UK National Screening Committee

Newborn screening for cytomegalovirus

External review against programme appraisal criteria for the UK National Screening Committee (UK NSC)

Version: Consultation document

Bazian Ltd.

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The UK NSC advises Ministers and the NHS in all four UK countries about all aspects of screening policy. Its policies are reviewed on a 3 yearly cycle. Current policies and the policy review process can be found at https://www.gov.uk/guidance/evidence-and-recommendations-nhs-population-screening#evidence-review-process

Abbreviations List

ABR	Auditory brainstem response
AUC	Area under the curve
CI	Confidence interval
(C)CMV	(Congenital) Cytomegalovirus
CNS	Central nervous system
CSF	Cerebrospinal fluid
CT	Computed tomography
DBS	Dried blood spot
FN	False negative
FP	False positive
IUGR	Intrauterine growth restriction
LOD	Limit of Detection
LR	Likelihood ratio
LSV	Lenticulostriated vasculopathy
MRI	Magnetic resonance imaging
NICU	Neonatal intensive care unit
NPV	Negative predictive value
OR	Odd ratio
PCR	Polymerase chain reaction
PPV	Positive predictive value
RCT	Randomised controlled trial
Sn	Sensitivity
SNHL	Sensorineural hearing loss
Sp	Specificity
SR	Systematic review
TN	True negative
TP	True positive

Plain English Summary

Cytomegalovirus is a common viral infection in children and adults but very rare in newborn babies. Around half of the UK population will be infected with it at some point. Cytomegalovirus infection spreads by contact of body fluids such as saliva, blood, urine and human milk. In a healthy person it causes no or few symptoms, and most people will recover without treatment. There is a risk of complications, however, if a woman has the infection during pregnancy and passes this onto the developing baby. This is called congenital cytomegalovirus infection. Research carried out in the 1970s suggested that around three in every thousand babies is born with congenital cytomegalovirus. That is around 2,400 babies each year in the UK

About 10 to 15% of newborns born with congenital cytomegalovirus infection (240 to 360 per year) have symptoms at birth. This includes problems with the brain or other organs. An additional 10 to 15% are well at birth, but go onto develop long-term hearing or developmental problems.

In 2011 the UK National Screening Committee (UK NSC) recommended against screening for cytomegalovirus.

The current review looks at research published since then to see if such decision is still valid.

The review confirmed that there is still:

- no reliable test to detect cytomegalovirus infection during pregnancy,
- no treatment that could prevent the developing baby in the womb getting cytomegalovirus infection from their mother,
- no reliable way of knowing which babies are going to develop long-term health problems from cytomegalovirus infection, and
- no treatment that could prevent babies from developing health problems from cytomegalovirus infection.

The review also found that:

1. One option for newborn screening would be to test a saliva sample. This approach looks promising but research is needed to understand more about it.
2. Many babies will have congenital cytomegalovirus infection, but there is still no way of knowing which will on to develop long-term problems and need medical treatment. This review found some research looking at how to identify babies at highest risk. This included looking at newborn blood test results or scanning the baby's brain. But this would also need more research to ensure that these approaches were reliable.
3. Finally, the best treatment for congenital cytomegalovirus infection is still not clear. Current treatment involves giving the newborn intravenous drugs (through a needle into a vein). This can have adverse effects and is only used in the most serious cases. Some research was found which looked at the effectiveness of an oral drug (given by mouth) to prevent hearing loss. The research was not clear if this was better than the intravenous drug that is in use.

The findings of this review indicate that, for now, the UK NSC should keep the recommendation of not screening newborns for congenital cytomegalovirus.

Executive Summary

Aim of the review

This review assesses whether newborn screening for congenital cytomegalovirus (CCMV) should be offered. The review considers literature published between January 2011 and February 2016, the date of the last UK NSC review.

Background

Cytomegalovirus (CMV) is a common viral infection of the herpes genus with estimated seroprevalence rates of around 50%* in the adult UK population. Though it causes no, or minimal, symptoms in healthy adults it can have serious consequences for foetuses/newborns exposed to infection during pregnancy. Research carried out in the 1970s suggested that around three in every thousand babies is born with congenital CMV. That is around 2,400 babies each year in the UK .An estimated 10-15% of newborns with CCMV (240 to 360 per year) have symptoms at birth including neurological and focal organ involvement (in particular spleen, liver, lungs, lymph nodes and central nervous system). Another 10-15% of asymptomatic newborns will develop late sequelae, mostly sensorineural hearing loss (SNHL). CCMV has been estimated to account for around one in five cases of SNHL.

To minimise the impact of CCMV several strategies have been proposed by experts in the field.[1] [2-6]These strategies include: earlier identification through maternal screening, vaccination, preventative and behavioural interventions, treatment for infected pregnant women, and screening and treatment of affected newborns.

Previous/ Current UK NSC Review

The UK NSC currently recommend against antenatal or newborn screening for CMV. This recommendation dates from the previous external review in 2011, which highlighted several uncertainties.

Antenatal screening

The uncertainties relating to antenatal screening included:

- a lack of clarity on the risks to the fetus associated with primary and non-primary infection
- screening and diagnostic strategies – there is not a test or combination of tests that can predict outcome in the infant
- no interventions to prevent mother-to-child transmission or minimise the severity of infection

Antenatal screening is only discussed briefly as part of the introduction to this review. This is because very little information on these issues was found in the literature search and the focus of attention relating to screening, has shifted to the newborn period.

* This prevalence data is based on data from 1980s and 1990s and therefore may not account for recent demographic changes that may affect the underlying infection rates

Newborn screening

The 2011 UK NSC review concluded that both newborn dried blood spots (DBS) and saliva swabs had potential for CCMV screening. DBS would be the obvious choice as the sample is routinely collected to screen for other conditions. However, this approach had not been demonstrated to be sensitive enough for use in a large-scale newborn screening programme.

Only one treatment was available, six weeks of intravenous ganciclovir, which should only be use in symptomatic newborns with neurological manifestations. A trial was underway investigating an oral formulation, valganciclovir, as an alternative. No treatment was available for asymptomatic infants or those with transient or non-specific symptoms.

The previous review also highlighted a need to better define risk in newborns and identify specific diagnostic signs or markers that could predict which newborns were likely to develop long-term sequelae.

The current review considers whether the volume and direction of the evidence produced since the 2011 review addresses these questions and indicates that the previous recommendation not to screen newborns for CCMV should be reconsidered.

Findings of current review

a. The main candidate for a newborn screening test appears to be polymerase chain reaction (PCR) evaluation of saliva samples.

   This approach was assessed by two cohort studies: One large US cohort including 73,239 newborns (reported by two publications), and an Irish cohort of 1044 newborns. Both of these studies assessed PCR assay of a one-off liquid or dried saliva sample. However, there were concerns about verification bias in both of these studies, as they did not perform confirmatory testing (saliva and urine re-testing) of the full study sample, only screen positives. Therefore test performance cannot be known with accuracy. They also have the limitation that they do not consider the test in the context of a diagnostic pathway and its ability to change the management of newborns found to have CCMV.

b. There is still a lack of clarity about how to identify newborns that will develop long-term sequelae, and therefore may benefit from medical intervention.

   One UK guideline recommends treating newborns with central nervous system (CNS) involvement or severe focal organ involvement. However, these recommendations are drawn from the single RCT of intravenous gancilovir combined with expert opinion. The guideline authors acknowledged the need for large studies of predictive markers.

   Three small cohort studies assessed the potential of specific CNS signs or viral load to predict the likelihood of long-term sequelae. However, symptomatic definitions varied widely across these studies and it is difficult to know how relevant or applicable these potential predictive markers may be to a population of newborns with CCMV identified through universal screening.

c. The treatment to be offered to babies with screen detected CCMV remains unclear.

One RCT assessed valganciclovir as oral alternative to ganciclovir in symptomatic newborns, comparing six weeks with six months of treatment. The trial found that six months treatment with valganciclovir had a weak, but not statistically significant effect on the primary outcome (best-ear hearing at six months) compared to six weeks treatment. There was some evidence that valganciclovir had a moderate, statistically significant positive effect on longer-term hearing (total-ear hearing) and neurodevelopmental outcomes at 12 to 24 months. There were some concerns in relation to the side effects of the drug; in particular severe neutropenia. The study was small, and its relevance to a screen-detected population was uncertain.

In addition no evidence was identified to establish the safety or effectiveness of oral valganciclovir compared to intravenous ganciclovir in terms of severity of hearing impairment or other complications at birth. No studies have assessed treatment for asymptomatic newborns.

No studies were available to inform whether long-term outcomes, such as hearing, differ in screened vs. non-screened populations.

Screening recommendations based on the current review

The findings of the this review indicate that the current recommendation not to perform universal newborn screening for CCMV should be maintained.

The issues identified in this review are unlikely to be resolved without further research aiming to:

- detect markers that can predict the severity of the condition in a screened detected population; and
- identify an effective intervention that could be beneficial to a screen detected population

Introduction

Cytomegalovirus (CMV) is a common viral infection of the herpes genus (called also human herpes virus 5, HHV-5). Previous estimates suggest that seropositivity rates among the general adult population in the UK are around 50%,[†] CMV infection usually causes no or minimal flu-like symptoms in healthy adults, but it can have serious consequences for immunocompromised individuals, and newborns exposed to infection during pregnancy or, in the case of preterm infants, during the postnatal period.

The 2011 UK NSC review on screening for CMV[7] during the antenatal and/or postnatal period considered CCMV an important health issue. It was reported that the birth prevalence of CCMV (CCMV) in Europe was around three to five per 1000, and in the UK was estimated to be three per 1000 in the late 1970s. This may have changed as a result of shifts in population characteristics over the last few decades, but there were no more recent estimates.

The review stated that 10-15% of neonates with CCMV present with symptoms, including petechiae, hepatomegaly, splenomegaly, hepatitis, and/or neurological signs such as microcephaly, chorioretinitis and intracranial calcification. About half of these children develop permanent sequelae with adverse outcomes higher in those with neurological presentations at birth. A further 10-15% of those who are initially asymptomatic develop neurological manifestations, mainly sensorineural hearing loss (SNHL), which may be bilateral, moderate or severe, or unilateral. Congenital CMV was estimated to account for 15-20% of moderate to profound, permanent bilateral hearing loss, but the true burden of the disease within the UK was unknown.

