaly (head circumference <3SD) Symptomatic CMV disease (at least one petechiae, hepatomegaly, splenomegaly, IUGR, microcephaly or chorioretinitis). Motor deficit (need for physiotherapy later in life) early psychomotor development (global and fine motor abilities, language skills and social skills) hearing loss, visual impairment, learning disability (repeating a class) behavioural problems (analysed by child psychiatrists)                                                                                                                                                      |



|                      |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |                                                 |                                                  |                               |
|----------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------|--------------------------------------------------|-------------------------------|
|                      | Outcomes at birth: Standardised CMV follow-up: examination by paediatrician, cranial ultrasound, eye fundus, ear examination (standard and BERA), this is for babies that had a positive amniocentesis and for all babies with a positive NU PCR even if amniocentesis negative<br>Outcomes long term: Purpose made questionnaire distributed to patients (parents). Patient reported information on neonatal health, neurological outcomes, hearing outcomes and educational progress. |                                                 |                                                  |                               |
| Outcome measurements | Outcomes at birth: Standardised CMV follow-up: examination by pediatrician, cranial ultrasound, eye fundus, ear examination (standard and BERA), this is for babies that had a positive amniocentesis and for all babies with a positive NU PCR even if amniocentesis negative<br>Outcomes long term: Purpose made questionnaire distributed to patients (parents). Patient reported information on neonatal health, neurological outcomes, hearing outcomes and educational progress.  |                                                 |                                                  |                               |
|                      | Results                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |                                                 |                                                  |                               |
|                      | <b>Outcome</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                          | <b>CMV at birth with positive amniocentesis</b> | <b>CMV at birth with negative amniocentesis*</b> | <b>OR (95%CI)<sup>a</sup></b> |
|                      | Termination of pregnancy                                                                                                                                                                                                                                                                                                                                                                                                                                                                | 46/116 (39.6%)                                  | 0/7 (0%)                                         | 5.98 (0.33 to 106.96)         |
|                      | At birth                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |                                                 |                                                  |                               |
|                      | Microcephaly                                                                                                                                                                                                                                                                                                                                                                                                                                                                            | 1/62 (1.6%)                                     | 0/7 (0%)                                         | 0.36 (0.01 to 9.65)           |
|                      | Abnormal neurological examination                                                                                                                                                                                                                                                                                                                                                                                                                                                       | 0/ 62 (0%)                                      | 0/7 (0%)                                         | /                             |
|                      | Jaundice                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | 11/62 (17.7%)                                   | 4/7 (57.1%)                                      | 0.31 (0.07 to 1.24)           |
|                      | Petechiae                                                                                                                                                                                                                                                                                                                                                                                                                                                                               | 8/62 (12.9%)                                    | 2/7 (28.6%)                                      | 0.45 (0.07 to 2.56)           |
|                      | Hepatosplenomegaly                                                                                                                                                                                                                                                                                                                                                                                                                                                                      | 1/62 (1.6%)                                     | 0/7 (0%)                                         | 0.36 (0.01 to 9.65)           |
|                      | Chorioretinitis                                                                                                                                                                                                                                                                                                                                                                                                                                                                         | 1/62 (1.6%)                                     | 0/7 (0%)                                         | 0.36 (0.01 to 9.65)           |
|                      | Symptomatic at birth                                                                                                                                                                                                                                                                                                                                                                                                                                                                    | 12/62 (19.4%)                                   | 2/7 (28.6%)                                      | 0.67 (0.12 to 3.66)           |
|                      | Short term follow-up                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |                                                 |                                                  |                               |
|                      | Abnormal early psychomotor development                                                                                                                                                                                                                                                                                                                                                                                                                                                  | 2/51 (3.9%)                                     | 0/6(0%)                                          | 0.63 (0.02 to 14.64)          |
|                      | Need of logopaedics                                                                                                                                                                                                                                                                                                                                                                                                                                                                     | 9/51 (17.6%)                                    | 0/6(0%)                                          | 2.39 (0.12 to 46.2)           |
|                      | Need of physiotherapy                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | 5/51 (9.8%)                                     | 0/6(0%)                                          | 1.38 (0.06 to 28.1)           |
|                      | Overall motor deficit*                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | 11/51 (21.6%)                                   | 0/6(0%)                                          | 2.9 (0.15 to 55.27)           |
|                      | Hearing impairment                                                                                                                                                                                                                                                                                                                                                                                                                                                                      | 12/51 (23.5%)                                   | 0/6(0%)                                          | 3.15 (0.16 to 59.81)          |



|                                                                                                                                          |                                                                             |                                                 |                                                    |                               |
|------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------|-------------------------------------------------|----------------------------------------------------|-------------------------------|
| Other results: comparison between patients with CMV at birth with positive amniocentesis and no CMV at birth with negative amniocentesis | Subnormal vision                                                            | 8 /51 (15.7%)                                   | 0/6(0%)                                            | 2.14 (0.11 to 41.68)          |
|                                                                                                                                          | Scholastic backwardness                                                     | 1 /51 (2%)                                      | 0/6(0%)                                            | 0.37 (0.01 to 10.29)          |
|                                                                                                                                          | Behavioural problems                                                        | 5 /51 (9.8%)                                    | 0/6(0%)                                            | 1.38 (0.06 to 28.1)           |
|                                                                                                                                          | *Abnormal psychomotor development and need of logopaedics and physiotherapy |                                                 |                                                    |                               |
|                                                                                                                                          | <b>Outcome</b>                                                              | <b>CMV at birth with positive amniocentesis</b> | <b>No CMV at birth with negative amniocentesis</b> | <b>OR (95%CI)<sup>a</sup></b> |
|                                                                                                                                          | Termination of pregnancy                                                    | 46/116 (39.6%)                                  | 0/ 220 (0%)                                        | 176.02 (10.74 to 2882.33)     |
|                                                                                                                                          | At birth                                                                    |                                                 |                                                    |                               |
|                                                                                                                                          | Microcephaly                                                                | 1/62 (1.6%)                                     | 1/ 160 (0.6%)                                      | 2.58 (0.15 to 41.9)           |
|                                                                                                                                          | Abnormal neurological examination                                           | 0/ 62 (0%)                                      | 0/160 (0%)                                         | /                             |
|                                                                                                                                          | Jaundice                                                                    | 11/62 (17.7%)                                   | 31/160 (19.3%)                                     | 0.91 (0.43 to 1.93)           |
|                                                                                                                                          | Petechiae                                                                   | 8/62 (12.9%)                                    | 6/160 (3.8%)                                       | 3.44 (1.14 to 10.32)          |
|                                                                                                                                          | Hepatosplenomegaly                                                          | 1/62 (1.6%)                                     | 0/160 (0%)                                         | 7.7 (0.3 to 191.65)           |
|                                                                                                                                          | Chorioretinitis                                                             | 1/62 (1.6%)                                     | 0/160 (0%)                                         | 7.7 (0.3 to 191.65)           |
|                                                                                                                                          | Symptomatic at birth                                                        | 12/62 (19.4%)                                   | 10/ 160 (6.2%)                                     | 3.09 (1.27 to 7.53)           |
|                                                                                                                                          | Short term follow-up                                                        |                                                 |                                                    |                               |
|                                                                                                                                          | Abnormal early psychomotor development                                      | 2/51 (3.9%)                                     | 2/ 122 (1.6%)                                      | 2.39 (0.32 to 17.44)          |
|                                                                                                                                          | Need of logopaedics                                                         | 9/51 (17.6%)                                    | 3/122 (2.5%)                                       | 7.17 (1.86 to 27.59)          |
|                                                                                                                                          | Need of physiotherapy                                                       | 5/51 (9.8%)                                     | 3/122 (3.5%)                                       | 3.98 (0.91 to 17.3)           |
|                                                                                                                                          | Overall motor deficit*                                                      | 11/51 (21.6%)                                   | 7/122 (5.7)                                        | 3.75 (1.37 to 10.24)          |
|                                                                                                                                          | Hearing impairment                                                          | 12/51 (23.5%)                                   | 1/122 (0.8%)                                       | 28.7 (3.63 to 226.59)         |
|                                                                                                                                          | Subnormal vision                                                            | 8 /51 (15.7%)                                   | 5/122 (4.1%)                                       | 3.82 (1.19 to 12.26)          |
|                                                                                                                                          | Scholastic backwardness                                                     | 1/51 (2%)                                       | 3/122 (2.6%)                                       | 0.79 (0.08 to 7.84)           |
|                                                                                                                                          | Behavioural problems                                                        | 5 / 51 (9.8%)                                   | 4/122 (3.3%)                                       | 2.99 (0.77 to 11.59)          |
|                                                                                                                                          | *Abnormal psychomotor development and need of logopaedics and physiotherapy |                                                 |                                                    |                               |







## 9.6 Metanalysis for critical and important outcomes for amniocentesis to predict the risk of congenital CMV-related sequelae if cCMV is detected at birth

For some critical and important outcomes, we could perform a metanalysis.

All metanalysis are all performed using Stata.

The forest plot displays the results of a meta-analysis on binary outcomes, using Odds Ratios (OR) to compare event rates across studies. Each study's OR is shown with a square, and horizontal lines represent the 95% confidence intervals (CIs). The vertical line indicates an OR of 1, the point of no effect. The pooled effect is represented by a diamond at the bottom. To account for the heterogeneity of methods (case control, cohort studies, RCTs, different methods for measuring the outcome) a random effect has been applied to all metanalysis. A continuity correction of 0.5 was applied in case of zero events.

A subgroup analysis was dome when possible distinguishing patients that were treated and untreated with valaciclovir.

**Figure 23 – Forest plot of the meta-analysis on termination of pregnancy according to amniocentesis status in case of transmission at birth.**

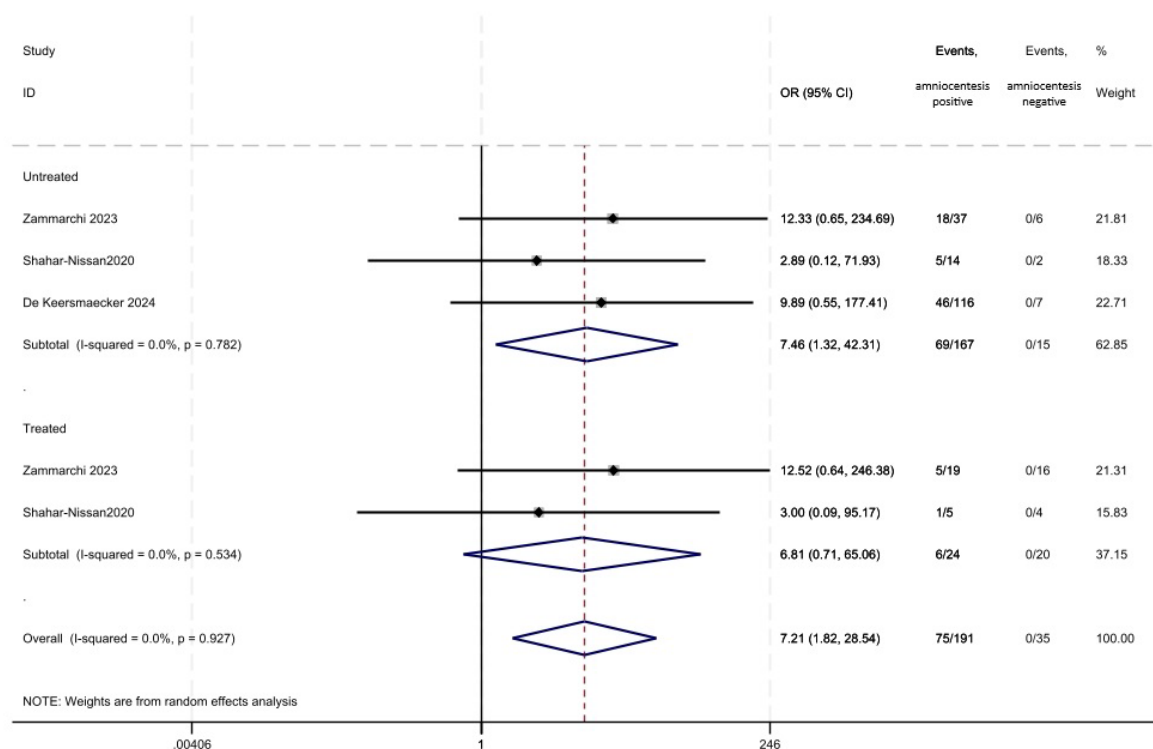


**Figure 24 – Forest plot of the meta-analysis on Severely symptomatic newborns according to amniocentesis status in case of transmission at birth.**

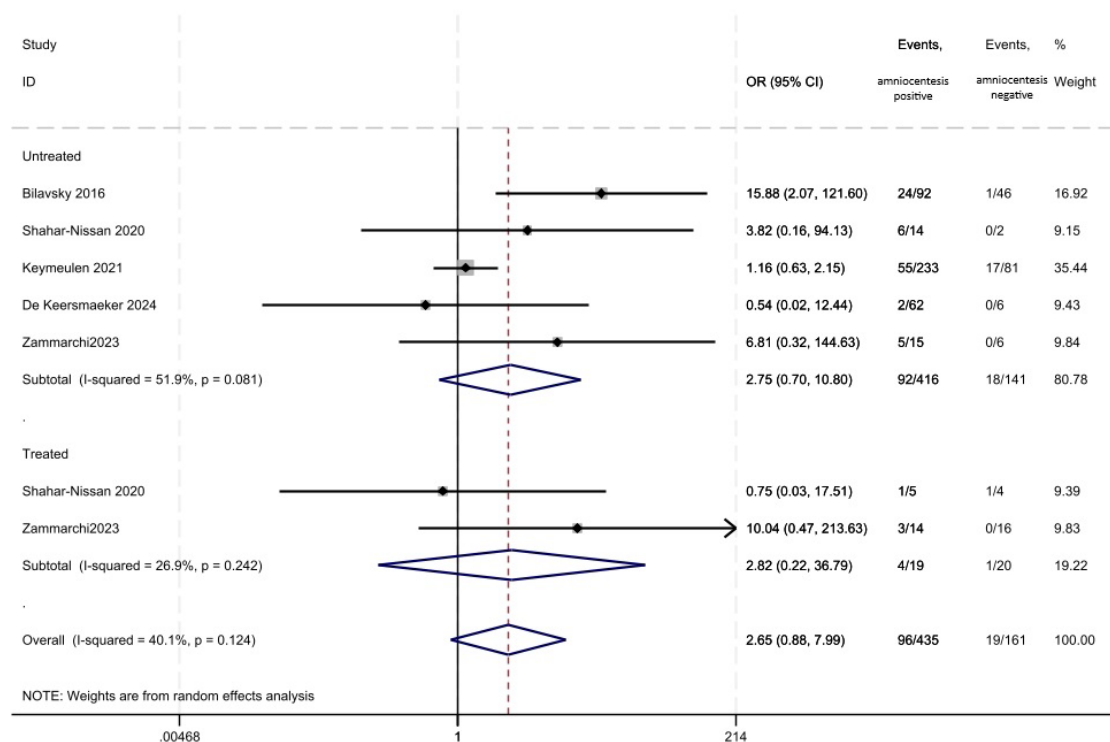




**Figure 25 – Forest plot of the meta-analysis on Severely symptomatic newborns, sensitivity analysis without Zammarchi 2023, according to amniocentesis results in case of transmission at birth**