To minimise the impact of CCMV several approaches have been proposed by experts in the field. [1] [2-6] These approaches include: earlier identification of infection through antenatal screening, vaccination of pregnant women, preventative and behavioural interventions in pregnancy, treatment for newly infected pregnant women, and screening and treatment of affected newborns.

The 2011 UK NSC review[7] considered universal screening in the antenatal and/or newborn periods. The conclusion was that:

a. In pregnancy, there were uncertainties regarding natural history. Primary or new infection in the mother was thought to carry highest risk of transmission to the fetus, but there were uncertainties over the contribution of non-primary CMV infection (i.e. reactivation or reinfection in seropositive women) to the disease burden. There was a need to better understand how to refine the risk of adverse outcomes in the fetus/newborn depending on the type of maternal infection. Antenatal screening tests lacked sufficient sensitivity and no interventions had been shown to be effective in preventing acquisition of maternal infection or reducing the risk of transmission to the

[†] This prevalence data is based on data from 1980s and 1990s and therefore may not account for recent demographic changes that may affect the underlying infection rates

fetus. Although progress has been reported towards the development of a vaccine to prevent maternal primary CMV infection, more research was needed to evaluate this.

b. In the newborn period, the tests available had not been shown to be sufficiently reliable for screening, in particular newborn dried blood spot (DBS), which would be the obvious platform for CMV screening as this is routinely taken to screen for other conditions. There was also no clear evidence of benefit from the available intravenous or oral antiviral therapies.

Due to these uncertainties the UK National Screening Committee concluded that screening for CMV in pregnancy or in the newborn period was not recommended.

Antenatal screening for cytomegalovirus

Cytomegalovirus infection during pregnancy

Congenital infection in the newborn can be acquired through transplacental transmission from mothers with primary or non-primary CMV. Primary CMV occurs in women who are seronegative at the time of conception and then acquire CMV infection during pregnancy. Non-primary infection occurs when a woman who is already seropositive either has reactivation of the dormant virus during pregnancy or is infected by a different viral strain. The risk of transmission to the fetus is thought to be greater with primary than with non-primary maternal infection.[8, 9]

Placental transmission has been estimated to occur in around one third of cases of primary infection, with just over one in 10 cases resulting in symptomatic congenital infection.[8] However, transmission to the fetus and the effects that this might have on the baby vary with gestational age at infection.[10, 11] The 2011 UK NSC review[7] stated: “although early studies suggested no differences in rates of intrauterine transmission by trimester of maternal infection, there is increasing evidence that seroconversion in late pregnancy is associated with a higher rate of congenital infection. However, transmission later in pregnancy appears to be associated with a lower risk of damage to the fetus.”

There is less clarity over the risk of congenital infection and fetal sequelae following non-primary infection. There are no current figures on CMV seroprevalence among pregnant women in the UK, though it is likely that prevalence rates would reflect those of the general population at around 50%.* The 2011 UK NSC review[7] noted evidence that socioeconomic status and ethnicity may have an influence on CMV seroprevalence. One study from the 1980s reported higher seropositive rates in older, parous women, and in those who were unmarried. Seropositivity was also reported to range from 46% in White women to 88% in women of Asian origin. Therefore, changes in the demographic profile of the UK population in recent decades, and the consequential changes in some practices such as breastfeeding, may have had an influence on seropositivity rates.

Though the risk from non-primary maternal infection is uncertain, the 2011 review[7] also highlighted how geographic areas with high seropositive rates (in general, communities of lower socioeconomic status and developing countries) tend to have high prevalence of CCMV (above 1%). As such, non-primary infection could contribute more to the prevalence of the disease than previously thought.

As part of the current review, a literature search and appraisal was first conducted to review new evidence published since the 2011 UK NSC review[7] regarding antenatal CMV. The aim was to see whether there was new evidence that better described the natural history of CMV in pregnancy (in particular the risks from primary vs. non-primary infection), and in addition whether there was new evidence on screening tests and antenatal treatment. The evidence found is summarised in the tables in Appendix B.

Natural history of maternal infection

No large studies were identified that clarify whether risk of fetal transmission or severity of outcomes differs between primary and non-primary infection.

One Japanese single-centre cohort (Ebina et al. 2014,[12] Appendix B.i) provided some evidence to further support the understanding that risk of fetal transmission is higher with primary infection (as indicated by low IgG avidity index) than with non-primary infection. However, recent evidence was not able to clarify whether the risk of fetal/newborn complications differs between primary and non-primary infection. Two small cohort studies of children with CCMV (Yamamoto 2011[13] and Townsend 2013[14], Appendices ii and iii) found that more children with SNHL were born to mothers with non-primary infection. This again seemed to confirm the pattern found in previous reviews that in populations with high seropositivity, a greater number of CCMV complications may be attributed to non-primary infection. However, the size of these studies and other applicability issues restricted meaningful analysis.

Meanwhile other systematic reviews (de Vries 2013[15] and Goderis 2014,[16] Appendices iv and v) suggested that the risk of SNHL may be more or less equivalent following both primary and non-primary infections. However, again, there were various quality limitations and potential sources of bias in the studies informing these reviews.

Similarly, a limited body of evidence was also found that looked at the timing of onset of maternal primary infection. Again these confirmed the pattern of results from earlier reviews that transmission risk may be higher for infections acquired during the third trimester, but early pregnancy infections may be more likely to result in infant with CCMV related-symptoms (Appendix B.vi).

Screening for maternal infection

Maternal infection is diagnosed usually through CMV IgM or IgG serology. IgG avidity is an indicator of the strength with which IgG antibodies bind to the antigen. Avidity is low in the weeks following acute infection and then progressively increases. Therefore a low IgG avidity index result in combination with a positive CMV IgM antibody is indicative of infection within the preceding three months. However, despite the availability of IgG avidity testing, the test cannot confirm exact timing of infection, because the cut-off for low avidity is not well established.

Considering the antenatal period, several serology based strategies to reduce the burden of CCMV have been suggested:

1. Universal screening of all women in early pregnancy: this approach would identify the seronegative women who could then be offered serial serology tests during pregnancy to identify seroconversions.

2. Performing 'one off' serology on all pregnant women at around 20 weeks (including avidity testing) to identify primary early pregnancy infections considered to be more likely to result in symptomatic CCMV.
3. Testing only women at increased risk of primary infection, such as those with frequent or prolonged contact with young children (under three years of age), for example, women living with young children or working in a day-care setting.
4. Performing targeted assessment at the second trimester ultrasound for features of CCMV (such as ventriculomegaly, intracerebral calcifications, microcephaly, echogenic bowel, intra-uterine growth restriction), and secondary maternal serology screening if positive features are identified.

These strategies are limited as they mainly focus on primary CMV infections. Antenatal screening strategies aimed at identifying seroconversions would by definition exclude women whose babies may be at risk of CCMV due to non-primary maternal infection.

The search identified only a single prospective cohort study (Yoshida et al. 2013,[17] Appendix B.vii) which shared similarities with strategies 1 and 2 above, but was not an exact match to either. This study screened all women for CMV IgM during the first trimester, and those who screened positive then received testing for CMV DNA in the amniotic fluid. The study demonstrated that serological CMV IgM screening has an extremely low positive predictive value to indicate fetal infection (4.8%). Otherwise the study could give no further screening test performance data due to the lack of follow-up of screen negatives.

Antenatal interventions

In addition, the evidence on options for intervention in the antenatal period to prevent acquisition of infection, mother-to-child transmission or attenuation of complications in the newborn remains limited.

Primary prevention: although behavioural interventions (as suggested by the NHS[18]) may be effective in improving hygienic practices and preventing acquisition of primary infection in pregnant women, the 2011 UK NSC review[7] found no evidence to support this and highlighted that such interventions had not been tested under controlled conditions.

The updated search did not identify new evidence related to primary prevention methods.

Secondary prevention: two interventions have been proposed for the prevention of CMV transmission or complications in the newborn following primary maternal infection. These are antiviral therapy and CMV hyperimmune globulin treatment. The 2011 UK NSC review[7] noted some evidence from a small study suggesting that CMV hyperimmune globulin was associated with a reduced risk of fetal transmission and that this was being investigated in randomised controlled trials.

The updated search identified a single phase II RCT, the results of which contrasted with this early finding. Revello et al. (2014)[19] (Appendix B.viii) found that hyperimmune globulin was ineffective compared with placebo at preventing transmission of maternal primary infection acquired during the 1st or 2nd trimester. However, the sample size was relatively small and the overall transmission rate low. The authors calculated that triple the sample size would have been needed to have sufficient power to detect a treatment effect. The RCT also provided no

information on whether treatment prevents complications in the newborn. No evidence was found to inform whether hyperimmune globulin may have a different effect following non-primary infection. Information on use of antiviral drugs for CMV infection in pregnancy to prevent transmission remains very limited.

Conclusion regarding antenatal screening

The 2011 UK NSC review[7] noted that due to the complexities around diagnosis of maternal and fetal CMV infection and the lack of available interventions to prevent transmission or development of CCMV disease, the focus of attention had shifted in recent years towards neonatal screening instead of antenatal screening.

The updated literature search found no significant new evidence to suggest that the UK NSC should reconsider screening in the antenatal period. Evidence on natural history continues to suggest that primary maternal infection carries higher risk of fetal transmission, but that the role of non-primary infection in the burden of CCMV morbidity may be considerable due to high population seroprevalence rates. The optimum antenatal screening strategy remains unclear. The review identified no primary studies exploring the effectiveness or practicality of the strategies previously proposed in discussion papers. Evidence from a single RCT did not confirm the effectiveness of antenatal hyperimmune globulin suggested in previous studies. No further evidence was identified on primary or secondary preventative strategies.

Therefore, the decision was made to focus the 2016 review on the postnatal period, with the aim to establish if the evidence produced since the previous review, is sufficient to gauge whether the UK NSC should revisit the 2011 recommendation about a national neonatal screening programme for CCMV.

Newborn screening for cytomegalovirus

Congenital infection in the newborn can occur with different levels of severity from asymptomatic with no signs or symptoms[‡] of the disease (including normal hearing), to moderate and severe manifestations with multiple signs or symptoms and central nervous system (CNS) involvement.

Babies with severe and moderate symptoms in the first two weeks of life (10 to 15 % of newborns with CCMV; 240 to 360 per year) are likely to be identified without screening. However, around 10% of CCMV infected babies that are asymptomatic at birth, or who have mild unspecified sign or symptoms, can ,later develop hearing loss if they are not treated.[16] This group of babies is most likely to be the potential target and beneficiary of newborn screening.

Conclusion from the 2011 UK NSC review

The UK NSC currently recommend against newborn screening for CMV. This followed the previous external review in 2011,[7] which highlighted several key uncertainties including the lack of a sensitive newborn screening test and no clear evidence that newborn treatments are

[‡] Thrombocytopenia, petechiae, hepatomegaly, splenomegaly, intrauterine growth restriction and hepatitis (raised transaminases or bilirubin)

effective. The current review considers whether the volume and direction of the evidence produced since the 2011 review indicates that the previous recommendation should be reconsidered.

The 2011 review[7] conclusions in relation to newborn screening were:

a. Both neonatal dried blood spots (DBS) and saliva swabs had the potential of becoming strategies for CCMV newborn screening in the future. DBS would be the obvious platform as it is routinely used to screen for other conditions. However, this approach had not been demonstrated to be sensitive enough for use in a large-scale newborn screening programme.
b. Intravenous ganciclovir was the only recommended treatment for infants with CCMV. However, it had only been tested in infants with neurological manifestations who were at risk of developing adverse sequelae. For asymptomatic infants or those with transient or non-specific symptoms, it was not possible to predict whether adverse outcomes would develop to make treatment worth the risk. An oral formulation, valganciclovir, was being investigated as an alternative to ganciclovir in a clinical trial of short- versus long-term treatment in infants with any symptoms (not restricted to neurological).

Current update review

The current review considers whether the volume and direction of the evidence produced since the 2011 external review indicates that the previous recommendation should be reconsidered. Five main criteria will be considered, with particular focus given to areas the 2011 review identified as uncertain, or supported by insufficient evidence. The main criteria and key questions reviewed are:

Table 1. Key questions for current CMV update review

Criterion	Key Questions (KQ)	# KQ Studies Included
4. There should be a simple, safe, precise and validated screening test.	1. What is the performance of screening strategies for detecting CCMV infection in newborns using tests based on dried blood spot, saliva or urine samples?	1 SR and 2 primary studies
9. There should be an effective intervention for patients identified through screening, with evidence that intervention at a pre-symptomatic phase leads to better outcomes for the screened individual compared with usual care. Evidence relating to wider	2. What is the effectiveness and safety of treatments for CCMV? Treatments of interest are: a) Ganciclovir b) Valganciclovir c) Combination therapy of the above d) [Any licensed treatment that can be offered to newborns – can include off-label use of licensed treatments]	1 primary study

benefits of screening, for example those relating to family members, should be taken into account where available. However, where there is no prospect of benefit for the individual screened then the screening programme shouldn't be further considered.	3. Is there evidence that treatment is effective in newborns with different signs and symptoms of CCMV? For example, children with bilateral or unilateral hearing impairment?	0 studies
10. There should be agreed evidence based policies covering which individuals should be offered treatment and the appropriate treatment to be offered.	4. Has an evidence based pathway been identified which can distinguish babies that are likely to be adversely affected by CMV and that may benefit from treatment?	1 guideline, 9 primary studies
11. There should be evidence from high quality randomised controlled trials that the screening programme is effective in reducing mortality or morbidity. Where screening is aimed solely at providing information to allow the person being screened to make an "informed choice" (such as Down's syndrome or cystic fibrosis carrier screening), there must be evidence from high quality trials that the test accurately measures risk. The information that is provided about the test and its outcome must be of value and readily understood by the individual being screened.	5. Is there evidence that screening for CCMV impacts on morbidity (e.g. hearing) outcomes?	0 studies

Each criterion was summarised as 'met', 'not met' or 'uncertain' by considering the results of the included studies in light of the volume, quality and consistency of the body of evidence. Several factors were assessed to determine the quality of the identified evidence, including study design and methodology, risk of bias, directness and applicability of the evidence. Factors that were determined to be pertinent to the quality of the body of evidence identified for each criterion are outlined in the results section as well as the comment section of the Appendix tables.

For Criterion 4, quality assessment focused on four main domains: patient selection, the index test, the reference standard, and flow and timing of index test and reference standard. Each domain was assessed for risk of bias, and the first three domains were assessed for applicability to a potential UK screening programme population. Details of these assessments can be found in the comment section of the Appendix tables.

A systematic literature search of three databases was carried out looking for studies published between January 2011 and 19 February 2016. The search strategy is detailed in the appendix. Overall, the search yielded 2239 references addressing CMV. Of these, 289 were assessed as being potentially relevant to antenatal or newborn CCMV and were further filtered at title and abstract level. A total of 68 were selected for full text appraisal.

Each section below provides additional information on the results of the evidence selection process for the given criterion. Across all questions, we excluded studies not available in English language, conference abstracts, letters, editorials and other communications, grey literature, and studies of any design with sample size of less than 20 people. There were also six publications where the full text could not be identified.

Selection and appraisal of studies was predominantly undertaken by one reviewer, any queries were resolved through discussion with a second reviewer, or with the UK NSC evidence team. The review was checked using Bazian Ltd's quality assurance process.

Appraisal against UK NSC Criteria

These criteria are available online at https://www.gov.uk/government/publications/evidence-review-criteria-national-screening-programmes/criteria-for-appraising-the-viability-effectiveness-and-appropriateness-of-a-screening-programme.

4. There should be a simple, safe, precise and validated screening test.

Description of the previous UK NSC evidence review conclusion

The 2011 UK NSC review[7] described how diagnosis of CCMV requires a sample to be collected within the first two weeks of life, as testing after this time would not be able to distinguish congenital from postnatally acquired infection. However, few cases of CCMV are currently diagnosed clinically because most infected newborns are either asymptomatic or have non-specific symptoms. Therefore universal screening for CCMV would need to be conducted as soon as possible after birth.

Newborns with CCMV shed large amounts of the virus in saliva and urine. Diagnosis can be confirmed by viral culture of these specimens, or by polymerase chain reaction (PCR) analysis, which is rapidly replacing culture as the diagnostic method of choice due to greater efficiency and sensitivity.[20] Isolation of the virus in a urine sample has often been regarded as the gold standard method,[20] though urine collection from newborns has practical difficulties, which may preclude this from being considered for universal screening purposes. Collection of saliva, meanwhile, is relatively easy and non-invasive, though it would still require an additional sample to be taken from the newborn. Dried blood spot (DBS) screening would be the most obvious platform for CCMV screening as this sample is already routinely collected as part of the UK

Newborn Screening Programme. However, previous studies had demonstrated sensitivity of the DBS test to be low as the viral load in blood is much lower than in saliva and urine.

Therefore the 2011 review considered that either DBS or saliva swab could have potential roles in universal newborn CCMV screening.

Current UK NSC key question

The current review aimed to assess whether new evidence has been published since the last review that has assessed the performance of universal screening strategies for CCMV (involving either DBS, saliva or urine sample) conducted in all newborns.

Description of the evidence

Overall, 118 studies were identified as potentially relevant to CMV screening during title and abstract sifting. These studies were reviewed in more depth and 22 were accessed at full text.

Priority was given to prospective studies including large, unselected samples of newborns (e.g. consecutively enrolled newborns) who would be representative of universal screening practice, or to any systematic reviews of these cohorts.

Studies were excluded that performed screening or diagnostic testing of selective populations of newborns thought to be at high risk of CMV. This included studies testing only symptomatic newborns (including those with SNHL), those born to mothers with active CMV infection or diagnosed with HIV, or fetuses/infants with other indications such as neurological-imaging findings, restricted growth, low birthweight or prematurity.

As the question concerned screening for CCMV, rather than CMV that may be acquired during the later postnatal period, only studies where testing was performed within the first 2-3 weeks of life were included.

Of the reviewed studies, one systematic review and two prospective cohort studies (one covered by two publications) met eligibility criteria for universal newborn CCMV screening.

Wang et al. (2015)[21] conducted a systematic review and meta-analysis of newborn CMV screening by PCR assay of dried blood spot (Table 2, Appendix 1). This review included the results of two prospective cohort studies of DBS screening that have been published since the 2011 UK NSC review. As the cohorts were pooled in this meta-analysis, the primary publications were not reviewed separately.

Boppana et al. (2011)[22] was a prospective cohort study of newborn evaluating the usefulness of real-time PCR assay of dried or liquid saliva samples as a screening test (Table 3, Appendix 2). Further results of the continuation of this screening programme were subsequently published in a brief report by Pinninti[23] (Appendix 3).

The second prospective cohort, by Waters et al. (2014)[24] (Table 4, Appendix 4), examined newborn screening by PCR assay of liquid saliva sample.