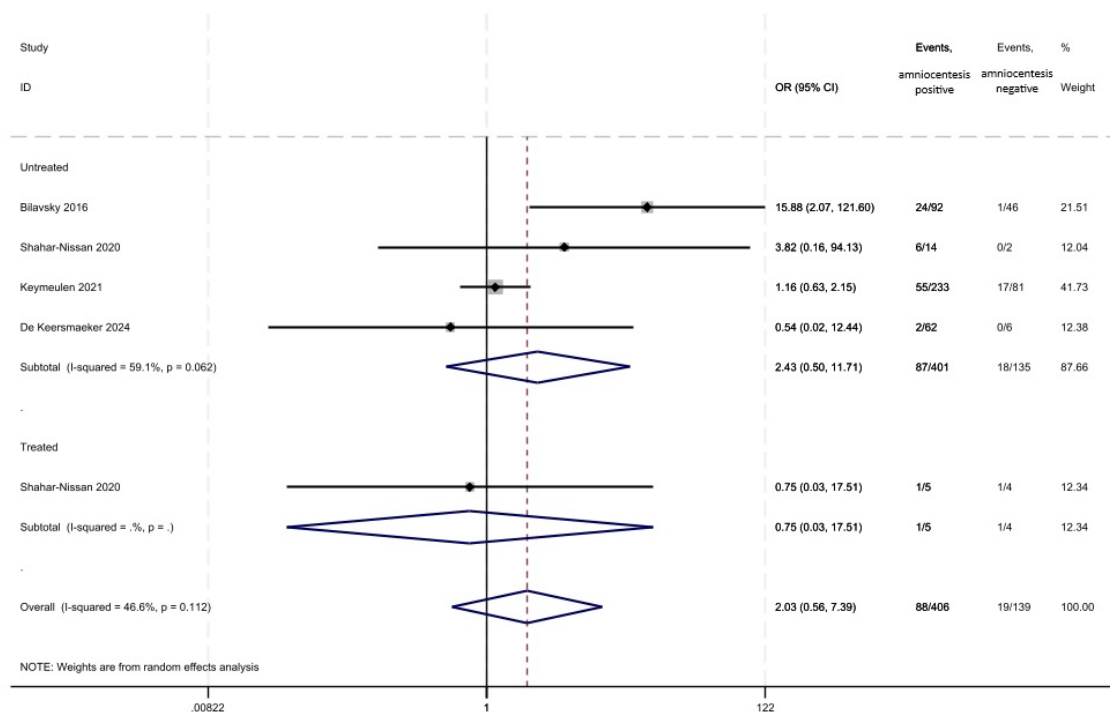


**Figure 26 – Forest plot of the meta-analysis on Hearing loss at birth, according to amniocentesis results in case of transmission at birth**

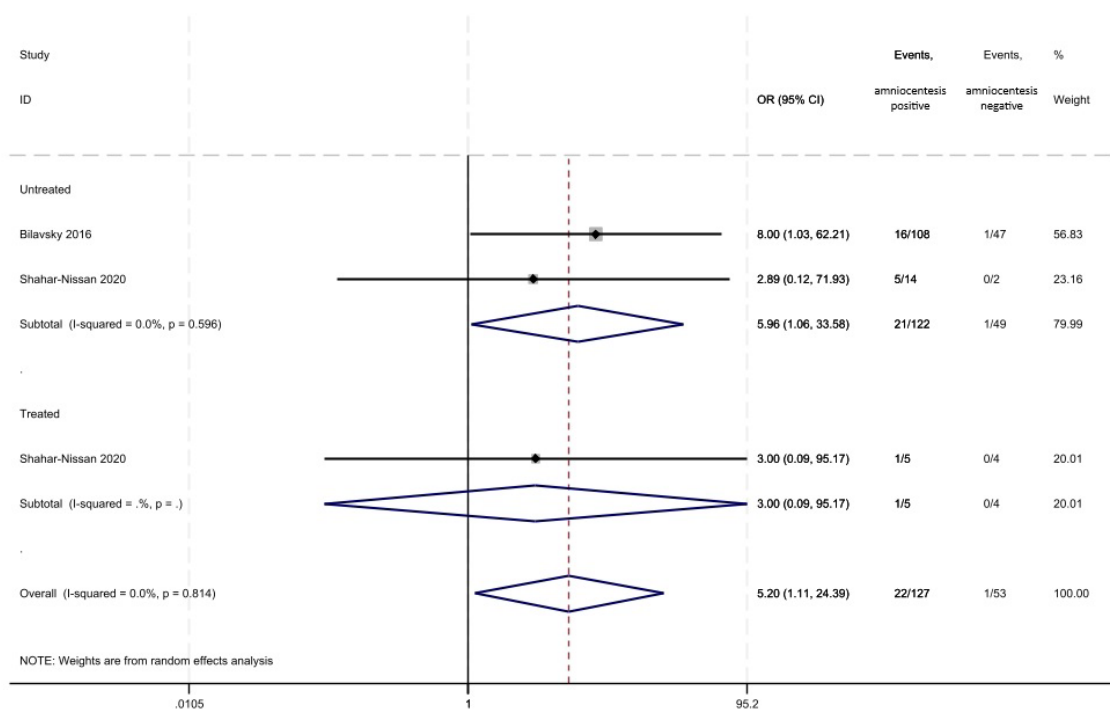


**Figure 27 – Forest plot of the meta-analysis on Hearing loss at follow-up, according to amniocentesis results in case of transmission at birth**

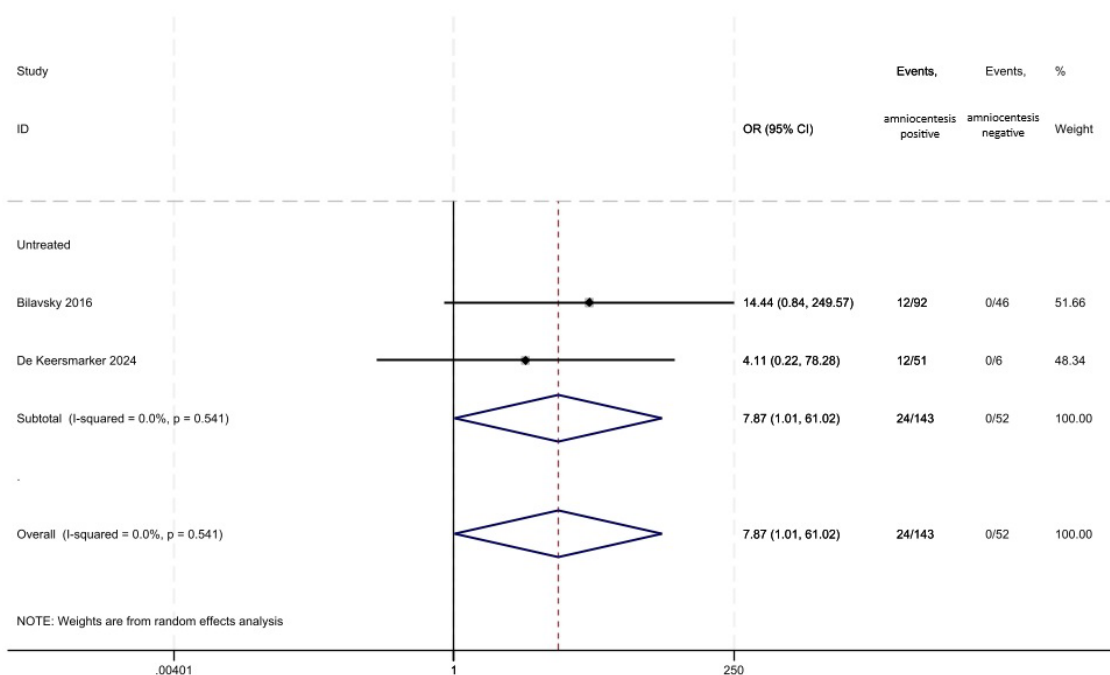




**Table 22 – critical and important outcomes coming for single studies only (according to amniocentesis results in case of transmission at birth)**

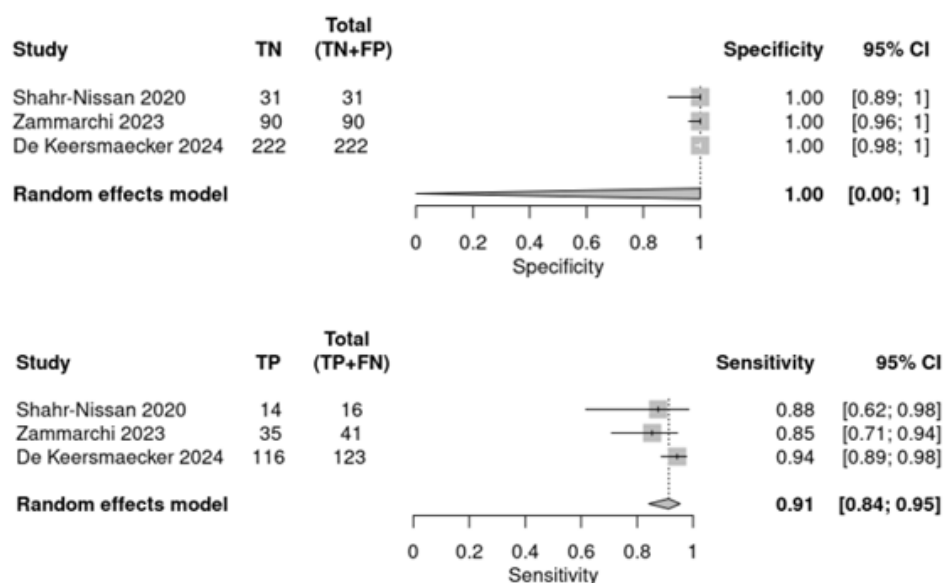
| Outcome                                                               | Study                | CMV at birth with positive amniocentesis | CMV at birth with negative amniocentesis | OR (95%CI) <sup>a</sup> |
|-----------------------------------------------------------------------|----------------------|------------------------------------------|------------------------------------------|-------------------------|
| Miscarriage (untreated)                                               | Zammarchi 2023       | 2/37 (5.4%)                              | 0/6 (0%)                                 | 0.86 (0.03 to 20.19)    |
| Miscarriage (treated)                                                 | Zammarchi 2023       | 0/19 (0%)                                | 0/16 (0%)                                | /                       |
| Motor developmental delay (Overall motor deficit) at follow-up        | De Keersmaecker 2024 | 11/51 (21.6%)                            | 0/6(0%)                                  | 2.9 (0.15 to 55.27)     |
| Visual impairment at follow-up                                        | De Keersmaecker 2024 | 8 /51 (15.7%)                            | 0/6(0%)                                  | 2.14 (0.11 to 41.68)    |
| Cognitive developmental delay (neurodevelopmental delay) at follow-up | Bilavsky 2016        | 2/92 (2.17%)                             | 0/46 (0%)                                | 2.51 (0.12 to 53.43)    |
| Behavioural problems at follow-up                                     | De Keersmaecker 2024 | 5 /51 (9.8%)                             | 0/6(0%)                                  | 1.38 (0.06 to 28.1)     |

## 9.7 Metanalysis for critical and important outcomes for prognostic accuracy of amniocentesis predict the risk of congenital CMV-related sequelae

Three of the included study had sufficient data to compute prognostic accuracy of amniocentesis. The Metanalysis has been performed using Meta-DiSc 2.0, a web-based application developed using the R Shiny package(37). Due to the low number of studies, a univariate model with random effects was used.

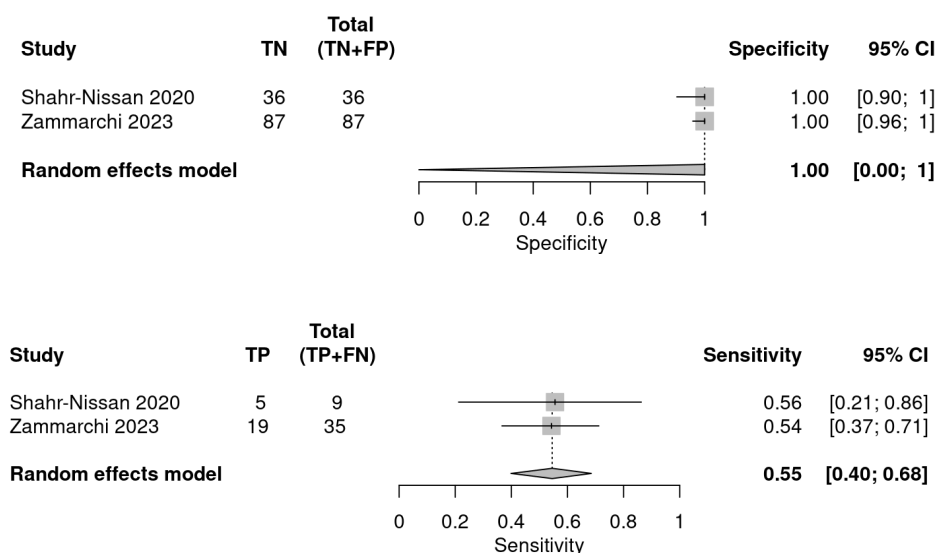
It is to be noted that the included studies had a high risk of bias, prognostic accuracy was not their primary aim, and the low event counts made it difficult to estimate prevalence of the outcomes reliably. In treated patients especially, confidence intervals for accuracy statistics are large. As a consequence individual and summary measures need to be evaluated with caution. The overall certainty of the evidence for this body of evidence is considered very low (risk of bias of the studies, imprecision of the measurements especially in treated patients, indirectness of the evidence to produce guidelines for follow-up management)

**Figure 28 – Accuracy of amniocentesis to detect cCMV at birth in patients not treated with valaciclovir**



In non-treated patients the pooled prevalence of cCMV at birth is 165/ 358 (46%), as a consequence the PPV is 100% and the NPV is 92.9%

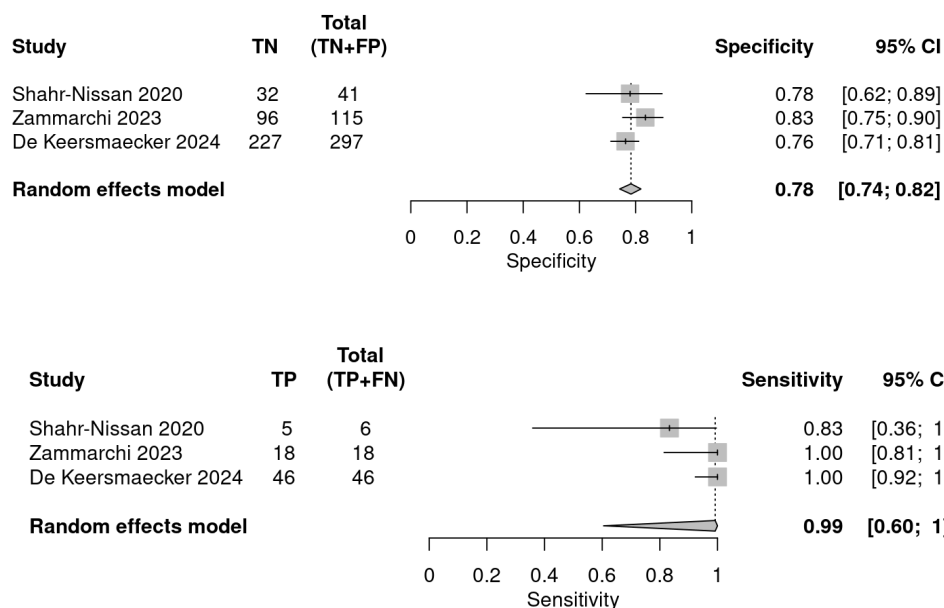
**Figure 29 – Accuracy of amniocentesis to detect cCMV at birth in patients treated with valaciclovir**



In patients that are treated with valaciclovir the pooled prevalence of cCMV at birth is 44/167 (26.3%), the PPV is 100% and the NPV is 86.2%