Results

Table 2: Newborn CMV screening by PCR assay of DBS vs. PCR or culture of urine or saliva sample

Study	Population characteristics	Screen test/ Reference test	Meta-analysis	Sensitivity (95% CI)	Specificity (95% CI)	NPV (95% CI)	PPV (95% CI)
Wang et al. (2015)[21] (Appendix 1) Systematic review with meta-analysis (Search date: 1990 to Jan 2014)	15 cohorts n=26,007; 583 CMV positive 7 prospective, 8 retrospective 6 studies of universal screening; 9 selection based on suspected or confirmed infection. Individual study sample size range 19 to 11,407	DBS PCR assay vs. viral isolation or PCR DNA detection in urine and/or saliva (Method of DNA extraction/PCR amplification and reference standard used variable across studies) Collection age: DBS within the first week of life; urine/saliva within the first three weeks of life.	All studies (n=15)	0.844 (0.812 to 0.872)	0.999 (0.998 to 0.999)	0.991 (0.972 to 0.997)	0.906 (0.835 to 0.948)
			Prospective studies (n=7)	0.623 (0.548 to 0.693)	0.999 (0.999 to 1.000)	NR	NR
			Retrospective studies (n=8)	0.945 (0.918 to 0.965)	0.983 (0.974 to 0.989)	NR	NR
			High assay LOD ≥1500 copies/ml (n=4)	0.853 (0.773 to 0.914)	0.983 (0.960 to 0.994)	NR	NR
			Low assay LOD <1500 copies/ml (n=4)	0.612 (0.534 to 0.658)	1.000 (0.999 to 1.000)	NR	NR
			Large DBS area >25mm^2 (n=5)	0.861 (0.792 to 0.914)	0.999 (0.997 to 1.000)	NR	NR
			Small DBS area ≤25mm^2 (n=5)	0.632 (0.557 to 0.702)	1.000 (0.999 to 1.000)	NR	NR

Abbreviations: DBS, dried blood spot; LOD, limit of detection; NPV, negative predictive value; NR, not reported; PCR, polymerase chain reaction; PPV positive predictive value

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Table 3: Newborn CMV screening by PCR assay vs. culture of liquid/dried saliva sample

Study	Population characteristics	Screen test/ Reference test	Specimen	Sensitivity (95% CI)	Specificity (95% CI)	NPV (95% CI)	PPV (95% CI)
Boppana et al. (2011)[22] (Appendix 2) Prospective cohort 7 US centres June 08 to Nov 09	n=34,989 n=177 CMV positive by index and/or reference standard* Unselected sample: 98% from well-baby nurseries, 2% NICU	Real-time PCR assay vs. rapid viral culture of liquid/dry saliva* Collection age: 1.0+/-1.2 days	Liquid saliva (n=17,662)	100 (95.8 to 100)	99.9 (99.9 to 100)	100 (99.9 to 100)	91.4 (83.8 to 96.2)
			Dried saliva (n=17,327)	97.4 (90.8 to 99.7)	99.9 (99.9 to 100)	99.9 (99.9 to 100)	90.2 (81.7 to 95.7)
* 79/93 screen-positives by PCR and/or culture liquid saliva received confirmatory testing (PCR and culture of both saliva and urine): calculated PPV for PCR assay 91.1% and PPV for rapid culture 98.6%. 74/88 screen-positives by PCR and/or culture dry saliva received confirmatory testing (PCR and culture of both saliva and urine): calculated PPV for PCR assay 94.4% and PPV for rapid culture 100%.							

Abbreviations: Cl, confidence interval; NICU, neonatal intensive care unit; NPV, negative predictive value; PCR, polymerase chain reaction; PPV positive predictive value

Table 4: Newborn CMV screening by PCR assay of saliva sample vs. confirmatory re-testing with PCR and culture of urine and saliva

Study	Population characteristics	Screen / Reference test	Sensitivity (95% CI)	Specificity (95% CI)	PPV	NPV
Waters et al. (2014)[24] (Appendix 4) Prospective cohort Single Centre, Ireland June 2011 to May 2012	n=1044 asymptomatic infants n=4 screen positives; n=2 confirmed positive by reference standard Excluded: symptomatic infants, gestation <35 weeks	Real-time PCR assay of saliva sample vs. confirmatory re-testing with PCR assay of both urine and saliva and serology Collection age: first week of life	100% (95% CI 54.07 to 100.00)	99.74% (95% CI 99.23 to 99.93)	Reviewer calculated: 2 TPs/(2TPs + 2FPs) = 50%	Cannot be calculated: Only screen-positives received confirmatory testing. FNs unknown

Abbreviations: Cl, confidence interval; NICU, NPV, Negative predictive value; PPV positive predictive value; PCR, polymerase chain reaction

Dried blood spot testing

The systematic review by Wang et al.[21] (Table 2, Appendix 1) examined the performance of PCR assay of DBS. It confirmed the conclusions of the 2011 UK NSC review that DBS is not reliable enough to be used for universal screening purposes.

Compared with the standard diagnostic methods of viral culture or PCR assay of newborn saliva or urine, the specificity of PCR assay of DBS is very high, suggesting there would be few false positives. However, the sensitivity is too low to make this a reliable screening test, particularly when considering the pooled results of the prospective studies only. The retrospective studies included in the review gave better performance results, but these may have the potential for selection bias, including a higher proportion of suspected or confirmed cases than may occur in a general population sample.

There were other limitations to quality and applicability of the evidence. The review specified that all index and reference test samples had to be collected within the first three weeks of life. Such practice ensures that the infection detected is congenital. However, the individual cohorts varied in the length of DBS storage (from days to years in some retrospective studies) and method of DNA extraction and PCR amplification. These variations may affect screening test performance. For example, subgroup analysis revealed that sensitivity was significantly affected by the limit of DNA detection on PCR assay, and DBS surface area.

The individual cohort sample sizes also varied widely from 19 to 11,407, which could considerably affect the reliability of test performance results as CCMV would be relatively rare in a general newborn sample.

There was reported to be low risk of bias around the reference standard used across studies. However, blinding to the result of the corresponding index or reference standard when interpreting the alternative test was not reported. In fact for 2/15 studies it was reported that the reference standard was known when interpreting the DBS result. Therefore there was the potential for reviewer bias when interpreting the index test.

None of the 15 included studies came from the UK and it is unclear what method of DBS PCR assay or reference standard, PCR or culture of saliva or urine, would preferentially be used here.

The review reports that five of the cohorts found no significant difference in test performance between samples taken from asymptomatic and symptomatic newborns.

The review does not assess the value of DBS testing in identifying newborns at different risk of adverse outcomes from CCMV.

Otherwise there was no indication from this review of whether DBS screen test results correlate with the risk of complications from CCMV. That is, whether the test is more likely to detect newborns at risk of adverse outcomes.

Saliva testing

Two prospective cohort studies assessed the diagnostic performance PCR assay of saliva samples.

The first was a US cohort by Boppana et al.[22] (Table 3, Appendix 2). It reported on the performance of real-time PCR assay of dried or liquid saliva specimens in the detection of CCMV compared with the standard clinical test, rapid culture of saliva specimens. The study included 34,989 newborns (less than two days old) tested between June 2008 to December 2009. A

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second briefer report (Pinninti et al.[23], Appendix 3) included data on the longer term continuation of this study through to March 2012, including a final sample of 73,239 newborns.

Boppana et al.[22] aimed to establish if using real-time PCR assay on saliva samples could be an effective, high-throughput and convenient method suitable for population screening. The study reported that dried saliva samples had slightly a lower sensitivity (>97%) than liquid samples (100%) when both were compared with the standard clinical test, saliva rapid culture.

However, there are limitations to this study:

a. The potential for over-detection from the test, or its consequences, was not explored. As a proof-of-concept study, the evidence shows that the test is clinically valid in accurately identifying the presence of CCMV infection. However, the test cannot identify those babies that will suffer from adverse long term outcomes.

b. The clinical utility of the test was not fully explored in the study. The way in which the test affects management of the identified babies is a key evidence requirement for an evaluation of its usefulness as a screening tool, but was beyond the objectives of the study. The authors state that follow-up of the positive cases is ongoing but do not provide details.

c. There is some concern about verification bias in the study. The study was primarily assessing the value of PCR assay of saliva compared with saliva viral culture which is used as standard clinical test. However gold standard confirmatory testing with viral culture of both saliva and urine samples, was only performed for babies who tested positive by either saliva PCR or culture. This gold standard testing demonstrated some misclassification from both PCR and viral culture of saliva. The possibility that there may have also been misclassification among those who tested negative by both saliva PCR and culture was not explored.

d. Generalisability of the test and applicability to the UK population is unclear. The study was conducted in the US and the sample was taken at two days of life. If implemented in the UK the screening would probably be performed alongside the bloodspot screening visit at five days of life. The accuracy of the test on samples taken at different time was not explored. The majority of the study population (63.4%) was from ethnic minority groups (Asian 3.9%, Black 23.7%, White Hispanic 36.7% and other 3.3%) and so not representative of the UK sociodemographic.

The continuation of this study (reported by Pinninti et al.[23], Appendix 3) contained evidence suggesting that PCR assay of saliva was superior to viral culture. The limited quality of the reporting in this brief publication prevents firm conclusions being drawn. However, due to the apparent superiority of PCR, the researchers stopped performing viral culture for those who screened negative by PCR assay of saliva. This publication therefore adds to the concerns of verification bias raised in point c above.

While this US cohort does not definitively establish PCR assay of saliva samples as a valid screening test, they do suggest PCR assay of saliva, particularly dried sample, as a practical candidate worthy of evaluation in future studies as a potential screening test.

The second cohort by Waters et al.[24] (Table 4, Appendix 4) provides little additional information in this regard. It initially aimed to assess the performance of one-off PCR assay of saliva or urine sample; however, due to feasibility issues around collecting urine, the study reverted to collecting saliva samples only. PCR assay of saliva was compared against the

reference standard of confirmatory re-testing of both saliva and urine, along with serology for CMV viral load and CMV IgM.

The study has several applicability and quality issues. It was a relatively small sample from a single Irish centre. The samples were all collected within the first week of life so are relevant to CCMV screening, but only asymptomatic and full-term infants were sampled. Therefore it may have excluded newborns at highest risk from complications of CCMV.

Sensitivity and specificity of one-off PCR assay of saliva were reported at near 100%, but there were only four screen positives. Two of these were negative on confirmatory re-testing, which gives a poor PPV of one-off PCR assay of only 50%. However, the very low prevalence rate in this asymptomatic sample, which may also differ from the rest of Ireland or the UK, limits the value of the PPV.

Similar to the Boppana et al.[22] there was potential for verification bias. Confirmatory re-testing of saliva and urine was only performed for screen-positives. The number of false negatives is unknown and so NPV could not be calculated. It is unclear how the researchers calculated sensitivity and specificity. If the full cohort had received confirmatory re-testing this would have given a better indication of the reliability of one-off PCR assay of saliva as a potential screen test.

Similar to the Wang et al.[21] review of DBS screening, neither the Boppana or Waters cohorts assessing PCR assay of saliva samples measured longer term outcomes in screen positives. Therefore it is not possible to know how well positive screen results by saliva sample correlate with health outcomes in the infant.

Summary: Criterion 4 not met.

One systematic review provides further evidence that newborn dried blood spot sampling is not sufficiently sensitive to be a reliable screening test for CCMV when compared with diagnostic methods of PCR assay or viral culture of saliva or urine.

PCR assay of saliva samples has been identified as the main candidate for a newborn screening test. This was based on the evidence from two cohorts that addressed screening by PCR assay on a one-off dried or liquid saliva sample taken from newborns. However, these studies have potential for verification bias as they do not perform confirmatory testing of the full cohort, only screen positives. There is also uncertain applicability to the UK screening population.

An overriding issue concerning both DBS and saliva testing is that even if these approaches are able to detect CCMV with sufficient reliability, none of the studies reviewed here have yet reported longer-term disease outcomes. It is therefore not known how screening test results correlate with the likelihood of adverse outcomes.

9. **There should be an effective intervention for patients identified through screening, with evidence that intervention at a pre-symptomatic phase leads to better outcomes for the screened individual compared with usual care. Evidence relating to wider benefits of screening, for example those relating to family members, should be taken into account where available. However, where there is no prospect of benefit for the individual screened then the screening programme shouldn't be further considered**

Description of the previous UK NSC evidence review conclusion

The previous UK NSC review[7] concluded that intravenous ganciclovir was the only recommended treatment for infants with CCMV, but this had only been tested in those with neurological manifestations. This followed a single trial[25] which found that intravenous ganciclovir improved hearing and developmental outcomes compared with no treatment in infants with neurological manifestations. However, the review reported that the need for prolonged hospital stays precluded longer duration of treatment, particularly in the absence of clear evidence of benefit. An oral formulation, valganciclovir was reportedly being investigated in a clinical trial, as past observational studies had demonstrated that outcomes were improved by six weeks of ganciclovir followed by six months of oral valganciclovir.

The previous review noted that there was no treatment currently approved for asymptomatic infants with CCMV as many children would remain healthy and the toxicity risks from currently available treatments may outweigh any benefit.

Current UK NSC key question

The current review aimed to address two key questions in relation to newborn treatment:

2. Is there evidence on the effectiveness and safety of treatments for CCMV? This could include ganciclovir, valganciclovir or any other treatment licensed for use in newborns.
3. Is there evidence that treatment is effective in newborns with different signs or symptoms of CCMV, for example bilateral or unilateral deafness?

Description of the evidence

Overall, 40 studies were considered potentially relevant to this question at first pass appraisal and 23 were selected for full text appraisal. A corresponding publication could not be identified for three of these studies.

For question 2 the reviewer prioritised randomised controlled trials assessing any treatment compared with no treatment/placebo or alternative treatment in newborns with CCMV, and systematic reviews of RCTs. If RCT evidence was not available they would move down the hierarchy of evidence to look at comparative cohorts assessing outcomes in a sample of treated newborns compared with a sample of untreated/alternatively treated newborns.

For question 3 the reviewer would look at any RCTs or observational cohorts that reported treatment outcomes in relation to baseline complications/adverse outcomes. For example, they would look at RCTs or prospective cohorts that assessed treatment outcomes in a sample of newborns with bilateral deafness and a sample with unilateral deafness. They would also look at cohorts of treated newborns that retrospectively assessed how treatment outcomes were related to newborn characteristics.

The reviewer did not include studies that solely reported outcomes for a group of treated newborns but contained no comparison either to a group of untreated/alternatively treated newborns, or comparing treatment outcomes by baseline symptoms.

A single randomised controlled trial Kimberlin et al. (2015)[26] met the inclusion criteria for question 2. The study assessed the effect of six weeks treatment with valganciclovir[§] compared with six months treatment with the same drug. This study is summarised in Table 5, Appendix 5.

No other studies addressing questions 2 or 3 were identified. Additional cohorts reporting outcomes in treated compared with untreated newborns included samples of less than 20 newborns and were considered too small to give reliable treatment effects. No studies had assessed treatment outcomes in relation to baseline characteristics, such as bilateral deafness or unilateral deafness.

[§] Valganciclovir is a ganciclovir prodrug and it is used as ganciclovir oral versions

Results

Table 5: RCT of short vs. prolonged oral valganciclovir treatment

Study	Population	Intervention	Comparator	Overall results
Kimberlin et al. (2015)[26] (Appendix 5) RCT Multicentre, US, 2008 to 2011	n=96 newborns with symptomatic CCMV* (with or without CNS involvement) Exclusions: very preterm (born at <32 weeks), current weight <1800g, over 30 days postnatal	Total six months oral valganciclovir 6 weeks + 4.5 month continuation (16 mg per kilogram body weight, twice daily)	Total six weeks oral valganciclovir 6 weeks + 4.5 month placebo	Primary outcome: change in best-ear hearing to six months • aOR 1.75, 95% CI 0.69 to 4.43 (p=0.24) Other outcomes: • change in best-ear hearing to 12 months: aOR 2.81, 95% CI 0.99 to 7.99 (p=0.05) • change in best-ear hearing to 24 months: aOR 3.28, 95% CI 0.91 to 11.9 (p=0.07) • change in total-ear hearing † to six months: aOR 1.69, 95% CI 0.76 to 3.73 (p=0.20) • change in total-ear hearing to 12 months: aOR 3.04, 95% CI 1.26 to 7.35 (p=0.01) • change in total-ear hearing to 24 months: aOR 2.61, 95% CI 1.05 to 6.43 (p=0.04) • neurological impairment on Bayley-III at 24 months: significant improvement in language-composite scores (p=0.005) and receptive-communication scale scores (p=0.003) • Grade 3-4 neutropenia: 19% in first six weeks; 21% intervention vs. 27% placebo six weeks to six months (p=0.64)

Abbreviations: aOR, adjusted odds ratio; CCMV, congenital CMV; CI confidence interval; CNS, central nervous system

* Symptomatic disease was defined as one or more of the following: thrombocytopenia, petechiae, hepatomegaly, splenomegaly, intrauterine growth restriction, hepatitis, or CNS involvement such as microcephaly, intracranial calcifications, abnormal cerebrospinal fluid indexes, chorioretinitis, sensorineural hearing loss, or the detection of CMV DNA in cerebrospinal fluid.

† Hearing in one or both ears that could be evaluated

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Kimberlin et al. (2015)[26] evaluated the short and longer-term treatment effects of an oral formulation of valganciclovir in newborns with symptomatic CCMV.[**] All the study participants were treated with the drug for six weeks followed by either 4.5 months of continued valganciclovir or placebo.

The trial found that six months treatment with valganciclovir had a weak, but not statistically significant effect, on the primary outcome of the study (best-ear hearing at six months) compared to six weeks treatment.

The study reported some evidence that prolonged treatment had a moderate and statistically significant effect on longer-term hearing (total-ear hearing)[††] and neurodevelopmental outcomes at 12 to 24 months. The authors caution that this effect could be due to statistical artefacts, but they did not provide more information.

This US multicentre study had strengths in its double-blind, placebo-controlled design and was adequately powered to detect differences in the primary outcome. The findings should be applicable to the UK setting. However, there are some concerns about the applicability of the study to this review's target population.

Firstly, the study recruited babies with symptomatic CCMV infection, which may not represent the population detected by screening. Secondly, 47% of the participants in the six month treatment group and 61% of the participants in the six weeks treatment group, entered the study at 15 days of age or older. Diagnosis of CCMV requires a sample to be collected within the first two weeks of life as testing after this time would not distinguish congenital from postnatally acquired infection.

The study showed that during the first six weeks of the open-label treatment there were three times fewer cases of severe neutropenia compared with their previous study.[25] In the following 4.5 months, the rate of severe neutropenia remained constant (around one in five cases), with no difference in rates between the valganciclovir and placebo groups. Nevertheless neutropenia remains a safety concern with valganciclovir.

No further adverse effects were reported, and there were no deaths or treatment withdrawals due to adverse effects in either group. The study did not report on other toxicity concerns that have been raised with ganciclovir, including possible carcinogenic and reproductive effects.

In summary this single trial provided some evidence of a potential longer-term benefit of six months compared with six weeks of treatment with oral valganciclovir, but did not provide evidence that these findings were applicable to the general screening population. Universal screening for CCMV would expand the spectrum of detected disease to include mildly symptomatic (many with only one of the signs or symptoms that were listed as inclusion criteria in the study) or asymptomatic babies. In the majority of these babies the condition improves without antiviral treatment[27] and with no long lasting effects and caution needs to be taken in advising antiviral treatment in such group to avoid overtreatment and potential harms. Moreover, the study did not provide evidence on the safety or effectiveness of short or long-

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[**] Symptomatic disease was defined as one or more of the following: thrombocytopenia, petechiae, hepatomegaly, splenomegaly, intrauterine growth restriction, hepatitis, or CNS involvement such as microcephaly, intracranial calcifications, abnormal cerebrospinal fluid indexes, chorioretinitis, sensorineural hearing loss, or the detection of CMV DNA in cerebrospinal fluid.

[††] Hearing in one or both ears that could be evaluated

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term treatment with valganciclovir compared with the current standard of six weeks of intravenous ganciclovir.

Summary: Criterion 9 not met.

Since the last UK NSC review a single placebo-controlled trial has been completed that compares six months of treatment with oral valganciclovir with six weeks of treatment in symptomatic newborns with CCMV with or without neurological manifestations.

The trial found no evidence that prolonged treatment with oral valganciclovir improved short-term hearing outcomes. There was some evidence, that a six months oral treatment may improve hearing and neurodevelopmental outcomes in the longer term at 12-24 months. However, there were some concerns on the safety of the drug.

Most importantly concerns remain on the applicability of this therapy to babies that have a mildly symptomatic disease or asymptomatic infection.

Finally, the population enrolled in the study was not enrolled through screening, limiting the applicability of the result to a screening programme.

10. There should be agreed evidence based policies covering which individuals should be offered treatment and the appropriate treatment to be offered.

Description of the previous UK NSC evidence review conclusion

The 2011 UK NSC review[7] noted that following detection of CCMV, newborns need to be assessed to identify symptoms and check for neurological manifestations. Signs of CNS involvement at birth indicate a risk of developing adverse sequelae, and therefore these babies may benefit from treatment.