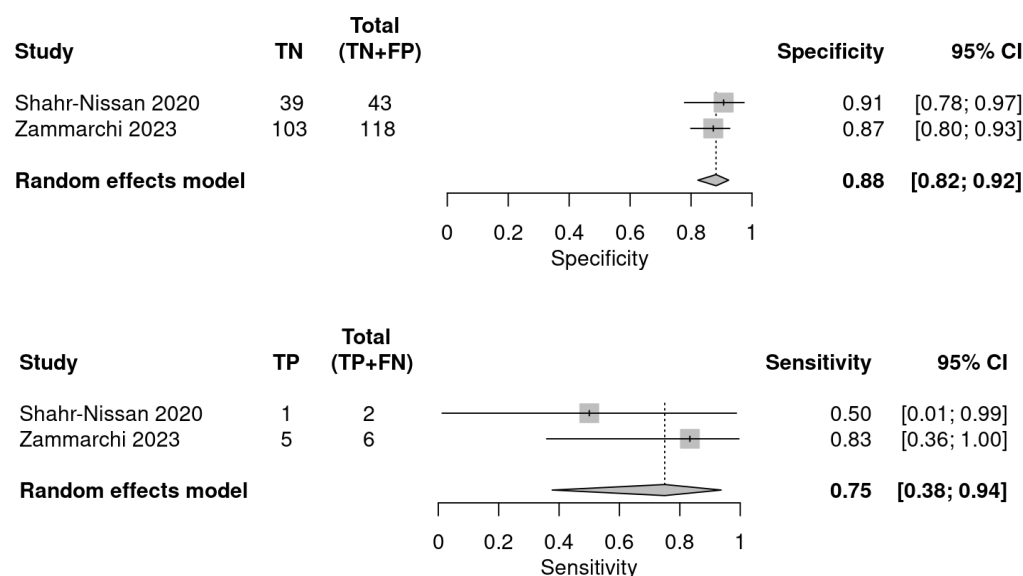


**Figure 30 – Accuracy of amniocentesis for all causes termination of pregnancy in patients not treated with valaciclovir**



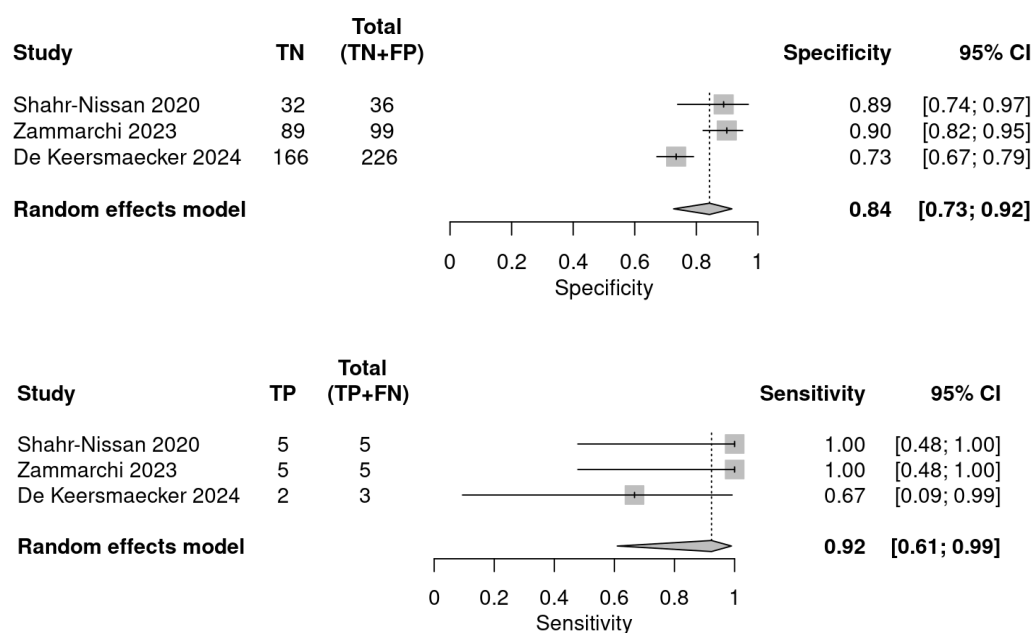
TOP treated

**Figure 31 – Accuracy of amniocentesis for all causes termination of pregnancy in patients treated with valaciclovir**



Severely symptomatic newborns treated

**Figure 32 – Accuracy of amniocentesis for severely symptomatic newborns at birth in patients not treated with valaciclovir**



Severely symptomatic newborns untreated

**Figure 33 – Accuracy of amniocentesis for severely symptomatic newborns in patients treated with valaciclovir**

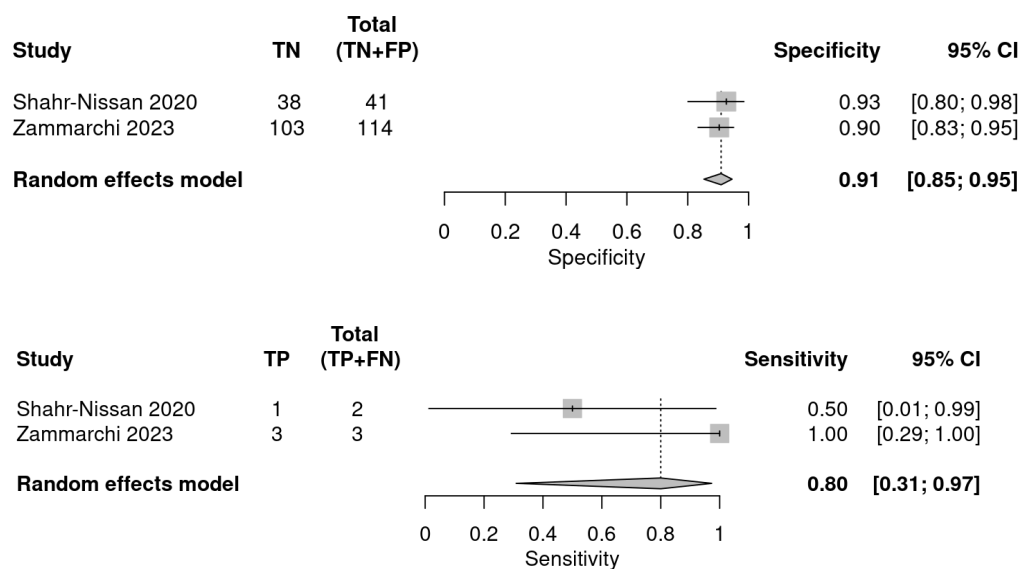




**Table 23 – Accuracy of amniocentesis to detect other critical and important outcomes, available only in patients not treated with valaciclovir**

[illegible]

## 9.8 GRADE tables for amniocentesis to predict the risk of congenital CMV-related sequelae if cCMV is detected at birth

### 9.8.1 In patients not treated with valaciclovir

| Certainty assessment                      |                        |                             |                          |                      |                           |                      | № of patients                                      |                                                    | Effect                     |                                                   | Certainty                             | Importance |
|-------------------------------------------|------------------------|-----------------------------|--------------------------|----------------------|---------------------------|----------------------|----------------------------------------------------|----------------------------------------------------|----------------------------|---------------------------------------------------|---------------------------------------|------------|
| № of studies                              | Study design           | Risk of bias                | Inconsistency            | Indirectness         | Imprecision               | Other considerations | Positive amniocentesis with positive cCMV at birth | Negative amniocentesis with positive cCMV at birth | Relative (95% CI)          | Absolute (95% CI)                                 |                                       |            |
| Severely symptomatic fetus - not reported |                        |                             |                          |                      |                           |                      |                                                    |                                                    |                            |                                                   |                                       |            |
| -                                         | -                      | -                           | -                        | -                    | -                         | -                    | -                                                  | -                                                  | -                          | -                                                 | -                                     | CRITICAL   |
| Termination of pregnancy                  |                        |                             |                          |                      |                           |                      |                                                    |                                                    |                            |                                                   |                                       |            |
| 3 <sup>2,3,4</sup>                        | non-randomised studies | very serious <sup>a</sup>   | not serious              | not serious          | very serious <sup>b</sup> | strong association   | 69/167 (41.3%)                                     | 0/15 (0.0%)                                        | OR 7.46<br>(1.32 to 42.31) | 0 fewer per 1,000<br>(from 0 fewer to 0 fewer)    | ⊕○○○<br>Very low <sup>a,b</sup>       | CRITICAL   |
| Miscarriage or stillbirth                 |                        |                             |                          |                      |                           |                      |                                                    |                                                    |                            |                                                   |                                       |            |
| 1 <sup>2</sup>                            | non-randomised studies | serious <sup>c</sup>        | not serious <sup>d</sup> | not serious          | very serious <sup>b</sup> | none                 | 2/56 (3.6%)                                        | 0/22 (0.0%)                                        | OR 1.99<br>(0.09 to 43.12) | 0 fewer per 1,000<br>(from 0 fewer to 0 fewer)    | ⊕○○○<br>Very low <sup>b,c,d</sup>     | IMPORTANT  |
| Severe symptoms at birth                  |                        |                             |                          |                      |                           |                      |                                                    |                                                    |                            |                                                   |                                       |            |
| 5 <sup>1,2,3,4,5</sup>                    | non-randomised studies | very serious <sup>c,e</sup> | serious <sup>f</sup>     | serious <sup>g</sup> | very serious <sup>h</sup> | none                 | 92/416 (22.1%)                                     | 18/135 (13.3%)                                     | OR 2.75<br>(0.70 to 10.80) | 164 more per 1,000<br>(from 36 fewer to 491 more) | ⊕○○○<br>Very low <sup>c,e,f,g,h</sup> | CRITICAL   |



| Hearing loss at follow up                  |                        |                               |                          |                      |                           |                    |                |             |                            |                                                |                                       |           |
|--------------------------------------------|------------------------|-------------------------------|--------------------------|----------------------|---------------------------|--------------------|----------------|-------------|----------------------------|------------------------------------------------|---------------------------------------|-----------|
| 24,5                                       | non-randomised studies | very serious <sup>c,e,j</sup> | not serious              | not serious          | serious <sup>b</sup>      | strong association | 24/143 (16.8%) | 0/52 (0.0%) | OR 7.87<br>(1.01 to 61.02) | 0 fewer per 1,000<br>(from 0 fewer to 0 fewer) | ⊕○○○<br>Very low <sup>b,c,e,j</sup>   | IMPORTANT |
| Motor developmental delay at follow-up     |                        |                               |                          |                      |                           |                    |                |             |                            |                                                |                                       |           |
| 14                                         | non-randomised studies | very serious <sup>c,e,j</sup> | not serious <sup>d</sup> | not serious          | very serious <sup>b</sup> | none               | 11/51 (21.6%)  | 0/6 (0.0%)  | OR 2.90<br>(0.15 to 55.27) | 0 fewer per 1,000<br>(from 0 fewer to 0 fewer) | ⊕○○○<br>Very low <sup>b,c,d,e,j</sup> | CRITICAL  |
| Visual impairment at follow-up             |                        |                               |                          |                      |                           |                    |                |             |                            |                                                |                                       |           |
| 14                                         | non-randomised studies | very serious <sup>c,e,j</sup> | not serious <sup>b</sup> | not serious          | very serious <sup>b</sup> | none               | 8/51 (15.7%)   | 0/6 (0.0%)  | OR 2.14<br>(0.11 to 41.68) | 0 fewer per 1,000<br>(from 0 fewer to 0 fewer) | ⊕○○○<br>Very low <sup>b,c,e,j</sup>   | IMPORTANT |
| Cognitive developmental delay at follow-up |                        |                               |                          |                      |                           |                    |                |             |                            |                                                |                                       |           |
| 15                                         | non-randomised studies | very serious <sup>c,e,j</sup> | not serious <sup>b</sup> | serious <sup>k</sup> | very serious <sup>b</sup> | none               | 2/92 (2.2%)    | 0/46 (0.0%) | OR 2.51<br>(0.12 to 53.43) | 0 fewer per 1,000<br>(from 0 fewer to 0 fewer) | ⊕○○○<br>Very low <sup>b,c,e,i,k</sup> | CRITICAL  |
| Behavioural problems at follow up          |                        |                               |                          |                      |                           |                    |                |             |                            |                                                |                                       |           |
| 14                                         | non-randomised studies | very serious <sup>c,e,j</sup> | not serious <sup>d</sup> | not serious          | very serious <sup>b</sup> | none               | 5/51 (9.8%)    | 0/6 (0.0%)  | OR 1.38<br>(0.06 to 28.10) | 0 fewer per 1,000<br>(from 0 fewer to 0 fewer) | ⊕○○○<br>Very low <sup>b,c,d,e,j</sup> | IMPORTANT |



|                                      |   |   |   |   |   |   |   |   |   |   |   |           |
|--------------------------------------|---|---|---|---|---|---|---|---|---|---|---|-----------|
| Cerebral palsy - not reported        |   |   |   |   |   |   |   |   |   |   |   |           |
| -                                    | - | - | - | - | - | - | - | - | - | - | - | CRITICAL  |
| Epilepsy - not reported              |   |   |   |   |   |   |   |   |   |   |   |           |
| -                                    | - | - | - | - | - | - | - | - | - | - | - | IMPORTANT |
| Vestibular difunction - not reported |   |   |   |   |   |   |   |   |   |   |   |           |
| -                                    | - | - | - | - | - | - | - | - | - | - | - | IMPORTANT |

**CI:** confidence interval; **OR:** odds ratio

#### Explanations

- In the retrospective studies, the reason for performing amniocentesis can introduce selection bias. Moreover, whether the indication for amniocentesis differed between those with positive and negative results (with maternal or fetal symptoms potentially more frequent among positives) is never reported. We have no systematic indication on whether the termination of pregnancy was due to severely symptomatic fetuses or to the fact that amniocentesis was positive alone.
- Large confidence interval, zero events in the negative amniocentesis group with small denominator
- In the retrospective studies, the reason for performing amniocentesis can introduce selection bias. Moreover, whether the indication for amniocentesis differed between those with positive and negative results (with maternal or fetal symptoms potentially more frequent among positives) is never reported
- Inconsistency was not assessed because evidence was available from a single study.
- The result for this outcome selects, by definition, only pregnancies that did not undergo termination of pregnancy. The most severe cases may be then excluded.
- Moderate statistical heterogeneity was observed between the studies with some variability in effect size although the direction of effect was generally consistent.
- Apart from Kaymeulen 2021, the definition of "severe outcomes" has been implied by the description in the paper and reclassified according to our own definition. In Bilavsky 2016, the number of CNS related outcomes have been considered severe outcomes, but it is possible that in the computation one patient may have had more than one outcome. In Zammarchi 2023 the description of most collected outcomes fits with our definition of "severe", but they are described without clarifying which patients did undergo amniocentesis.
- Large confidence interval, line of null effect crossed
- Methodology for follow up is mostly insufficiently described, length of follow up varies, lost to follow up are insufficiently addressed. asymmetrical outcome ascertainment between positive and negative amniocentesis at follow-up cannot be excluded
- Methodology for follow up is based on a questionnaire sent to parents that is verified only if symptoms are reported., length of follow up varies, lost to follow up are insufficiently addressed. asymmetrical outcome ascertainment between positive and negative amniocentesis at follow-up cannot be excluded



- k. The definition is "neurodevelopmental delay" we assume it refers to cognitive developmental delay