However, for the vast majority of newborns with CCMV (85-90%) who have transient or non-specific symptoms, or are asymptomatic, it is difficult to predict whether hearing loss or other adverse outcomes will develop. Due to the lack of trial data in this population, treatment is not currently recommended for asymptomatic babies.

A diagnostic pathway is necessary to identify a group screen positive babies who would develop adverse outcomes without intervention. These would be eligible for treatment to prevent the onset of sequelae or for treatment of early presenting symptoms.

Current UK NSC key question

The current review aimed to identify whether an evidence based pathway has been identified that can distinguish babies that are likely to be adversely affected by CCMV and that may benefit from treatment.

To this end, this review aimed to identify any evidence looking at whether there are specific clinical factors in newborns that may be predictive of adverse long term outcomes.

Description of the evidence

Ninety-eight studies were considered potentially relevant to this question at first pass sift and were reviewed in more depth at abstract level at second pass appraisal. Twenty-six were selected for full text appraisal.

The reviewers firstly aimed to identify any published guidelines or evidence-based pathways that had already established specific clinical findings that are associated with adverse outcomes and that indicate treatment. A single, UK evidence-based guideline on the management of CCMV was identified (Kadambari et al. 2011[20]), the recommendations of which are summarised in Table 6 below.

The reviewers then looked at other systematic reviews or primary studies (cohorts or case control studies) that had assessed whether specific signs or symptoms were predictive of adverse long-term outcomes.

Three cohort studies provided evidence of most direct relevance to this question. Alarcon et al. (2013)[28] (Appendix 6) assessed specific neurological findings in symptomatic newborns with CCMV as high-risk markers for later sequelae. Forner et al. (2015)[27] (Appendix 7) assessed viral load at birth in asymptomatic newborns with CCMV as a predictor of late onset sequelae. Bilavsky et al. (2015)[29] (Appendix 8) was a retrospective cohort study assessing an isolated neurological finding (lenticulostriated vasculopathy, LSV) in an otherwise asymptomatic

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newborn as a marker for later hearing impairment. These studies are summarised in Table 7 below.

The review identified no further studies that assessed the association between specific signs and symptoms and longer term outcomes.

Six further studies were identified (one systematic review and five cohort studies) that reinforced the previously established observation that symptomatic newborns (particularly those with neurological manifestations) are more likely to develop adverse outcomes than asymptomatic newborns. However, none of these studies explored the predictive value of specific signs or clinical markers, and they therefore provide no new information relevant to this key question. These studies are summarised in Table 8.

Two studies just fell short of meeting inclusion criteria. One proof-of-concept study (Matsuo et al. 2014[30]) aimed to establish a method for measuring ventricular dilatation using computed tomography in newborns with CCMV compared with healthy newborns. The authors further aimed to determine cut-off dimensions associated with current abnormal auditory brainstem response (ABR). As the study aimed to establish an indicator of current impairment, but did not assess whether the abnormal ABR thresholds were associated with longer term outcomes, it is not of sufficient relevance to the question considered here.

A second validity study (Capretti et al. 2014[31]) aimed to see how cerebral ultrasound and magnetic resonance imaging (MRI) in newborns with CCMV compared in terms of identifying neurological pathologies associated with longer term sequelae. For example, they found that of 10/40 newborns with long-term sequelae, ultrasound and MRI were both abnormal in six, both normal in one, and three had had normal ultrasound but abnormal MRI. Therefore, the findings, athough needing validation, suggest that MRI may be the better diagnostic tool. However, as this study assessed the predictive the ability of a tool, rather than specific signs or symptoms, this study was also of limited relevance.

Studies were excluded from this review that reported long-term outcomes for a cohort of newborns with CCMV but did not assess the likelihood of outcomes in relation to specific clinical features present at birth. Studies were also excluded comparing serology of symptomatic and asymptomatic newborns but not linking this with longer term outcomes; those comparing outcomes for infants with CMV and other congenital infections; and those examining later childhood features as predictors of longer-term outcome, as these would not be relevant to newborn screening decisions.

Results

Table 6: Evidence-based guideline recommendations

Guideline	Treatment Indications	Evidence base	Treatment	Treatment duration
Kadambari et al. (2011) [20] UK Recommendations based on systematic review with Medline and Embase (search 1990 to May 2011).	1. CNS disease – SNHL, cerebral disease, chorioretinitis	Single prospective study (RCT on ganciclovir treatment)	Intravenous ganciclovir 6 mg/kg twice daily Oral valganciclovir 16 mg/kg twice daily if clinically appropriate	Total six weeks (treatment started within the first four weeks of life)
	2. Severe focal organ disease – severe hepatitis, severe anaemia, neutropaenia, thrombocytopaenia, colitis, pneumonitis	Informal expert opinion		

Abbreviations: CNS, central nervous system; SNHL, Sensorineural hearing loss

Table 7: Cohorts examining newborn characteristics predictive of long-term neurodevelopment outcome

Study	Population	Newborn predictive factor(s)	Long-term outcome	Overall results
Alarcon et al. (2013)[28] (Appendix 6) Partially retrospective and prospective cohort Single centre, Spain 1993 to 2009	n=26 newborns with symptomatic CCMV Diagnosed during the first two weeks of life	Adjusted microcephaly CSF β2-microglobulin level Neuroimaging score	Global adverse outcome at mean 8.7 years (including SNHL, visual deficit, neurodevelopmental disorders and death)	All factors significantly associated. Best individual predictive factor: CSF β2-microglobulin >7.9mg/l: • Sn 69, Sp 100, PPV 100, NPV, 63 • OR 3.25 (95% CI 1.43 to 7.34) Best combination: CSF β2-microglobulin >7.9mg/l and neuroimaging score 2-3: • Sn 87, Sp 100, PPV 100, NPV, 77 • OR 8.00 (95% CI 2.18 to 29.24)
Forner et al. (2015)[27] (Appendix 7) Prospective cohort Single centre, Italy	n=33 newborns with asymptomatic CCMV from primary infection Diagnosed at birth.	CMV DNA load in blood (copies/ml)	Late-onset sequelae at up to six years (including SNHL, hemiparesis, hypertonia or hypotonia, psychomotor retardation)	10/33 (30%) developed late onset sequelae: mean viral load at birth 17,045 vs. 1770 in those who remained symptom-free (p=0 .0002) • Risk of late-onset disease >50% at viral load ≥12,000 copies/ml (p=0.0002)

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Study	Population	Newborn predictive factor(s)	Long-term outcome	Overall results
2004 to 2007				• Risk of SNHL >50% at viral load ≥17,000 copies/ml (p=0.0001)
Bilavsky et al. (2015)[29] (Appendix 8) Retrospective cohort Single centre, Israel 2005 to 2012.	Newborns with CCMV Diagnosed during the first two weeks of life n=52 asymptomatic n=13 with isolated LSV, untreated (before 2009) n=51 with isolated LSV, treated (after protocol change in 2009)	LSV as an isolated finding on cerebral ultrasound in an otherwise asymptomatic infant (no hearing impairment, microcephaly, chorioretinitis, other abnormalities on cranial US)	Hearing deterioration after one year of age. (as defined by increase of ≥10 dB in the auditory threshold in one or two ears during two consecutive assessments or two behavioural tests resulting in a change in hearing category).	Hearing deteriorated in 16/116: • 5/52 (9.6%) asymptomatic • 11/13 (84.6%) with isolated LSV, untreated • 0/51 (0%) with isolated LSV, treated Those otherwise asymptomatic with LSV and untreated deteriorated significantly more than the asymptomatic group (p<0.001). Those asymptomatic deteriorated more than those treated with isolated LSV (p=0.008).

Abbreviations: CCMV, congenital CMV; CFS, Cerebrospinal fluid; LSV, Lenticulostriated vasculopathy, OR, odds ratio; Sn, sensitivity; SNHL, Sensorineural hearing loss

Table 8: Summary of studies reporting frequency of long-term sequelae among symptomatic and asymptomatic newborns with CCMV

Study	Population	Long-term outcome	Outcome rate in symptomatic newborn	Outcome rate in asymptomatic newborn	Statistical analysis
Goderis et al. (2014)[16] Systematic review with meta-analysis	10 studies (design and sample size not reported)	SNHL (timing not reported)	32.8% (95% CI 23.2 to 43.2)	9.9% (95% CI 6.3 to 14.2)	Not reported
Goderis et al. (2016)[32] Prospective cohort Multicentre, Belgium 2007 to 2014	n=379	SNHL up to mean 18 months	62.6% (77/123)	8.2% (21/256)	Not reported
Royackers et al. (2011)[33] Prospective cohort Single centre, Belgium 2003 to 2009	n=97 (rate for 156 normal-hearing ears at baseline)	SNHL up to mean 2.4 years	17.4% (4/23 ears)	2.3% (3/133 ears)	Not reported

Study	Population	Long-term outcome	Outcome rate in symptomatic newborn	Outcome rate in asymptomatic newborn	Statistical analysis
Yamamoto et al. (2011)[13] Prospective cohort 2 centres, Brazil 2003 to 2009	n=85	SNHL up to median 56 months	60% (6/10)	5.3% (4/75)	OR 38.1 (95% CI 1.6 to 916.7)
Townsend et al. (2011)[34] British Surveillance System (UK and Ireland) 2001 to 2002	n=78	Moderate to severe outcome SNHL up to median 17.7 months	60% (36/60) 54% (25/46)	22% (4/18) 24% (4/17)	p=0.001 p=0.09
Townsend et al. (2013)[14] Retrospective review two cohorts: Sweden 1977-85 and London 1979-86	n=176	Any neurodevelopmental impairment up to five years	42.1% (8/19)	14.1% (25/176)	p=0.006

Abbreviations: Cl, confidence interval; OR odd ratio; SNHL, Sensorineural hearing loss

Evidence-based guidelines

The UK guideline by Kadambari et al.[20] demonstrates the limited evidence-base guiding the management of CCMV prior to 2011 from when the current review UK NSC review dates.