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| Certainty assessment                                      |                        |                           |                           |                      |                           |                      | № of patients                                      |                                                   | Effect                  |                                                | Certainty                                                                                                          | Importance |
|-----------------------------------------------------------|------------------------|---------------------------|---------------------------|----------------------|---------------------------|----------------------|----------------------------------------------------|---------------------------------------------------|-------------------------|------------------------------------------------|--------------------------------------------------------------------------------------------------------------------|------------|
| № of studies                                              | Study design           | Risk of bias              | Inconsistency             | Indirectness         | Imprecision               | Other considerations | Positive amniocentesis with positive cCMV at birth | Negative amniocentesis with positive CMV at birth | Relative (95% CI)       | Absolute (95% CI)                              |                                                                                                                    |            |
| Severely symptomatic fetus - not reported                 |                        |                           |                           |                      |                           |                      |                                                    |                                                   |                         |                                                |                                                                                                                    |            |
| -                                                         | -                      | -                         | -                         | -                    | -                         | -                    | -                                                  | -                                                 | -                       | -                                              | -                                                                                                                  | CRITICAL   |
| Termination of pregnancy                                  |                        |                           |                           |                      |                           |                      |                                                    |                                                   |                         |                                                |                                                                                                                    |            |
| 2                                                         | non-randomised studies | very serious <sup>a</sup> | not serious               | not serious          | very serious <sup>b</sup> | none                 | 6/24 (25.0%)                                       | 0/20 (0.0%)                                       | OR 6.81 (0.71 to 65.06) | 0 fewer per 1,000 (from 0 fewer to 0 fewer)    | <br>Very low <sup>a,b</sup>     | CRITICAL   |
| Miscarriage or stillbirth - not reported                  |                        |                           |                           |                      |                           |                      |                                                    |                                                   |                         |                                                |                                                                                                                    |            |
| -                                                         | -                      | -                         | -                         | -                    | -                         | -                    | -                                                  | -                                                 | -                       | -                                              | -                                                                                                                  | IMPORTANT  |
| Severe symptoms at birth                                  |                        |                           |                           |                      |                           |                      |                                                    |                                                   |                         |                                                |                                                                                                                    |            |
| 2                                                         | non-randomised studies | very serious <sup>a</sup> | very serious <sup>c</sup> | serious <sup>d</sup> | very serious <sup>a</sup> | none                 | 4/19 (21.1%)                                       | 1/10 (10.0%)                                      | OR 2.82 (0.22 to 36.79) | 139 more per 1,000 (from 76 fewer to 703 more) | <br>Very low <sup>a,c,d,e</sup> | CRITICAL   |
| Hearing loss at follow up - not reported                  |                        |                           |                           |                      |                           |                      |                                                    |                                                   |                         |                                                |                                                                                                                    |            |
| -                                                         | -                      | -                         | -                         | -                    | -                         | -                    | -                                                  | -                                                 | -                       | -                                              | -                                                                                                                  | IMPORTANT  |
| Motor developmental delay at follow-up - not reported     |                        |                           |                           |                      |                           |                      |                                                    |                                                   |                         |                                                |                                                                                                                    |            |
| -                                                         | -                      | -                         | -                         | -                    | -                         | -                    | -                                                  | -                                                 | -                       | -                                              | -                                                                                                                  | CRITICAL   |
| Visual impairment at follow-up - not reported             |                        |                           |                           |                      |                           |                      |                                                    |                                                   |                         |                                                |                                                                                                                    |            |
| -                                                         | -                      | -                         | -                         | -                    | -                         | -                    | -                                                  | -                                                 | -                       | -                                              | -                                                                                                                  | IMPORTANT  |
| Cognitive developmental delay at follow-up - not reported |                        |                           |                           |                      |                           |                      |                                                    |                                                   |                         |                                                |                                                                                                                    |            |



|                                                  |   |   |   |   |   |   |   |   |   |   |   |           |
|--------------------------------------------------|---|---|---|---|---|---|---|---|---|---|---|-----------|
| -                                                | - | - | - | - | - | - | - | - | - | - | - | CRITICAL  |
| Behavioural problems at follow up - not reported |   |   |   |   |   |   |   |   |   |   |   |           |
| -                                                | - | - | - | - | - | - | - | - | - | - | - | IMPORTANT |
| Cerebral palsy - not reported                    |   |   |   |   |   |   |   |   |   |   |   |           |
| -                                                | - | - | - | - | - | - | - | - | - | - | - | CRITICAL  |
| Epilepsy - not reported                          |   |   |   |   |   |   |   |   |   |   |   |           |
| -                                                | - | - | - | - | - | - | - | - | - | - | - | IMPORTANT |
| Vestibular disfunction - not reported            |   |   |   |   |   |   |   |   |   |   |   |           |
| -                                                | - | - | - | - | - | - | - | - | - | - | - | IMPORTANT |

**CI:** confidence interval; **OR:** odds ratio

#### Explanations

- In Zammarchi 2023 12/116 (10.3%) of newborns of mothers treated with valaciclovir with a negative amniocentesis were lost to follow-up. In Shahar-Nissan 2020 six participants refused amniocentesis (including one twin pregnancy). Three neonates were negative for CMV and their amniocentesis was presumed to be negative, and four neonates with positive CMV at birth were presumed to be positive
- Large confidence interval, zero events in the negative amniocentesis group with small denominator
- In the two included studies the point estimates are in different directions
- In Zammarchi 2023 the description of most collected outcomes fits with our definition of "severe", but they are described without clarifying which patients did undergo amniocentesis.
- Large confidence intervals

#### References

- Zammarchi L, Tomasoni LR, Liuzzi G, Simonazzi G, Dionisi C, Mazzarelli LL, Seidenari A, Maruotti GM, Ornaghi S, Castelli F, Abbate I, Bordi L, Mazzotta S, Fusco P, Torti C, Calò Carducci FI, Baccini M, Modi G, Galli L, Lilleri D, Furione M, Zavattoni M, Ricciardi A, Arossa A, Vimercati A, Lovatti S, Salomè S, Raimondi F, Sarno L, Sforza A, Fichera A, Caforio L, Trotta M, Lazzarotto T. MEGAL-ITALI Working Group. Treatment with valacyclovir during pregnancy for prevention of congenital cytomegalovirus infection: a real-life multicenter Italian observational study. *Am J Obstet Gynecol MFM*; 2023.
- Shahar-Nissan K, Pardo J, Peled O, Krause I, Bilavsky E, Wiznitzer A, Hadar E, Amir J. Valaciclovir to prevent vertical transmission of cytomegalovirus after maternal primary infection during pregnancy: a randomised, double-blind, placebo-controlled trial. *Lancet*; 2020.

Since there were zero events for some of the outcomes in the negative amniocentesis group, a continuity correction was applied to allow estimation of the odds ratios and 95% confidence intervals. 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 choice; therefore, absolute risks are not reported, and only the fact that there were zero events is noted. It is important to note that the absence of observed events does not imply absence of risk, especially with small samples.

### 9.9 GRADE tables for 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. Nonetheless, within the studies selected for the question on CMV-related sequelae in cases of negative amniocentesis with cCMV detected at birth, three studies were found that provided sufficient follow-up of pregnancies with a negative amniocentesis and negative CMV at birth—albeit differential follow-up cannot be excluded—to allow calculation of prognostic accuracy measurements in untreated patients, two of those studies included data on treated patients..

Prevalence of CMV-related outcomes was estimated as the minimum, maximum and pooled prevalence of the studies reporting important and critical outcomes in the treatment question ( see 8). All outcomes not reported in the question regarding the use of valaciclovir are those reported in De Keersmaecker et al(35) and are available only for not treated patients. All studies come with a high risk of bias, and they did not compare more versus less intensive antenatal post-amniocentesis follow-up in pregnancies with a negative amniocentesis. Accordingly, we consider these findings indirect evidence for the follow-up question.



### 9.9.1 Prognostic accuracy of amniocentesis in untreated patients

#### cCMV at birth

|                    |                             |
|--------------------|-----------------------------|
| <b>Sensitivity</b> | 0.91 (95% CI: 0.84 to 0.95) |
| <b>Specificity</b> | 1.00 (95% CI: 0.89 to 1.00) |

|             |       |     |     |
|-------------|-------|-----|-----|
| Prevalences | 30.7% | 28% | 39% |
|-------------|-------|-----|-----|

| cCMV at birth (untreated) | No studies (No of patients) | Study design      | Factors that may decrease certainty of evidence |                           |               |             |                  | Effect per 1,000 patients tested |                             |                             | Test accuracy CoE               |
|---------------------------|-----------------------------|-------------------|-------------------------------------------------|---------------------------|---------------|-------------|------------------|----------------------------------|-----------------------------|-----------------------------|---------------------------------|
|                           |                             |                   | Risk of bias                                    | Indirectness              | Inconsistency | Imprecision | Publication bias | pre-test probability of 30.7%    | pre-test probability of 28% | pre-test probability of 39% |                                 |
| True positives            | 3 studies 165 patients      | cohort type study | very serious <sup>a</sup>                       | very serious <sup>b</sup> | not serious   | not serious | none             | 279 (258 to 292)                 | 255 (235 to 266)            | 355 (328 to 371)            | ⊕○○○<br>Very low <sup>a,b</sup> |
| False negatives           |                             |                   |                                                 |                           |               |             |                  | 28 (15 to 49)                    | 25 (14 to 45)               | 35 (19 to 62)               |                                 |
| True negatives            | 3 studies 343 patients      | cohort type study | very serious <sup>a</sup>                       | very serious <sup>b</sup> | not serious   | not serious | none             | 693 (617 to 693)                 | 720 (641 to 720)            | 610 (543 to 610)            | ⊕○○○<br>Very low <sup>a,b</sup> |
| False positives           |                             |                   |                                                 |                           |               |             |                  | 0 (0 to 76)                      | 0 (0 to 79)                 | 0 (0 to 67)                 |                                 |

#### Explanations

a. Prognostic accuracy not the primary aim of the studies, many patients refused amniocentesis in the cohorts and the reason for performing amniocentesis can introduce selection bias, in Shahar-Nissan 2020 in case of refusal of amniocentesis the results were imputed with the results at birth

b. Indirect data for answering question on follow-up

**All cause termination of pregnancy**

|                    |                             |
|--------------------|-----------------------------|
| <b>Sensitivity</b> | 0.99 (95% CI: 0.60 to 1.00) |
| <b>Specificity</b> | 0.78 (95% CI: 0.74 to 0.82) |

|             |       |     |     |
|-------------|-------|-----|-----|
| Prevalences | 11.2% | 10% | 18% |
|-------------|-------|-----|-----|

| All cause termination of pregnancy (untreated) | No of studies (No of patients) | Study design      | Factors that may decrease certainty of evidence |                           |               |             |                  | Effect per 1,000 patients tested |                             |                             | Test accuracy CoE               |
|------------------------------------------------|--------------------------------|-------------------|-------------------------------------------------|---------------------------|---------------|-------------|------------------|----------------------------------|-----------------------------|-----------------------------|---------------------------------|
|                                                |                                |                   | Risk of bias                                    | Indirectness              | Inconsistency | Imprecision | Publication bias | pre-test probability of 11.2%    | pre-test probability of 10% | pre-test probability of 18% |                                 |
| True positives                                 | 3 studies 70 patients          | cohort type study | very serious <sup>a</sup>                       | very serious <sup>b</sup> | not serious   | serious     | none             | 111 (67 to 112)                  | 99 (60 to 100)              | 178 (108 to 180)            | ⊕○○○<br>Very low <sup>a,b</sup> |
| False negatives                                |                                |                   |                                                 |                           |               |             |                  | 1 (0 to 45)                      | 1 (0 to 40)                 | 2 (0 to 72)                 |                                 |
| True negatives                                 | 3 studies 383 patients         | cohort type study | very serious <sup>a</sup>                       | very serious <sup>b</sup> | not serious   | not serious | none             | 693 (657 to 728)                 | 702 (666 to 738)            | 640 (607 to 672)            | ⊕○○○<br>Very low <sup>a,b</sup> |
| False positives                                |                                |                   |                                                 |                           |               |             |                  | 195 (160 to 231)                 | 198 (162 to 234)            | 180 (148 to 213)            |                                 |

**Explanations**

a. Prognostic accuracy not the primary aim of the studies, many patients refused amniocentesis in the cohorts and the reason for performing amniocentesis can introduce selection bias, in Shahar-Nissan 2020 in case of refusal of amniocentesis the results were imputed with the results at birth

b. Indirect data for answering question on follow-up



### Severely symptomatic newborns

|                    |                             |
|--------------------|-----------------------------|
| <b>Sensitivity</b> | 0.92 (95% CI: 0.61 to 0.99) |
| <b>Specificity</b> | 0.84 (95% CI: 0.73 to 0.92) |

|             |    |    |     |
|-------------|----|----|-----|
| Prevalences | 9% | 8% | 12% |
|-------------|----|----|-----|

| Severely symptomatic newborns (untreated) | No of studies (No of patients) | Study design      | Factors that may decrease certainty of evidence |                           |               |             |                  | Effect per 1,000 patients tested |                            |                             | Test accuracy CoE               |
|-------------------------------------------|--------------------------------|-------------------|-------------------------------------------------|---------------------------|---------------|-------------|------------------|----------------------------------|----------------------------|-----------------------------|---------------------------------|
|                                           |                                |                   | Risk of bias                                    | Indirectness              | Inconsistency | Imprecision | Publication bias | pre-test probability of 9%       | pre-test probability of 8% | pre-test probability of 12% |                                 |
| True positives                            | 3 studies<br>13 patients       | cohort type study | very serious <sup>a</sup>                       | very serious <sup>b</sup> | not serious   | serious     | none             | 83 (55 to 89)                    | 74 (49 to 79)              | 110 (73 to 119)             | ⊕○○○<br>Very low <sup>a,b</sup> |
| False negatives                           |                                |                   |                                                 |                           |               |             |                  | 7 (1 to 35)                      | 6 (1 to 31)                | 10 (1 to 47)                |                                 |
| True negatives                            | 3 studies<br>361 patients      | cohort type study | very serious <sup>a</sup>                       | very serious <sup>b</sup> | not serious   | not serious | none             | 764 (664 to 837)                 | 773 (672 to 846)           | 739 (642 to 810)            | ⊕○○○<br>Very low <sup>a,b</sup> |
| False positives                           |                                |                   |                                                 |                           |               |             |                  | 146 (73 to 246)                  | 147 (74 to 248)            | 141 (70 to 238)             |                                 |

#### Explanations

- a. Prognostic accuracy not the primary aim of the studies, many patients refused amniocentesis in the cohorts and the reason for performing amniocentesis can introduce selection bias, in Shahar-Nissan 2020 in case of refusal of amniocentesis the results were imputed with the results at birth
- b. Indirect data for answering question on follow-up

Other outcomes

| Outcome in untreated                                     | No. of participants (studies) | Study design         | Prevalence | TP | FP | FN | TN  | Sensitivity (95% CI)      | Specificity (95% CI)      | Predictive value of positive test (95% CI) | Predictive value of negative test (95% CI) | Certainty of evidence (GRADE) | Importance |
|----------------------------------------------------------|-------------------------------|----------------------|------------|----|----|----|-----|---------------------------|---------------------------|--------------------------------------------|--------------------------------------------|-------------------------------|------------|
| Severely symptomatic foetus-not reported                 | -                             | -                    | -          | -  | -  | -  | -   | -                         | -                         | -                                          | -                                          | -                             | CRITICAL   |
| Miscarriage or stillbirth- not reported                  | -                             | -                    | -          | -  | -  | -  | -   | -                         | -                         | -                                          | -                                          | -                             | IMPORTANT  |
| Hearing loss at follow-up                                | 179 (1)                       | Retrospective cohort | 7.26 %     | 12 | 39 | 1  | 127 | 92.31% (63.97% to 99.81%) | 76.51% (69.31% to 82.73%) | 23.53% (18.32% to 29.68%)                  | 99.22% (95.07% to 99.88%)                  | Very low ⊕○○○ (a,b,c)         | IMPORTANT  |
| Motor developmental delay at follow up                   | 179 (1)                       | Retrospective cohort | 10.06 %    | 11 | 40 | 7  | 121 | 61.11% (35.75% to 82.70%) | 75.16% (67.74% to 81.62%) | 21.57% (14.84% to 30.26%)                  | 94.53% (90.59% to 96.88%)                  | Very low ⊕○○○ (a,b,c)         | CRITICAL   |
| Cognitive developmental delay at follow-up- not reported | -                             | -                    | -          | -  | -  | -  | -   | -                         | -                         | -                                          | -                                          | -                             | CRITICAL   |
| Visual impairment at follow-up                           | 179 (1)                       | Retrospective cohort | 7.26 %     | 8  | 43 | 5  | 123 | 61.54% (31.58% to 86.14%) | 74.10% (66.74% to 80.58%) | 15.69% (10.13% to 23.49%)                  | 96.09% (92.48% to 98.01%)                  | Very low ⊕○○○ (a,b,c)         | IMPORTANT  |
| Behavioural problems at follow up                        | 179 (1)                       | Retrospective cohort | 5.03 %     | 5  | 46 | 4  | 124 | 55.56% (21.20% to 86.30%) | 72.94% (65.61% to 79.46%) | 9.80% (5.45% to 17.01%)                    | 96.88% (93.69% to 98.48%)                  | Very low ⊕○○○ (a,b,c)         | IMPORTANT  |
| Cerebral palsy- not reported                             | -                             | -                    | -          | -  | -  | -  | -   | -                         | -                         | -                                          | -                                          | -                             | CRITICAL   |
| Epilepsy- not reported                                   | -                             | -                    | -          | -  | -  | -  | -   | -                         | -                         | -                                          | -                                          | -                             | IMPORTANT  |
| Vestibular disfunction-not reported                      | -                             | -                    | -          | -  | -  | -  | -   | -                         | -                         | -                                          | -                                          | -                             | IMPORTANT  |

a: Serious risk of bias (selection bias due to indication for amniocentesis; reasons for termination not reported; possible differential follow-up).

b: Serious indirectness (does not directly answer the follow-up question).

c: Serious imprecision (single study; wide CIs, sparse events).



### 9.9.2 Prognostic accuracy of amniocentesis in treated patients

#### cCMV at birth

|                    |                             |
|--------------------|-----------------------------|
| <b>Sensitivity</b> | 0.55 (95% CI: 0.40 to 0.68) |
| <b>Specificity</b> | 1.00 (95% CI: 0.90 to 1.00) |

|             |       |       |       |
|-------------|-------|-------|-------|
| Prevalences | 21.6% | 18.6% | 22.7% |
|-------------|-------|-------|-------|

| cCMV at birth (treated) | № of studies (№ of patients) | Study design      | Factors that may decrease certainty of evidence |                           |               |                           |                  | Effect per 1,000 patients tested |                               |                               | Test accuracy CoE                 |
|-------------------------|------------------------------|-------------------|-------------------------------------------------|---------------------------|---------------|---------------------------|------------------|----------------------------------|-------------------------------|-------------------------------|-----------------------------------|
|                         |                              |                   | Risk of bias                                    | Indirectness              | Inconsistency | Imprecision               | Publication bias | pre-test probability of 21.6%    | pre-test probability of 18.6% | pre-test probability of 22.7% |                                   |
| True positives          | 2 studies<br>44 patients     | cohort type study | very serious <sup>a</sup>                       | very serious <sup>b</sup> | not serious   | very serious <sup>c</sup> | none             | 119 (86 to 147)                  | 102 (74 to 126)               | 125 (91 to 154)               | ⊕○○○<br>Very low <sup>a,b,c</sup> |
| False negatives         |                              |                   |                                                 |                           |               |                           |                  | 97 (69 to 130)                   | 84 (60 to 112)                | 102 (73 to 136)               |                                   |
| True negatives          | 2 studies<br>155 patients    | cohort type study | very serious <sup>a</sup>                       | very serious <sup>b</sup> | not serious   | not serious               | none             | 784 (706 to 784)                 | 814 (733 to 814)              | 773 (696 to 773)              | ⊕○○○<br>Very low <sup>a,b</sup>   |
| False positives         |                              |                   |                                                 |                           |               |                           |                  | 0 (0 to 78)                      | 0 (0 to 81)                   | 0 (0 to 77)                   |                                   |

#### Explanations

a. Prognostic accuracy not the primary aim of the studies, many patients refused amniocentesis in the cohorts and the reason for performing amniocentesis can introduce selection bias, in Shahar-Nissan 2020 in case of refusal of amniocentesis the results were imputed with the results at birth

b. Indirect data for answering question on follow-up

c. Wide confidence intervals



### All cause termination of pregnancy

|                    |                             |
|--------------------|-----------------------------|
| <b>Sensitivity</b> | 0.75 (95% CI: 0.38 to 0.94) |
| <b>Specificity</b> | 0.88 (95% CI: 0.82 to 0.92) |

|             |      |    |    |
|-------------|------|----|----|
| Prevalences | 3.9% | 6% | 3% |
|-------------|------|----|----|

| All cause termination of pregnancy (treated) | No of studies (No of patients) | Study design      | Factors that may decrease certainty of evidence |                           |               |                      |                  | Effect per 1,000 patients tested |                            |                            | Test accuracy CoE                 |
|----------------------------------------------|--------------------------------|-------------------|-------------------------------------------------|---------------------------|---------------|----------------------|------------------|----------------------------------|----------------------------|----------------------------|-----------------------------------|
|                                              |                                |                   | Risk of bias                                    | Indirectness              | Inconsistency | Imprecision          | Publication bias | pre-test probability of 3.9%     | pre-test probability of 6% | pre-test probability of 3% |                                   |
| True positives                               | 2 studies<br>8 patients        | cohort type study | very serious <sup>a</sup>                       | very serious <sup>b</sup> | not serious   | serious <sup>c</sup> | none             | 29 (15 to 37)                    | 45 (23 to 56)              | 23 (11 to 28)              | ⊕○○○<br>Very low <sup>a,b,c</sup> |
| False negatives                              |                                |                   |                                                 |                           |               |                      |                  | 10 (2 to 24)                     | 15 (4 to 37)               | 7 (2 to 19)                |                                   |
| True negatives                               | 2 studies<br>361 patients      | cohort type study | very serious <sup>a</sup>                       | very serious <sup>b</sup> | not serious   | not serious          | none             | 846 (788 to 884)                 | 827 (771 to 865)           | 854 (795 to 892)           | ⊕○○○<br>Very low <sup>a,b</sup>   |
| False positives                              |                                |                   |                                                 |                           |               |                      |                  | 115 (77 to 173)                  | 113 (75 to 169)            | 116 (78 to 175)            |                                   |

#### Explanations

a. Prognostic accuracy not the primary aim of the studies, many patients refused amniocentesis in the cohorts and the reason for performing amniocentesis can introduce selection bias, in Shahar-Nissan 2020 in case of refusal of amniocentesis the results were imputed with the results at birth

b. Indirect data for answering question on follow-up

c. Wide confidence interval



### Severely symptomatic newborns

|                    |                             |
|--------------------|-----------------------------|
| <b>Sensitivity</b> | 0.80 (95% CI: 0.31 to 0.97) |
| <b>Specificity</b> | 0.91 (95% CI: 0.85 to 0.95) |

|             |      |    |    |
|-------------|------|----|----|
| Prevalences | 1.7% | 5% | 1% |
|-------------|------|----|----|

| Severely symptomatic newborns (treated) | No of studies (No of patients) | Study design      | Factors that may decrease certainty of evidence |                           |               |                      |                  | Effect per 1,000 patients tested |                            |                            | Test accuracy CoE                 |
|-----------------------------------------|--------------------------------|-------------------|-------------------------------------------------|---------------------------|---------------|----------------------|------------------|----------------------------------|----------------------------|----------------------------|-----------------------------------|
|                                         |                                |                   | Risk of bias                                    | Indirectness              | Inconsistency | Imprecision          | Publication bias | pre-test probability of 1.7%     | pre-test probability of 5% | pre-test probability of 1% |                                   |
| True positives                          | 2 studies<br>5 patients        | cohort type study | very serious <sup>a</sup>                       | very serious <sup>b</sup> | not serious   | serious <sup>c</sup> | none             | 14 (5 to 16)                     | 40 (16 to 49)              | 8 (3 to 10)                | ⊕○○○<br>Very low <sup>a,b,c</sup> |
| False negatives                         |                                |                   |                                                 |                           |               |                      |                  | 3 (1 to 12)                      | 10 (1 to 34)               | 2 (0 to 7)                 |                                   |
| True negatives                          | 2 studies<br>155 patients      | cohort type study | very serious <sup>a</sup>                       | very serious <sup>b</sup> | not serious   | not serious          | none             | 895 (836 to 934)                 | 864 (808 to 903)           | 901 (842 to 941)           | ⊕○○○<br>Very low <sup>a,b</sup>   |
| False positives                         |                                |                   |                                                 |                           |               |                      |                  | 88 (49 to 147)                   | 86 (47 to 142)             | 89 (49 to 148)             |                                   |

#### Explanations

a. Prognostic accuracy not the primary aim of the studies, many patients refused amniocentesis in the cohorts and the reason for performing amniocentesis can introduce selection bias, in Shahar-Nissan 2020 in case of refusal of amniocentesis the results were imputed with the results at birth

b. Indirect data for answering question on follow-up

c. Wide confidence intervals

[illegible]



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

### Appendix 1.2. PIRTS

**Table 24 – PIRTS diagnostic accuracy review of timing of amniocentesis for cCMV at**

|                    | Timing of amniocentesis for cCMV at birth                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
|--------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Objective          | To determine whether amniocentesis can be performed at an earlier date compared to current management habits                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
| Review Question    | In pregnant patients with primary CMV infection, should amniocentesis be performed at $\geq 17+0$ weeks and $\geq 6$ weeks after seroconversion, compared to later timing, for detecting vertical transmission of CMV at birth?                                                                                                                                                                                                                                                                                                                                                                |
| Population         | Pregnant patients with a primary CMV infection, who accepted amniocentesis                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
| Index test         | Amniocentesis with CMV PCR at $\geq 17+0$ weeks and $\geq 6$ weeks after seroconversion                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
| Comparator test    | Amniocentesis with CMV PCR at a later date                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
| Reference standard | CMV PCR positive in the newborn within the first 3 weeks of life                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
| Target condition   | Congenital CMV                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
| Outcomes           | Critical: Diagnostic accuracy<br>Important: Any test burden, complications<br>Other: clinical management after amniocentesis, congenital CMV disease                                                                                                                                                                                                                                                                                                                                                                                                                                           |
| Study design       | Good quality systematic reviews, diagnostic randomised controlled trials, prospective and retrospective diagnostic cohort studies, in which the index test and the reference standard are applied to the same patients, case-control studies.<br><b>Excluded:</b> 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 not include CMV PCR. |
| Analysis           | By trimester of seroconversion, by treatment status                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |

## 10.1 Search strategies

### 10.1.1 Medline through Pubmed

1. "Amniocentesis"[MeSH] OR amniocentesis[tiab] OR "amniotic fluid sampl\*" [tiab] OR "amniotic fluid test\*" [tiab] OR "amniotic fluid analys\*" [tiab] OR amnio\* [tiab] OR "prenatal diagnos\*" [tiab] OR "antenatal diagnos\*" [tiab] OR "antenatal screening\*" [tiab] OR "prenatal screening\*" [tiab] OR "fetal diagnos\*" [tiab] OR "foetal diagnos\*" [tiab] OR "intrauterine diagnos\*" [tiab]
2. "Cytomegalovirus Infections"[MeSH] OR "Cytomegalovirus"[MeSH] OR cytomegalovirus[tiab] OR CMV[tiab] OR hhv5[tiab] OR "hhv 5"[tiab] OR "HHV-5"[tiab] OR "herpesvirus 5"[tiab] OR "herpes virus 5"[tiab] OR hcmv[tiab] OR "salivary gland virus"[tiab] OR "salivary gland disease"[tiab] OR "inclusion disease"[tiab] OR "cytomegalic inclusion disease"[tiab] OR "congenital cytomegalovirus infection"[tiab]
3. #1 AND #2

### 10.1.2 Embase

1. 'amniocentesis'/exp OR amniocentesis:ti,ab,kw OR 'amniotic fluid sampl\*':ti,ab,kw OR 'amniotic fluid test\*':ti,ab,kw OR 'amniotic fluid analys\*':ti,ab,kw OR amnio\*:ti,ab,kw OR 'prenatal diagnos\*':ti,ab,kw OR 'antenatal diagnos\*':ti,ab,kw OR 'antenatal screening\*':ti,ab,kw OR 'prenatal screening\*':ti,ab,kw OR 'fetal diagnos\*':ti,ab,kw OR 'foetal diagnos\*':ti,ab,kw OR 'intrauterine diagnos\*':ti,ab,kw
2. 'congenital cytomegalovirus infection'/exp OR 'Human cytomegalovirus'/exp OR cytomegalovirus:ti,ab,kw OR CMV:ti,ab,kw OR hhv5:ti,ab,kw OR 'hhv 5':ti,ab,kw OR 'HHV-5':ti,ab,kw OR 'herpesvirus 5':ti,ab,kw OR 'herpes virus 5':ti,ab,kw OR hcmv:ti,ab,kw OR 'salivary gland virus':ti,ab,kw OR 'salivary gland disease':ti,ab,kw OR 'inclusion disease':ti,ab,kw OR 'cytomegalic inclusion disease':ti,ab,kw OR 'congenital cytomegalovirus infection':ti,ab,kw
3. #1 AND #2

### 10.1.3 Cochrane

1. [mh "Amniocentesis"] OR amniocentesis:ti,ab,kw OR ("amniotic fluid sample" OR "amniotic fluid samples"):ti,ab,kw OR ("amniotic fluid test" OR "amniotic fluid tests"):ti,ab,kw OR ("amniotic fluid analysis" OR "amniotic fluid analyses"):ti,ab,kw OR amnio\*:ti,ab,kw OR ("prenatal diagnosis" OR "prenatal diagnoses"):ti,ab,kw OR ("antenatal diagnosis" OR "antenatal diagnoses"):ti,ab,kw OR ("antenatal screening" OR "antenatal screenings"):ti,ab,kw OR ("prenatal screening" OR "prenatal screenings"):ti,ab,kw OR ("fetal diagnosis" OR "fetal diagnoses"):ti,ab,kw OR ("foetal diagnosis" OR "foetal diagnoses"):ti,ab,kw OR ("intrauterine diagnosis" OR "intrauterine diagnoses"):ti,ab,kw
2. [mh "Cytomegalovirus Infections, Congenital"] OR [mh "Cytomegalovirus Infections"] OR [mh "Cytomegalovirus"] OR cytomegalovirus:ti,ab,kw OR CMV:ti,ab,kw OR hhv5:ti,ab,kw OR "hhv 5":ti,ab,kw OR "HHV-5":ti,ab,kw OR "herpesvirus 5":ti,ab,kw OR "herpes virus 5":ti,ab,kw OR hcmv:ti,ab,kw OR "salivary gland virus":ti,ab,kw OR "salivary gland disease":ti,ab,kw OR "inclusion disease":ti,ab,kw OR "cytomegalic inclusion disease":ti,ab,kw OR "congenital cytomegalovirus infection":ti,ab,kw
3. #1 AND #2
4. #3 NOT ("case report" OR "case reports"):ti,ab,kw



### Appendix 1.3. Study selection

Figure 34 – Study flow selection of search for timing of amniocentesis for cCMV at birth