Kadambari et al.[20] recommend that a newborn with CCMV receives full clinical examination, serological, radiological, audiological and ophthalmological assessments to determine whether they have signs or symptoms. The recommendations are then to treat those with CNS involvement and/or severe focal organ involvement.

The recommendation to treat newborns with neurological involvement is drawn solely from the 2003 Kimberlin et al. trial of intravenous ganciclovir,[25] in which this group was treated. The recommendation to treat with ganciclovir comes from the same RCT, while the suggestion to use oral valganciclovir as an alternative follows initial study by Kimberlin et al. prior to their 2015 trial publication of oral valganciclovir [26] described in this review.

Therefore, these recommendations to treat symptomatic newborns with neurological manifestations are compatible with the previous UK NSC review. However, there are no explicit definitions of CNS involvement. For example, the presence of “cerebral disease” may be open to professional interpretation and is also dependent on which diagnostic tests are carried out.

The recommendation to also treat newborns with severe focal organ involvement (in the absence of CNS signs) is based on expert opinion only, rather than additional evidence. As with CNS signs, the listed factors considered as “focal organ disease”, such as severe hepatitis and serological findings, are not clearly defined and interpretation may vary depending on local practice and protocols.

The guideline highlights the lack of good evidence around which newborns to treat, and what specifically constitutes “symptomatic”. As stated by Kadambari et al.[20]: The guideline authors also highlighted the need for large studies to establish the predictive power of symptoms and signs.’

Since this 2011 guideline, few studies have been published that report on potential predictive signs and symptoms that could guide treatment.

Cohorts assessing potential predictive markers

Alarcon et al.[28] assessed the predictive power of specific neurological manifestations and found that high CSF β2-microglobulin level and high neuroimaging score predicted global adverse outcome in later childhood with excellent specificity and PPV. However, notably not all children who developed adverse outcomes had these markers at birth, suggesting that other signs and symptoms aside from these CNS indicators may be relevant to later outcomes.

Bilavsky et al.[29] also looked at CNS involvement, specifically at the potential predictive value of isolated lenticulostriated vasculopathy (LSV) in infants with no other neurological manifestations and normal hearing at birth. They found that newborns with this abnormality demonstrated significantly greater hearing decline than asymptomatic newborns without this sign. Consequently this hospital began treating otherwise asymptomatic infants with isolated LSV mid-way through their study period and found reduced hearing impairment in the children who were treated.

Forner et al.[27] looked at CMV DNA in asymptomatic newborns and identified threshold levels that were associated with late onset CNS disease and SNHL specifically.

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However, there are clear limitations to these studies due to the small size of the cohorts and considerable variation in the populations, predictive markers and outcomes that were assessed.

In all three studies newborns met the definition for CCMV, with valid diagnostic confirmation within the first 2-3 weeks of life. The populations are from Spain, Italy and Israel and so should also be broadly comparable to UK newborns with CCMV.

However, though all newborns had CCMV, the populations of the three studies otherwise varied considerably.

Definitions of "symptomatic" disease were not fully compatible with each other, or with the definition of "symptomatic" in the Kadambari[20] guideline. For example, Alarcon et al.[28] included symptoms not specified by Kadambari et al.[20], such as IUGR and petechiae. Forner et al.[27] included more specific descriptions of neurological involvement than the guideline, such as hypotonia and hemiparesis, but did not include serological indicators of anaemia, neutropenia or thrombocytopenia. Bilavsky et al.[29] documented only neurological assessments with no apparent assessment of other organ involvement, so newborns defined as asymptomatic (with or without isolated LSV) may not have been fully asymptomatic.

Furthermore, Forner et al.[27] included only newborns born to mothers with primary infection, and Bilavsky et al.[29] included a majority of newborns infected following primary maternal infection (which in itself they consider may account for the high prevalence of LSV observed in their study group). It is uncertain whether disease manifestations may differ following primary and non-primary infections, but these studies may not apply to non-primary infection. As discussed, it is possible that a considerable proportion of newborns with CCMV could follow non-primary infections.

There are other limitations to the reliability of the predictive markers identified. The studies were all single centre studies with very small sample size. The Alarcon et al.[28] and Forner et al.[27] studies included only around a total 30 newborns each. The Alarcon[28] study did not have CSF analysis and neuroimaging available for the full cohort, which further reduces power. Bilavsky et al.[29] had a comparison sample of only 13 untreated newborns with LSV. This reduces the reliability of the risk associations. Larger sample sizes examining the prospective association with these makers may have given different results, for example they may have identified different threshold levels for viral load or CSF β2-microglobulin.

The timing of assessment of the predictive markers could also have an influence. In the Alarcon et al.[28] study the age of neurological assessments ranged from birth to four weeks of age. The Forner et al.[27] study also highlighted that the timing of serology and quantification of viral DNA (in this cohort taken during the first days of life) could have a significant effect on the predictive power of the measure.

The cohorts also differed in the outcomes examined in association with these markers, and when these were assessed. Both Alarcon[28] and Forner et al.[27] studied neurodevelopmental outcomes at around six to eight years, but they differed in the assessment scales that they used. Bilavsky et al.[29] assessed hearing impairment only, and this was at any time above one year of age.

Another limitation to neurological markers such as high neuroimaging score and LSV is in knowing how specific they are to CCMV and how common they may be in other non-CMV populations.

Summary: Criterion 10 not met.

The available evidence provides only limited new information since the last UK NSC review about treatment indications for CCMV. There remains a lack of clarity about which newborns with CCMV will go on to develop adverse outcomes and might benefit from treatment.

One UK guideline was identified which recommends treating newborns presenting with CNS involvement or severe focal organ involvement. The guideline recommendations were based on very limited evidence.

Three small cohort studies have assessed the potential of specific CNS signs or viral load to predict the likelihood of long-term sequelae. However, definitions of 'symptomatic' varied widely across these cohorts and the studied populations weren't comparable. Long-term outcome assessments also differed. It is difficult to know how relevant or applicable these potential predictive markers may be to current practice and specifically to newborns with CCMV identified through universal screening.

11. There should be evidence from high quality randomised controlled trials that the screening programme is effective in reducing mortality or morbidity. Where screening is aimed solely at providing information to allow the person being screened to make an "informed choice" (such as Down's syndrome or cystic fibrosis carrier screening), there must be evidence from high quality trials that the test accurately measures risk. The information that is provided about the test and its outcome must be of value and readily understood by the individual being screened.

Current UK NSC key question

The 2011 UK NSC review[7] did not address whether evidence had been published that newborn screening was effective in reducing morbidity or mortality from CCMV infection.

The current review aimed to address this question and identify whether any evidence had been published since 2011 that screening for CMV impacts hearing outcomes.

Description of the evidence

The aim was to identify RCTs or, if unavailable, non-randomised comparative cohorts that compared a screened population with a non-screened population and examined long-term outcomes. All of the 289 studies identified at first pass title appraisal and abstract sifting were reviewed for potential relevance to this question. At second pass appraisal at abstract level, no studies were found that contained comparative information for screened and non-screened populations. The studies reviewed for criterion 1 on the validity of the screen test did not contain longer-term follow-up information for their screened newborns.

Results

No studies were identified that compared outcomes in screened with non-screened populations.

Summary: Criterion 11 not met.

Since the previous UK NSC review there is no evidence available on whether screening for CCMV has an impact on longer term outcomes compared with routine practice.

Conclusions

Implications for policy

This report assesses newborn screening for congenital cytomegalovirus (CCMV) infection against select UK National Screening Committee (UK NSC) criteria for appraising the viability, effectiveness and appropriateness of a screening programme.

This review sought to establish whether evidence relating to key questions informed by the last 2011 UK NSC review[7] suggests that the current recommendation not to screen for CCMV in newborns should be reconsidered.

The evidence identified does not answer the uncertainties raised by the last review:

a. One systematic review examined the performance of PCR assay of DBS and it confirmed the conclusions of the 2011 UK NSC review that DBS is not reliable enough to be used for universal screening method when compared with current diagnostic methods of PCR assay or viral culture of saliva or urine samples.

b. Two cohorts assessed PCR assay of a one-off saliva sample. The two studies do not definitively establish one-off PCR assay of dried or liquid saliva samples as a valid screening test as they do not perform confirmatory diagnostic testing of the full study sample. There is also uncertain applicability to the UK. However they do suggest that this approach should be considered as a candidate test in future studies of screening for CCMV.

c. One RCT has assessed the oral alternative to ganciclovir, valganciclovir, in symptomatic newborns, comparing six weeks with six months treatment. It found no evidence that prolonged treatment improved the primary outcome of short-term hearing, but some evidence that it could have an effect on outcomes in the longer term at 12-24 months. Oral valganciclovir caused severe neutropenia in about one in five infants. In summary this single trial provided some evidence of a potential longer-term benefit, but did not provide evidence that these findings were applicable to the general screening population.

d. No evidence was identified that could inform whether intravenous ganciclovir or oral valganciclovir may be differentially safe or effective in newborns according to severity of hearing impairment or other signs or symptoms at birth. No studies have assessed the benefits of early vs late treatment in symptomatic newborns.

e. There remains a lack of clarity over how to identify which newborns are at risk of long-term neurodevelopmental sequelae. Three small cohorts have assessed the potential of specific CNS signs or viral load to predict the likelihood of long-term sequelae. However, it is difficult to know how relevant or applicable these potential predictive markers are to a population of newborns with CCMV identified through universal screening.

f. No studies were available to inform whether long-term outcomes, such as hearing, differ in screened vs. non-screened populations.

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Overall the evidence required to inform and support a universal newborn screening programme for CCMV has not been published since 2011, which indicates that the current recommendation not to screen for this infection in the UK should be maintained at the present time.

Implications for research

The findings of the this review indicate that the current recommendation not to perform universal newborn screening for CCMV should be maintained.

The issues identified in this review are unlikely to be resolved without further research aiming to:

- detect markers that can predict the severity of the condition in the screened detected population; and
- identify an effective intervention that could be beneficial to a screen detected population

Methodology

The draft update report was prepared by Bazian Ltd., and then adapted in discussion with the National Screening Committee. Each criterion was summarised as 'met' or 'not met' by considering the results of the included studies in light of the volume, quality and consistency of the body of evidence. Several factors were assessed to determine the quality of the identified evidence, including study design and methodology, risk of bias, directness and applicability of the evidence. Factors that were determined to be pertinent to the quality of the body of evidence identified for each criterion are outlined in the results section as well as the comment section of the Appendix tables.