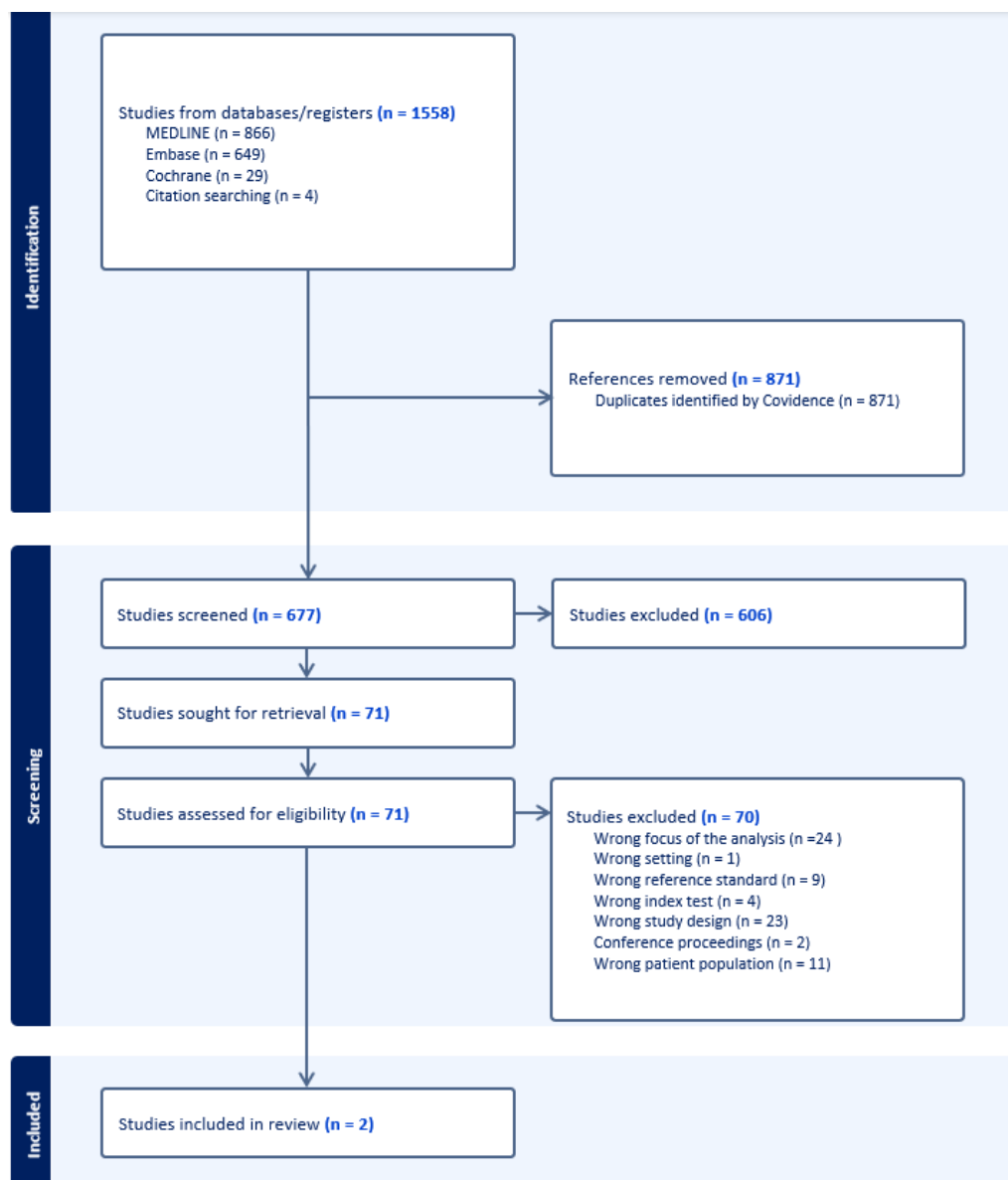


Figure provided by Covidence

### Appendix 1.3.1. Studies excluded with reason

**Table 25 – Excluded primary studies for timing of amniocentesis for cCMV at birth**

| Study and year         | Exclusion reason                                                                      |
|------------------------|---------------------------------------------------------------------------------------|
| Dinsmoor 2022          | Exclusion reason: Wrong focus of the analysis, no analysis on timing of amniocentesis |
| Revello 2014           | Exclusion reason: Wrong focus of the analysis, no analysis on timing of amniocentesis |
| Toti 2023              | Exclusion reason: Wrong study design                                                  |
| NCT02351102 2015       | Exclusion reason: Wrong study design                                                  |
| Shahar-Nissan 2019     | Exclusion reason: Conference proceedings                                              |
| Antsaklis 2000         | Exclusion reason: Wrong reference standard                                            |
| Azam 2001              | Exclusion reason: Wrong index test and reference standard                             |
| Bartek 2025            | Exclusion reason: Wrong patient population                                            |
| Bodeus 1999            | Exclusion reason: Wrong focus of the analysis, no analysis on timing of amniocentesis |
| Catanzarite 1993       | Exclusion reason: Wrong study design                                                  |
| Chatzakis 2023         | Exclusion reason: Wrong study design                                                  |
| D'Alberti 2024         | Exclusion reason: Wrong study design                                                  |
| DeConinck 2024         | Exclusion reason: Wrong focus of the analysis, no analysis on timing of amniocentesis |
| DeSantis 2020          | Exclusion reason: Wrong study design                                                  |
| Denef 2022             | Exclusion reason: Wrong study design                                                  |
| Donner 1994            | Exclusion reason: Wrong reference standard                                            |
| Donner 1993            | Exclusion reason: Wrong reference standard                                            |
| Fattuoni 2016          | Exclusion reason: Wrong study design                                                  |
| Fernicola 2024         | Exclusion reason: Wrong study design                                                  |
| Gabbay-Benziv 2012     | Exclusion reason: Wrong focus of the analysis, no analysis on timing of amniocentesis |
| Goegebuer 2009         | Exclusion reason: Wrong reference standard                                            |
| Gouarin 2001           | Exclusion reason: Wrong focus of the analysis, no analysis on timing of amniocentesis |
| Guerra 2000            | Exclusion reason: Wrong focus of the analysis, no analysis on timing of amniocentesis |
| Hui 2022               | Exclusion reason: Wrong study design                                                  |
| Kagan 2017             | Exclusion reason: Wrong study design                                                  |
| Kanengisser-Pines 2009 | Exclusion reason: Wrong study design                                                  |
| Lazzarotto 2014        | Exclusion reason: Wrong study design;                                                 |
| Leruez-Ville 2016      | Exclusion reason: Wrong patient population                                            |
| Lipitz 2002            | Exclusion reason: Wrong reference standard                                            |
| Lipitz 2020            | Exclusion reason: Wrong patient population                                            |
| Lipitz 1997            | Exclusion reason: Wrong focus of the analysis, no analysis on timing of amniocentesis |



|                         |                                                                                       |
|-------------------------|---------------------------------------------------------------------------------------|
| Mappa 2023              | Exclusion reason: Wrong patient population                                            |
| Margioulas-Siarkou 2015 | Exclusion reason: Wrong focus of the analysis, no confirmation of cCMV at birth       |
| Miller 2009             | Exclusion reason: Wrong patient population                                            |
| Minsart 2018            | Exclusion reason: Wrong focus of the analysis, no analysis on timing of amniocentesis |
| Moaven 1995             | Exclusion reason: Wrong study design                                                  |
| Naing 2016              | Exclusion reason: Wrong study design                                                  |
| Nigro 2005              | Exclusion reason: Wrong study design                                                  |
| Patel 2024              | Exclusion reason: Wrong study design                                                  |
| Picone 2013             | Exclusion reason: Wrong focus of the analysis, no analysis on timing of amniocentesis |
| Revello 1995            | Exclusion reason: Wrong study design                                                  |
| Revello 2008            | Exclusion reason: Wrong focus of the analysis, no analysis on timing of amniocentesis |
| Satilmis 2007           | Exclusion reason: Wrong study design                                                  |
| Seidenari 2025          | Exclusion reason: Wrong patient population                                            |
| Simonazzi 2017          | Exclusion reason: Wrong reference standard                                            |
| TugrulErsak 2023        | Exclusion reason: Wrong focus of the analysis, no analysis on timing of amniocentesis |
| VandenEede 2025         | Exclusion reason: Wrong patient population                                            |
| Visentin 2012           | Exclusion reason: Wrong reference standard                                            |
| Zavattoni 2016          | Exclusion reason: Wrong focus of the analysis                                         |
| Zhang 2025              | Exclusion reason: Wrong patient population                                            |
| Fourgeaud 2025          | Exclusion reason: Wrong focus of the analysis                                         |
| MedinaGarcía 2024       | Exclusion reason: Wrong study design                                                  |
| Buca 2021               | Exclusion reason: Wrong patient population                                            |
| Seidel 2020             | Exclusion reason: Wrong patient population                                            |
| Zavattoni 2014          | Exclusion reason: Wrong focus of the analysis                                         |
| Enders 2011             | Exclusion reason: Wrong study design                                                  |
| Feldman 2011            | Exclusion reason: Wrong focus of the analysis, no confirmation of cCMV at birth       |
| Daiminger 2005          | Exclusion reason: Wrong study design                                                  |
| Avidor 2004             | Exclusion reason: Wrong study design                                                  |
| Lazzarotto 1998         | Exclusion reason: Wrong patient population                                            |
| Hughes 2019             | Exclusion reason: Conference proceedings                                              |
| Shahar-Nissan 2020      | Exclusion reason: Wrong focus of the analysis, no analysis on timing of amniocentesis |
| Bilavsky 2016           | Exclusion reason: Wrong focus of the analysis, no analysis on timing of amniocentesis |
| DeKeersmaecker 2024     | Exclusion reason: Wrong focus of the analysis, no analysis on timing of amniocentesis |



|                |                                                                                       |
|----------------|---------------------------------------------------------------------------------------|
| Liesnard 2000  | Exclusion reason: Wrong reference standard                                            |
| Zammarchi 2023 | Exclusion reason: Wrong focus of the analysis, no analysis on timing of amniocentesis |
| Enders 2001    | Exclusion reason: Wrong focus of the analysis, no analysis on timing of amniocentesis |
| Keymeulen 2021 | Exclusion reason: Wrong focus of the analysis, no analysis on timing of amniocentesis |



## Appendix 1.4. Appraisal

The two selected studies are a retrospective cohort study (Enders 2017a) and a subset of the same population (Enders 2017b on which different analysis are being performed. Enders 2017 a is the study with the largest sample size but is published in a research letter, a more complete methodology is published in Enders 2017b. We make the assumption that the methodology described in Enders 2017 a also applies to Enders 2017b

**Table 26 – QUADAS-2 assessment for timing of amniocentesis for cCMV at birth**

| Question                                                                                               | Enders 2017a                                                                                                                                                                                                                                                                                    | Enders 2017b                                                                                                                                                                                                                                                                                                       |
|--------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Selection of participants</b>                                                                       |                                                                                                                                                                                                                                                                                                 |                                                                                                                                                                                                                                                                                                                    |
| 1. Was a consecutive or random sample of <b>participants</b> enrolled?                                 | Unclear, this study expands Enders 2017b for a 16 years period. Reason for testing is unclear, it is unknown whether they are referrals for symptoms/anomalies or patients with positive systematic screening. We don't know the timing of maternal seroconversion. The study is retrospective. | Unclear, we know that the study investigated 132 women with primary CMV infection. Reason for testing is unclear, it is unknown whether they are referrals for symptoms/anomalies or patients with positive systematic screening. We don't know the timing of maternal seroconversion. The study is retrospective. |
| 2. Was a case-control design avoided?                                                                  | Yes                                                                                                                                                                                                                                                                                             | Yes                                                                                                                                                                                                                                                                                                                |
| 3. Did the study avoid inappropriate exclusions?                                                       | Yes                                                                                                                                                                                                                                                                                             | Yes                                                                                                                                                                                                                                                                                                                |
| <b>Could selection of participants have introduced bias?</b>                                           | <b>Unclear</b>                                                                                                                                                                                                                                                                                  | <b>Unclear</b>                                                                                                                                                                                                                                                                                                     |
| <b>Is there concern that the included participants do not match the review question?</b>               | <b>Unclear</b>                                                                                                                                                                                                                                                                                  | <b>Unclear</b>                                                                                                                                                                                                                                                                                                     |
| <b>Index test</b>                                                                                      |                                                                                                                                                                                                                                                                                                 |                                                                                                                                                                                                                                                                                                                    |
| 1. Were the index test results interpreted without knowledge of the results of the reference standard? | Yes                                                                                                                                                                                                                                                                                             | Yes                                                                                                                                                                                                                                                                                                                |
| 2. If a threshold was used, was it pre-specified?                                                      | Yes                                                                                                                                                                                                                                                                                             | Yes                                                                                                                                                                                                                                                                                                                |
| <b>Could the conduct or interpretation of the index test have introduced bias?</b>                     | <b>Low</b>                                                                                                                                                                                                                                                                                      | <b>Low</b>                                                                                                                                                                                                                                                                                                         |
| <b>Could the conduct or interpretation of the index test have introduced bias?</b>                     | <b>Low</b>                                                                                                                                                                                                                                                                                      | <b>Low</b>                                                                                                                                                                                                                                                                                                         |

| Reference standard                                                                                                    |                                                                                                                                                                                                                                                                                                                                                                                                                                        |                                                                                                                                                                                                                                                                                                                                                                                             |
|-----------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 1. Is the reference standard likely to correctly classify the target condition?                                       | Yes                                                                                                                                                                                                                                                                                                                                                                                                                                    | Yes                                                                                                                                                                                                                                                                                                                                                                                         |
| 2. Were the reference standard results interpreted without knowledge of the results of the index test?                | Unclear                                                                                                                                                                                                                                                                                                                                                                                                                                | Unclear                                                                                                                                                                                                                                                                                                                                                                                     |
| <b>Could the reference standard, its conduct, or its interpretation have introduced bias?</b>                         | <b>Unclear</b>                                                                                                                                                                                                                                                                                                                                                                                                                         | <b>Unclear</b>                                                                                                                                                                                                                                                                                                                                                                              |
| <b>Are there concerns that the reference standard, its conduct, or interpretation differ from the review question</b> | <b>Unclear, reference standard is not specified in the paper. However, Enders 2017b precise that testing after delivery were done with CMV DNA PCR and/(or) virus detection</b>                                                                                                                                                                                                                                                        | <b>Unclear</b>                                                                                                                                                                                                                                                                                                                                                                              |
| Flow and timing                                                                                                       |                                                                                                                                                                                                                                                                                                                                                                                                                                        |                                                                                                                                                                                                                                                                                                                                                                                             |
| 1. Was there an appropriate interval between index test(s) and reference standard?                                    | Yes                                                                                                                                                                                                                                                                                                                                                                                                                                    | Yes                                                                                                                                                                                                                                                                                                                                                                                         |
| 2. Did all participants receive the same reference standard?                                                          | Unclear, testing after delivery were done with CMV DNA PCR and/(or) virus detection. Our PICO aims to a reference standard with CMV DNA PCR.                                                                                                                                                                                                                                                                                           | Unclear, testing after delivery were done with CMV DNA PCR and/(or) virus detection. Our PICO aims to a reference standard with CMV DNA PCR.                                                                                                                                                                                                                                                |
| 3. Were all participants included in the analysis?                                                                    | High: Of the 531 primary infections, accuracy analyses is restricted to 338 with virological outcome available, in 28 cases time between seroconversion and amniocentesis could not be established. Only 264 patients with seroconversion occurring more than 8 weeks after amniocentesis were included in the analysis. Some of the patients received hyperimmune immunoglobulins if amniocentesis was positive (proportion unknown). | High, of the 132 pregnant patients included, prenatal diagnosis with amniocentesis was done in 128 and the analysis was performed in 105 pregnancies with known virological outcome and more than 17 weeks of gestation. In further 28 cases, time between seroconversion and amniocentesis could not be established. 49 patients received hyperimmune immunoglobulins after amniocentesis; |
| <b>Could the participant flow have introduced bias?</b>                                                               | <b>High</b>                                                                                                                                                                                                                                                                                                                                                                                                                            | <b>High</b>                                                                                                                                                                                                                                                                                                                                                                                 |



## Appendix 1.5. Evidence tables

**Enders 2017b: Enders M, Daiminger A, Exler S, Ertan K, Enders G, Bald R. Prenatal diagnosis of congenital cytomegalovirus infection in 115 cases: a 5 years' single center experience. Prenat Diagn. 2017 Apr;37(4):389-398.(38)**

|                                                 |                                                                                                                                                                                                                                                |
|-------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Design</b>                                   | Retrospective, single-center cohort (diagnostic accuracy study) of pregnancies with <i>primary</i> CMV infection undergoing invasive prenatal diagnosis                                                                                        |
| <b>Source of funding and competing interest</b> | Funding not reported; no conflict of interests reported.                                                                                                                                                                                       |
| <b>Setting</b>                                  | Single centre in Germany from 2010 to 2014                                                                                                                                                                                                     |
| <b>Sample size</b>                              | N=115, (of which 111 were tested with amniocentesis) with known virological outcome (from 132 who underwent invasive prenatal diagnosis)                                                                                                       |
| <b>Time interval between tests</b>              | Key analytic factor was interval from estimated maternal seroconversion to amniocentesis (AC): ≤8 weeks vs >8 weeks; gestational age strata ≥17+0 t and ≥20+0 weeks.                                                                           |
| <b>Statistical analysis</b>                     | diagnostic accuracy (sensitivity, specificity, PPV, NPV with 95% CI); Fisher's exact test and chi-square for group comparisons                                                                                                                 |
| <b>Patient characteristics</b>                  | Pregnant patients with primary CMV infection who underwent invasive prenatal diagnosis at the study center                                                                                                                                     |
| <b>Eligibility criteria</b>                     | Primary CMV infection during pregnancy                                                                                                                                                                                                         |
| <b>Exclusion criteria</b>                       | No invasive diagnosis performed, no virological outcome available                                                                                                                                                                              |
| <b>Patient &amp; disease characteristics</b>    | Prevalence of cCMV 39.6% (44/111), among the patients who underwent amniocentesis. The majority of patients received Hyperimmune Immunoglobulin therapy if prenatal diagnosis (amniocentesis or other prenatal diagnosis) was positive (n=49). |
| <b>Index test</b>                               | Amniocentesis test: CMV DNA PCR (in-house or RealStar®; LoD 125 IU/mL) and/ or rapid cell culture (shell vial).                                                                                                                                |
| <b>Target condition</b>                         | Congenital CMV infection at birth                                                                                                                                                                                                              |
| <b>Reference standard</b>                       | Confirmation by CMV DNA and/or virus detection in fetal or placental tissue at termination or neonatal urine within the first 2 weeks of life                                                                                                  |
| <b>Results</b>                                  |                                                                                                                                                                                                                                                |

### Diagnostic accuracy (sensitivity, specificity)

#### Amniocentesis PCR All Timings >=17GW

| Study        | TP | FP | FN | TN | Sensitivity (95% CI) | Specificity (95% CI) | Sensitivity (95% CI) | Specificity (95% CI) |
|--------------|----|----|----|----|----------------------|----------------------|----------------------|----------------------|
| Enders 2017b | 32 | 0  | 12 | 67 | 0.73 [0.57, 0.85]    | 1.00 [0.95, 1.00]    |                      |                      |

#### Amniocentesis PCR >=17GW (<=8weeks after seroconversion)

| Study        | TP | FP | FN | TN | Sensitivity (95% CI) | Specificity (95% CI) | Sensitivity (95% CI) | Specificity (95% CI) |
|--------------|----|----|----|----|----------------------|----------------------|----------------------|----------------------|
| Enders 2017b | 5  | 0  | 6  | 14 | 0.45 [0.17, 0.77]    | 1.00 [0.77, 1.00]    |                      |                      |

#### Amniocentesis PCR >=17GW (>8weeks after seroconversion)

| Study        | TP | FP | FN | TN | Sensitivity (95% CI) | Specificity (95% CI) | Sensitivity (95% CI) | Specificity (95% CI) |
|--------------|----|----|----|----|----------------------|----------------------|----------------------|----------------------|
| Enders 2017b | 20 | 0  | 2  | 30 | 0.91 [0.71, 0.99]    | 1.00 [0.88, 1.00]    |                      |                      |

#### Amniocentesis PCR >=20GW (<=8weeks after seroconversion)

| Study        | TP | FP | FN | TN | Sensitivity (95% CI) | Specificity (95% CI) | Sensitivity (95% CI) | Specificity (95% CI) |
|--------------|----|----|----|----|----------------------|----------------------|----------------------|----------------------|
| Enders 2017b | 3  | 0  | 6  | 10 | 0.33 [0.07, 0.70]    | 1.00 [0.69, 1.00]    |                      |                      |

#### Amniocentesis PCR >=20GW (>8weeks after seroconversion)

| Study        | TP | FP | FN | TN | Sensitivity (95% CI) | Specificity (95% CI) | Sensitivity (95% CI) | Specificity (95% CI) |
|--------------|----|----|----|----|----------------------|----------------------|----------------------|----------------------|
| Enders 2017b | 18 | 0  | 2  | 17 | 0.90 [0.68, 0.99]    | 1.00 [0.80, 1.00]    |                      |                      |

Sensitivity and negative predictive value are statistically significantly different if amniocentesis is done more than 8 weeks after seroconversion compared to at 8 weeks or less.

### Other

No results for any outcomes other than diagnostic accuracy, related to timing of amniocentesis

### Limitations and other comments

Single centre, retrospective, most patients received immunoglobulins if amniocentesis was positive (n=49)



**Enders 2017a :Enders M, Daiminger A, Exler S, Enders G. Amniocentesis for prenatal diagnosis of cytomegalovirus infection: challenging the 21 weeks' threshold. Prenat Diagn. 2017 Sep;37(9):940-942 (39)**

|                                                 |                                                                                                                                                                                                                                                                                                                                        |
|-------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Design</b>                                   | Retrospective single-center cohort (research letter) expanding on the analysis done by Enders2017b                                                                                                                                                                                                                                     |
| <b>Source of funding and competing interest</b> | Funding not reported; no conflict of interests reported.                                                                                                                                                                                                                                                                               |
| <b>Setting</b>                                  | Single centre in Germany from 2001 to 2016                                                                                                                                                                                                                                                                                             |
| <b>Sample size</b>                              | N=338 women of known virological outcome and tested with amniocentesis after CMV primary infection (264 with amniocentesis performed more than 8 weeks after seroconversion). From a total of 2706 patients who underwent amniocentesis, 531 with known primary infection.                                                             |
| <b>Time interval between tests</b>              | Interval from maternal seroconversion to amniocentesis categorized as $\leq 8$ weeks (n = 46), > 8 weeks (n = 264), indeterminate (n = 28); interval inferred from symptoms, serial IgG/IgM titers, avidity assays, and prior serostatus. Comparison of amniocentesis performed between 17 and 20.6 weeks or between 21 and 22+6 weeks |
| <b>Statistical analysis</b>                     | diagnostic accuracy (sensitivity, specificity, PPV, NPV with 95% CI); Fisher's exact test and chi-square for group comparisons                                                                                                                                                                                                         |
| <b>Patient characteristics</b>                  | Pregnant patients with primary CMV infection who underwent amniocentesis at the study center                                                                                                                                                                                                                                           |
| <b>Eligibility criteria</b>                     | Primary CMV infection during pregnancy                                                                                                                                                                                                                                                                                                 |
| <b>Exclusion criteria</b>                       | No invasive diagnosis performed, no virological outcome available, multiple pregnancies                                                                                                                                                                                                                                                |
| <b>Patient &amp; disease characteristics</b>    | Prevalence of cCMV 32,1%% (82/264), among the patients who underwent amniocentesis. Some patients received Hyperimmune Immunoglobulin therapy if prenatal diagnosis (amniocentesis or other prenatal diagnosis) was positive (n unknown).                                                                                              |
| <b>Index test</b>                               | Amniocentesis test: CMV DNA PCR (in-house or RealStar®; LoD 125 IU/mL) and/ or rapid cell culture (shell vial). For other prenatal diagnosis testing: fetal blood tests: CMV DNA PCR, CMV-specific IgM (ELISA), plus non-virological markers (platelets, beta-2 microglobulin, GGT, AST). Ultrasound performed concurrently.           |
| <b>Target condition</b>                         | Congenital CMV in the newborn                                                                                                                                                                                                                                                                                                          |
| <b>Reference standard</b>                       | Confirmation by CMV DNA and/or virus detection in fetal or placental tissue at termination or neonatal urine within the first 2 weeks of life                                                                                                                                                                                          |
| <b>Results</b>                                  |                                                                                                                                                                                                                                                                                                                                        |

### Diagnostic accuracy (sensitivity, specificity)

#### Amniocentesis PCR $\geq 17+0$ to $20+6$ GW ( $>8$ weeks after seroconversion)

| Study        | TP | FP | FN | TN | Sensitivity (95% CI) | Specificity (95% CI) | Sensitivity (95% CI) | Specificity (95% CI) |
|--------------|----|----|----|----|----------------------|----------------------|----------------------|----------------------|
| Enders 2017a | 41 | 0  | 6  | 88 | 0.87 [0.74, 0.95]    | 1.00 [0.96, 1.00]    |                      |                      |

#### Amniocentesis PCR $\geq 21+0$ to $22+6$ GW ( $>8$ weeks after seroconversion)

| Study        | TP | FP | FN | TN | Sensitivity (95% CI) | Specificity (95% CI) | Sensitivity (95% CI) | Specificity (95% CI) |
|--------------|----|----|----|----|----------------------|----------------------|----------------------|----------------------|
| Enders 2017a | 35 | 0  | 3  | 91 | 0.92 [0.79, 0.98]    | 1.00 [0.96, 1.00]    |                      |                      |

#### Amniocentesis PCR at 17 GW ( $>8$ weeks after seroconversion)

| Study        | TP | FP | FN | TN | Sensitivity (95% CI) | Specificity (95% CI) | Sensitivity (95% CI) | Specificity (95% CI) |
|--------------|----|----|----|----|----------------------|----------------------|----------------------|----------------------|
| Enders 2017a | 2  | 0  | 2  | 14 | 0.50 [0.07, 0.93]    | 1.00 [0.77, 1.00]    |                      |                      |

#### Amniocentesis PCR at 18 GW ( $>8$ weeks after seroconversion)

| Study        | TP | FP | FN | TN | Sensitivity (95% CI) | Specificity (95% CI) | Sensitivity (95% CI) | Specificity (95% CI) |
|--------------|----|----|----|----|----------------------|----------------------|----------------------|----------------------|
| Enders 2017a | 6  | 0  | 1  | 20 | 0.86 [0.42, 1.00]    | 1.00 [0.83, 1.00]    |                      |                      |

#### Amniocentesis PCR at 19 GW ( $>8$ weeks after seroconversion)

| Study        | TP | FP | FN | TN | Sensitivity (95% CI) | Specificity (95% CI) | Sensitivity (95% CI) | Specificity (95% CI) |
|--------------|----|----|----|----|----------------------|----------------------|----------------------|----------------------|
| Enders 2017a | 8  | 0  | 0  | 16 | 1.00 [0.63, 1.00]    | 1.00 [0.79, 1.00]    |                      |                      |

#### Amniocentesis PCR at 20 GW ( $>8$ weeks after seroconversion)

| Study        | TP | FP | FN | TN | Sensitivity (95% CI) | Specificity (95% CI) | Sensitivity (95% CI) | Specificity (95% CI) |
|--------------|----|----|----|----|----------------------|----------------------|----------------------|----------------------|
| Enders 2017a | 25 | 0  | 3  | 38 | 0.89 [0.72, 0.98]    | 1.00 [0.91, 1.00]    |                      |                      |

#### Amniocentesis PCR at 21 GW ( $>8$ weeks after seroconversion)

| Study        | TP | FP | FN | TN | Sensitivity (95% CI) | Specificity (95% CI) | Sensitivity (95% CI) | Specificity (95% CI) |
|--------------|----|----|----|----|----------------------|----------------------|----------------------|----------------------|
| Enders 2017a | 23 | 0  | 2  | 68 | 0.92 [0.74, 0.99]    | 1.00 [0.95, 1.00]    |                      |                      |

#### Amniocentesis PCR at 22 GW ( $>8$ weeks after seroconversion)

| Study        | TP | FP | FN | TN | Sensitivity (95% CI) | Specificity (95% CI) | Sensitivity (95% CI) | Specificity (95% CI) |
|--------------|----|----|----|----|----------------------|----------------------|----------------------|----------------------|
| Enders 2017a | 12 | 0  | 1  | 23 | 0.92 [0.64, 1.00]    | 1.00 [0.85, 1.00]    |                      |                      |

No statistically significant difference if amniocentesis is performed between 17 and 20.6 weeks or between 21 and 22+6 weeks

### Other

No results for any outcomes other than diagnostic accuracy, related to timing of amniocentesis

### Limitations and other comments

Single centre, retrospective, some patients received immunoglobulins if amniocentesis was positive



## Appendix 1.6. Summary tables for GRADE

**Table 27 – Summary of findings for different timings of amniocentesis: Numbers in a cohort of 1000 primary CMV infections in pregnancy**

| Test                                                                          | No. of studies <sup>a</sup><br>(No. of patients) | Median prevalence % | Sensitivity (95% CI)*     | Specificity (95% CI)* | Predictive value of positive test (95% CI)* | Predictive value of negative test (95% CI)* | True positive | False negative | False positive | True negative | GRADE LoE |
|-------------------------------------------------------------------------------|--------------------------------------------------|---------------------|---------------------------|-----------------------|---------------------------------------------|---------------------------------------------|---------------|----------------|----------------|---------------|-----------|
| <i>Amniocentesis at &gt;= to 17 GW and &lt;= 8 weeks after seroconversion</i> | 1(25)                                            | 39.6                | 45.45<br>(16.75 to 76.62) | 100<br>(76.84 to 100) | 100<br>(47.82 to 100)                       | 73.66<br>(61.98 to 82.75)                   | 5             | 6              | 0              | 14            | Very Low  |
| <i>Amniocentesis at &gt;= to 17 GW and &gt; 8 weeks after seroconversion</i>  | 1(52)                                            | 39.6                | 90.91<br>(70.84 to 98.88) | 100<br>(88.43 to 100) | 100<br>(83.16 to 100)                       | 94.38%<br>(81.74 to 98.43)                  | 20            | 2              | 0              | 30            | Very low  |
| <i>Amniocentesis at 17 weeks and &gt; 8 weeks after seroconversion</i>        | 1(18)                                            | 32                  | 50<br>(6.76 to 93.24)     | 100<br>(76.84 to 100) | 100<br>(15.81 to 100)                       | 80.95 (61.47 to 91.89)                      | 2             | 2              | 0              | 14            | Very low  |
| <i>Amniocentesis at 18 weeks &gt; 8 weeks after seroconversion</i>            | 1(27)                                            | 32                  | 85.71<br>(42.13 to 99.64) | 100<br>(83.16 to 100) | 100<br>(54.07 to 100)                       | 93.7<br>(70.79 to 98.92)                    | 6             | 1              | 0              | 20            | Very low  |
| <i>Amniocentesis at 19 weeks &gt; 8 weeks after seroconversion</i>            | 1(24)                                            | 32                  | 100<br>(63.06 to 100)     | 100<br>(79.41 to 100) | 100<br>(63.06 to 100)                       | 100<br>(79.41 to 100)                       | 8             | 0              | 0              | 16            | Very low  |
| <i>Amniocentesis at 20 weeks &gt; 8 weeks after seroconversion</i>            | 1(66)                                            | 32                  | 89.29<br>(71.77 to 97.73) | 100<br>(90.75 to 100) | 100 (86.28 to 100)                          | 95.2<br>(87.19 to 98.3)                     | 25            | 3              | 0              | 38            | Low       |
| <i>Amniocentesis at 21 weeks &gt; 8 weeks after seroconversion</i>            | 1(93)                                            | 32                  | 92<br>(73.97 to 99.02)    | 100<br>(94.72 to 100) | 100<br>(85.18 to 100)                       | 96.37<br>(87.55 to 99.01)                   | 23            | 2              | 0              | 68            | Low       |
| <i>Amniocentesis at 22 weeks &gt; 8 weeks after seroconversion</i>            | 1(36)                                            | 32                  | 92.31<br>(63.97 to 99.81) | 100<br>(85.18 to 100) | 100<br>(73.54 to 100)                       | 96.51<br>(80.78 to 99.45)                   | 12            | 1              | 0              | 23            | Very low  |

\*[https://www.medcalc.org/calc/diagnostic\\_test.php](https://www.medcalc.org/calc/diagnostic_test.php); <sup>a</sup> There is only one study available, Enders 2017a, of which Enders 2017b is a subset that allows to perform analysis according to time interval between the moment of seroconversion and the moment of amniocentesis

**Table 28 – GRADE table accuracy for amniocentesis performed ≤ 8 weeks after seroconversion vs amniocentesis performed >8 weeks after seroconversion**

| Outcome                                                                       | № of studies (№ of patients) | Study design                                 | Factors that may decrease certainty of evidence |              |                          |                           |                  | Effect per 1,000 patients tested                                 |                                                 | Test accuracy CoE                 |
|-------------------------------------------------------------------------------|------------------------------|----------------------------------------------|-------------------------------------------------|--------------|--------------------------|---------------------------|------------------|------------------------------------------------------------------|-------------------------------------------------|-----------------------------------|
|                                                                               |                              |                                              |                                                 |              |                          |                           |                  | pre-test probability of 39.6%                                    |                                                 |                                   |
|                                                                               |                              |                                              | Risk of bias                                    | Indirectness | Inconsistency            | Imprecision               | Publication bias | Amniocentesis at <= 8 weeks after seroconversion                 | Amniocentesis at > 8 weeks after seroconversion |                                   |
| True positives (patients with cCMV at birth)                                  | 1 studies<br>77 patients     | cross-sectional (cohort type accuracy study) | serious <sup>a</sup>                            | not serious  | not serious <sup>b</sup> | very serious <sup>c</sup> | none             | 178 (63 to 301)                                                  | 356 (277 to 388)                                | ⊕○○○<br>Very low <sup>a,b,c</sup> |
| False negatives (patients incorrectly classified as not having cCMV at birth) |                              |                                              |                                                 |              |                          |                           |                  | 178 fewer TP in Amniocentesis at <= 8 weeks after seroconversion |                                                 |                                   |
|                                                                               |                              |                                              |                                                 |              |                          |                           |                  | 218 (95 to 333)                                                  | 40 (8 to 119)                                   |                                   |
|                                                                               |                              |                                              |                                                 |              |                          |                           |                  | 178 more FN in Amniocentesis at <= 8 weeks after seroconversion  |                                                 |                                   |
| True negatives (patients without cCMV at birth)                               | 1 studies<br>77 patients     | cross-sectional (cohort type accuracy study) | serious <sup>a</sup>                            | not serious  | not serious <sup>b</sup> | very serious <sup>c</sup> | none             | 604 (459 to 604)                                                 | 604 (532 to 604)                                | ⊕○○○<br>Very low <sup>a,b,c</sup> |
| False positives (patients incorrectly classified as having cCMV at birth)     |                              |                                              |                                                 |              |                          |                           |                  | 0 fewer TN in Amniocentesis at <= 8 weeks after seroconversion   |                                                 |                                   |
|                                                                               |                              |                                              |                                                 |              |                          |                           |                  | 0 (0 to 145)                                                     | 0 (0 to 72)                                     |                                   |
|                                                                               |                              |                                              |                                                 |              |                          |                           |                  | 0 fewer FP in Amniocentesis at <= 8 weeks after seroconversion   |                                                 |                                   |

a. Patients were retrospectively selected from patients' records. PD was performed either by PCR or viral culture. of the 132 pregnant patients included, only 111 could be included for analysis

b. Not applicable, only one study

c. Only 25 patients included that have less or equal to 8 weeks after seroconversion



Table 29 – GRADE table Amniocentesis at 17 weeks and &gt; 8 weeks after seroconversion

|                                                                                      | No of studies<br>(No of patients) | Study design                                 | Factors that may decrease certainty of evidence |              |                            |                                |                  | Effect per 1,000 patients tested | Test accuracy CoE               |
|--------------------------------------------------------------------------------------|-----------------------------------|----------------------------------------------|-------------------------------------------------|--------------|----------------------------|--------------------------------|------------------|----------------------------------|---------------------------------|
|                                                                                      |                                   |                                              | Risk of bias                                    | Indirectness | Inconsistency <sup>b</sup> | Imprecision                    | Publication bias | pre-test probability of 32%      |                                 |
| <b>True positives (patients with cCMV at birth)</b>                                  | 1 studies<br>18 patients          | cross-sectional (cohort type accuracy study) | serious <sup>a</sup>                            | not serious  | not serious                | extremely serious <sup>b</sup> | none             | 160 (22 to 298)                  | ⊕○○○<br>Very low <sup>a,b</sup> |
| <b>False negatives (patients incorrectly classified as not having cCMV at birth)</b> |                                   |                                              |                                                 |              |                            |                                |                  | 160 (22 to 298)                  |                                 |
| <b>True negatives (patients without cCMV at birth)</b>                               | 1 studies<br>18 patients          | cross-sectional (cohort type accuracy study) | serious <sup>a</sup>                            | not serious  | not serious                | extremely serious <sup>b</sup> | none             | 680 (524 to 680)                 | ⊕○○○<br>Very low <sup>a,b</sup> |
| <b>False positives (patients incorrectly classified as having cCMV at birth)</b>     |                                   |                                              |                                                 |              |                            |                                |                  | 0 (0 to 156)                     |                                 |

a. Patients were retrospectively selected from patients records, PD was performed either by PCR or viral culture. Of the total sample of 2706 amniocentesis performed, 531 were confirmed primary infections, but only in 338 cases adequate specimen was available for establishing diagnosis of cCMV

b. There is only one study including only 18 patients for this analysis

Table 30 – GRADE table Amniocentesis at 18 weeks and &gt; 8 weeks after seroconversion

|                                                                                      | Nº of studies<br>(Nº of patients) | Study design                                 | Factors that may decrease certainty of evidence |              |               |                                |                  | Effect per 1,000 patients tested | Test accuracy CoE               |
|--------------------------------------------------------------------------------------|-----------------------------------|----------------------------------------------|-------------------------------------------------|--------------|---------------|--------------------------------|------------------|----------------------------------|---------------------------------|
|                                                                                      |                                   |                                              | Risk of bias                                    | Indirectness | Inconsistency | Imprecision                    | Publication bias | pre-test probability of 32%      |                                 |
| <b>True positives (patients with cCMV at birth)</b>                                  | 1 studies<br>27 patients          | cross-sectional (cohort type accuracy study) | serious <sup>a</sup>                            | not serious  | not serious   | extremely serious <sup>b</sup> | none             | 275 (134 to 320)                 | ⊕○○○<br>Very low <sup>a,b</sup> |
| <b>False negatives (patients incorrectly classified as not having cCMV at birth)</b> |                                   |                                              |                                                 |              |               |                                |                  | 45 (0 to 186)                    |                                 |
| <b>True negatives (patients without cCMV at birth)</b>                               | 1 studies<br>27 patients          | cross-sectional (cohort type accuracy study) | serious <sup>a</sup>                            | not serious  | not serious   | extremely serious <sup>b</sup> | none             | 680 (564 to 680)                 | ⊕○○○<br>Very low <sup>a,b</sup> |
| <b>False positives (patients incorrectly classified as having cCMV at birth)</b>     |                                   |                                              |                                                 |              |               |                                |                  | 0 (0 to 116)                     |                                 |

a. Patients were retrospectively selected from patients records, PD was performed either by PCR or viral culture. Of the total sample of 2706 amniocentesis performed, 531 were confirmed primary infections, but only in 338 cases adequate specimen was available for establishing diagnosis of cCMV.

b. There is only one study including only 27 patients for this analysis



Table 31 – GRADE table Amniocentesis at 19 weeks and &gt; 8 weeks after seroconversion

| Outcome                                                                              | № of studies (№ of patients) | Study design                                 | Factors that may decrease certainty of evidence |              |               |                                |                  | Effect per 1,000 patients tested | Test accuracy CoE               |
|--------------------------------------------------------------------------------------|------------------------------|----------------------------------------------|-------------------------------------------------|--------------|---------------|--------------------------------|------------------|----------------------------------|---------------------------------|
|                                                                                      |                              |                                              | Risk of bias                                    | Indirectness | Inconsistency | Imprecision                    | Publication bias | pre-test probability of 32%      |                                 |
| <b>True positives (patients with cCMV at birth)</b>                                  | 1 studies<br>24 patients     | cross-sectional (cohort type accuracy study) | serious <sup>a</sup>                            | not serious  | not serious   | extremely serious <sup>b</sup> | none             | 320 (202 to 320)                 | ⊕○○○<br>Very low <sup>a,b</sup> |
| <b>False negatives (patients incorrectly classified as not having cCMV at birth)</b> |                              |                                              |                                                 |              |               |                                |                  | 0 (0 to 118)                     |                                 |
| <b>True negatives (patients without cCMV at birth)</b>                               | 1 studies<br>24 patients     | cross-sectional (cohort type accuracy study) | serious <sup>a</sup>                            | not serious  | not serious   | extremely serious <sup>b</sup> | none             | 680 (537 to 680)                 | ⊕○○○<br>Very low <sup>a,b</sup> |
| <b>False positives (patients incorrectly classified as having cCMV at birth)</b>     |                              |                                              |                                                 |              |               |                                |                  | 0 (0 to 143)                     |                                 |

a. Patients were retrospectively selected from patients records, PD was performed either by PCR or viral culture. Of the total sample of 2706 amniocentesis performed, 531 were confirmed primary infections, but only in 338 cases adequate specimen was available for establishing diagnosis of cCMV

b. There is only one study including only 24 patients for this analysis

Table 32 – GRADE table Amniocentesis at 20 weeks and &gt; 8 weeks after seroconversion

| Outcome                                                                              | № of studies<br>(№ of patients) | Study design                                 | Factors that may decrease certainty of evidence |              |               |                      |                  | Effect per 1,000 patients tested | Test accuracy CoE          |
|--------------------------------------------------------------------------------------|---------------------------------|----------------------------------------------|-------------------------------------------------|--------------|---------------|----------------------|------------------|----------------------------------|----------------------------|
|                                                                                      |                                 |                                              | Risk of bias                                    | Indirectness | Inconsistency | Imprecision          | Publication bias | pre-test probability of 32%      |                            |
| <b>True positives (patients with cCMV at birth)</b>                                  | 1 studies<br>66 patients        | cross-sectional (cohort type accuracy study) | serious <sup>a</sup>                            | not serious  | not serious   | serious <sup>b</sup> | none             | 285 (230 to 314)                 | ⊕⊕○○<br>Low <sup>a,b</sup> |
| <b>False negatives (patients incorrectly classified as not having cCMV at birth)</b> |                                 |                                              |                                                 |              |               |                      |                  | 35 (6 to 90)                     |                            |
| <b>True negatives (patients without cCMV at birth)</b>                               | 1 studies<br>66 patients        | cross-sectional (cohort type accuracy study) | serious <sup>a</sup>                            | not serious  | not serious   | serious <sup>b</sup> | none             | 680 (619 to 680)                 | ⊕⊕○○<br>Low <sup>a,b</sup> |
| <b>False positives (patients incorrectly classified as having cCMV at birth)</b>     |                                 |                                              |                                                 |              |               |                      |                  | 0 (0 to 61)                      |                            |

a. Patients were retrospectively selected from patients records, PD was performed either by PCR or viral culture. Of the total sample of 2706 amniocentesis performed, 531 were confirmed primary infections, but only in 338 cases adequate specimen was available for establishing diagnosis of cCMV

b. There is only one study including 66 patients for this analysis



Table 33 – GRADE table Amniocentesis at 21 weeks and &gt; 8 weeks after seroconversion

| Outcome                                                                              | Nº of studies<br>(Nº of patients) | Study design                                 | Factors that may decrease certainty of evidence |              |               |                      |                  | Effect per 1,000 patients tested | Test accuracy CoE          |
|--------------------------------------------------------------------------------------|-----------------------------------|----------------------------------------------|-------------------------------------------------|--------------|---------------|----------------------|------------------|----------------------------------|----------------------------|
|                                                                                      |                                   |                                              | Risk of bias                                    | Indirectness | Inconsistency | Imprecision          | Publication bias | pre-test probability of 32%      |                            |
| <b>True positives (patients with cCMV at birth)</b>                                  | 1 studies<br>93 patients          | cross-sectional (cohort type accuracy study) | serious <sup>a</sup>                            | not serious  | not serious   | serious <sup>b</sup> | none             | 294 (237 to 317)                 | ⊕⊕○○<br>Low <sup>a,b</sup> |
| <b>False negatives (patients incorrectly classified as not having cCMV at birth)</b> |                                   |                                              |                                                 |              |               |                      |                  | 26 (3 to 83)                     |                            |
| <b>True negatives (patients without cCMV at birth)</b>                               | 1 studies<br>93 patients          | cross-sectional (cohort type accuracy study) | serious <sup>a</sup>                            | not serious  | not serious   | serious <sup>b</sup> | none             | 680 (646 to 680)                 | ⊕⊕○○<br>Low <sup>a,b</sup> |
| <b>False positives (patients incorrectly classified as having cCMV at birth)</b>     |                                   |                                              |                                                 |              |               |                      |                  | 0 (0 to 34)                      |                            |

a. Patients were retrospectively selected from patients records, PD was performed either by PCR or viral culture. Of the total sample of 2706 amniocentesis performed, 531 were confirmed primary infections, but only in 338 cases adequate specimen was available for establishing diagnosis of cCMV

b. There is only one study including 93 patients for this analysis

Table 34 – GRADE table Amniocentesis at 22 weeks and &gt; 8 weeks after seroconversion

| Outcome                                                                              | No of studies<br>(No of patients) | Study design                                 | Factors that may decrease certainty of evidence |              |               |                           |                  | Effect per 1,000 patients tested | Test accuracy CoE               |
|--------------------------------------------------------------------------------------|-----------------------------------|----------------------------------------------|-------------------------------------------------|--------------|---------------|---------------------------|------------------|----------------------------------|---------------------------------|
|                                                                                      |                                   |                                              | Risk of bias                                    | Indirectness | Inconsistency | Imprecision               | Publication bias | pre-test probability of 32%      |                                 |
| <b>True positives (patients with cCMV at birth)</b>                                  | 1 studies<br>36 patients          | cross-sectional (cohort type accuracy study) | serious <sup>a</sup>                            | not serious  | not serious   | very serious <sup>b</sup> | none             | 294 (205 to 320)                 | ⊕○○○<br>Very low <sup>a,b</sup> |
| <b>False negatives (patients incorrectly classified as not having cCMV at birth)</b> |                                   |                                              |                                                 |              |               |                           |                  | 26 (0 to 115)                    |                                 |
| <b>True negatives (patients without cCMV at birth)</b>                               | 1 studies<br>36 patients          | cross-sectional (cohort type accuracy study) | serious <sup>a</sup>                            | not serious  | not serious   | very serious <sup>b</sup> | none             | 680 (578 to 680)                 | ⊕○○○<br>Very low <sup>a,b</sup> |
| <b>False positives (patients incorrectly classified as having cCMV at birth)</b>     |                                   |                                              |                                                 |              |               |                           |                  | 0 (0 to 102)                     |                                 |

a. Patients were retrospectively selected from patients records, PD was performed either by PCR or viral culture. Of the total sample of 2706 amniocentesis performed, 531 were confirmed primary infections, but only in 338 cases adequate specimen was available for establishing diagnosis of cCMV

b. There is only one study including only 36 patients for this analysis



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