The review was performed using a search strategy that would identify all evidence of relevance to maternal or antenatal screening for CCMV.

The evidence on maternal screening, treatment and natural history of transmission to the fetus/newborn was reviewed initially to see whether evidence in this area had changed since the last UK NSC review and would indicate that maternal screening could be reconsidered.

As the evidence base in this area had changed little, the decision was then made to focus the screening on key questions around newborn screening. All evidence of relevance to newborn screening, treatment and outcomes was then reviewed.

Search strategy

- CMV AND Vertical transmission (line #8)
- CMV AND (Population (maternal/newborn) OR Vertical transmission) AND Baby outcomes/symptoms (line #10)
- CMV AND Screening/testing AND Population (maternal/newborn) (line #7)
- CMV AND Population (maternal/newborn) AND Treatment (line #9)
- CMV AND Population (maternal/newborn) AND Baby outcomes/symptoms (line #11)

1. cytomegalovirus:ab,ti OR 'cytomegalovirus infection':ab,ti OR cmv:ab,ti OR 'human herpesvirus-5':ab,ti OR 'hhv-5':ab,ti OR 'hhv 5':ab,ti OR 'human cytomegalovirus'/exp OR 'cytomegalovirus'/exp OR 'cytomegalovirus infection'/exp AND [2011-2016]/py
2. test*:ab,ti OR 'screening'/exp OR 'screen*':ab,ti OR 'dried blood spot testing'/exp OR 'dried blood spot testing':ab,ti OR 'predictive value'/exp OR 'predictive value':ab,ti OR 'sensitivity and specificity'/exp OR 'sensitivity and specificity':ab,ti OR 'diagnostic accuracy'/exp OR 'diagnostic accuracy':ab,ti AND [2011-2016]/py
3. ante*natal:ab,ti OR pre*natal:ab,ti OR maternal:ab,ti OR pregnancy:ab,ti OR pregnant:ab,ti OR newborn:ab,ti OR neonat*:ab,ti OR 'pregnancy'/exp OR 'newborn'/exp OR 'vertical transmission'/exp OR 'vertical transmission':ab,ti OR congenital:ab,ti AND [2011-2016]/py
4. 'maternal-to-fetal':ab,ti OR 'vertical transmission':ab,ti OR 'transplacental infection':ab,ti OR 'maternal infection':ab,ti OR 'intrauterine transmission':ab,ti OR 'congenital infection':ab,ti OR 'vertical transmission'/exp OR congenital:ab,ti OR 'maternal-to-foetal':ab,ti AND [2011-2016]/py
5. 'hyperimmune globulin'/exp OR 'antivirus agent'/exp OR 'hyperimmune globulin':ab,ti OR 'antiviral medicine*':ab,ti OR 'antiviral agent*':ab,ti OR 'ganciclovir':ab,ti OR 'cymevene':ab,ti OR 'valganciclovir':ab,ti OR 'valcyte':ab,ti OR 'foscarnet':ab,ti OR 'foscavir':ab,ti OR 'cidofovir':ab,ti OR 'vistide':ab,ti OR 'off-label':ab,ti OR 'off label':ab,ti OR 'unlicensed':ab,ti OR 'immunoglobulin' OR 'treatment' OR 'drug therapy' AND [2011-2016]/py
6. 'symptomatology'/exp OR 'physical disease by body function'/exp OR 'newborn jaundice'/exp OR 'pneumonia'/exp OR 'rash'/exp OR 'hepatomegaly'/exp OR 'splenomegaly'/exp OR 'low birth weight'/exp OR 'seizure, epilepsy and convulsion'/exp OR 'small for date infant'/exp OR 'microcephaly'/exp OR 'symptom*':ab,ti OR 'sign*':ab,ti OR 'jaundice':ab,ti OR 'pneumonia':ab,ti OR 'rash':ab,ti OR 'enlarged liver':ab,ti OR 'hepatomegaly':ab,ti OR 'enlarged spleen':ab,ti OR 'splenomegaly':ab,ti OR 'low birth weight':ab,ti OR 'seizure*':ab,ti OR 'small size':ab,ti OR 'small for gestational age:ab,ti' OR 'small for date':ab,ti OR 'small head size':ab,ti OR 'microcephaly':ab,ti OR 'disease course'/exp OR 'prognosis'/exp OR 'follow up'/exp OR 'follow-up':ab,ti OR 'outcome*':ab,ti OR 'survival':ab,ti OR 'prognos*':ab,ti AND [2011-2016]/py
7. #1 AND #2 AND #3
8. #1 AND #4
9. #1 AND #3 AND #5
10. #1 AND (#3 OR #4) AND #6
11. #1 AND #3 AND #6

Database	Number of references
Embase/Medline	2223
The Cochrane Library	16

289 citations were deemed to be relevant at first pass appraisal. These citations were classified into the broad categories below. The citations were reviewed in these groups as a general guide,

though the groups were not exclusive. They were not an exact match to the key questions covered by the review, and there was some overlap of studies.

Category	Citations
Background	14
Maternal or newborn screening	118
Maternal-fetal transmission	21
Signs and symptoms in newborns	22
Treatment of the mother or baby	40
Outcomes in the baby/child	76
TOTAL	289

Appendix A: studies of newborn screening

Appendix number	1
Relevant criteria	4
Publication details	Wang L, Xu X, Zhang H, et al. Dried blood spots PCR assays to screen congenital cytomegalovirus infection: A meta-analysis. Virology Journal. 2015;12(1).[21]
Study details	Systematic review with meta-analysis
Study objectives	To review the diagnostic performance of dried blood spot (DBS) PCR assays for congenital CMV infection to see whether they are sufficiently effective to be used to screen neonates.
Inclusions	Studies published in Medline, Cochrane and the Science Citation Index (1990 to January 2014) fulfilling the criteria: • Studies that compared DBS PCR assays with the reference standard for detecting congenital CMV infection – normally viral isolation from, or PCR detection, in urine and/or saliva samples collected within the first 3 weeks of life. • DBS had to be collected within the first week of life, and the protocol for DBS PCR assays had to include DNA extraction from DBS samples and PCR amplification of CMV DNA. • Studies had to have data available to determine true positive (TP), false positive (FP), false negative (FN) and true negative (TN) rates.
Exclusions	Exclusion criteria: • Studies that did not compare DBS PCR testing with standard diagnostic tests for congenital CMV (viral isolation from urine and/or saliva). • Studies that overlapped with the studies selected (same study group, institution, and period of inclusion). • Letters, editorials, expert opinions, reviews without original data, and case reports.
Population	15 studies met inclusion criteria (covered by 14 articles). Total n=26,007 neonates, 583 diagnosed with congenital CMV by reference standard (individual study sample size range 19 to 11,407). 7 prospective cohorts, 8 retrospective cohorts (including 2 studies published post NSC review search date: Leruez-Ville [2011][35] and Paradiz [2012][36]) 6 studies assessed universal screening; 5 studies included those with compatible symptoms/suspected infection; 3 studies included infants confirmed with/without CMV; 1 study included infants of very low birth weight/small for gestational age.

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	No UK study: 3 America, 3 Italy, 2 France, Canada, Sweden, Portugal, Slovenia, Brussels, Brazil, Argentina. Studies QUADAS-2 quality assessed. The majority of studies had low applicability concerns and low risk of bias for the reference standard. However, 7 had high or unknown risk of bias for the index test and 9 had high risk of patient selection bias.
Intervention/test	DBS PCR assays (DNA extraction and PCR amplification). DBS collected within first week of life. Method of DNA extraction: heat shock (6 studies), Qiagen M48 robotic system (2 studies), QiAmpl DNA Blood Micro/Mini kit (4 studies), MagaZorb DNA extraction kit (1 study), and phenol-chloroform (2 studies). Type of PCR amplification: nested PCR (5 studies), real-time PCR (5 studies), single-primer real-time (1 study), two-primer real-time (1 study), PCR + hybridisation test (1 study), conventional PCR + nested PCR (1 study), CMV LC-real time PCR (1 study).
Comparator	Standard diagnostic test – viral isolation or PCR DNA detection from urine and/or saliva collected within the first 3 weeks of life. Length of storage DBS: varied 14 days to 18 years. Reference standard described: viral isolation from saliva or urine (2 studies); viral isolation from saliva (1 study); viral isolation from urine (2 studies); viral isolation from throat swab (1 study); urine sample PCR (2 studies); urine culture/PCR (1 study); urine culture (2 studies); urine shell-viral culture (1 study); urine sample viral culture and/or PCR (1 study); DEAFF assay on follow-up saliva/urine sample (2 studies).
Results/outcomes	15 studies pooled in meta-analysis using fixed effects model: • Sensitivity: 0.844 (95%CI 0.812 to 0.872) (I^2 94.6%) • Specificity: 0.999 (95%CI 0.998 to 0.999) (I^2 91.5%) • NPV: 0.991 (95%CI 0.972 to 0.997) • PPV: 0.906 (95%CI 0.835 to 0.948) • Negative LR: 0.110 (95%CI 0.0424 to 0.289) • Positive LR: 99.437 (95%CI 45.666 to 216.523) • AUC: 0.9953 (standard error 0.0023) • Diagnostic odds ratio: 1362.10 (95%CI 566.91 to 3272.60)

	Subgroup analysis of the 7 prospective studies (I^2 0%): • Sensitivity: 0.623 (95%CI 0.548 to 0.693) • Specificity: 0.999 (95%CI 0.999 to 1.000) • Negative LR: 0.374 (95%CI 0.182 to 0.768) • Positive LR: 280.72 (95%CI 60.026 to 1312.8) • Diagnostic odds ratio: 1573.9 (95%CI 699.17 to 3543.00) Subgroup analysis of the 8 retrospective studies (I^2 64%): • Sensitivity: 0.945 (95%CI 0.918 to 0.965) • Specificity: 0.983 (95%CI 0.974 to 0.989) • Negative LR: 0.043 (95%CI 0.007 to 0.280) • Positive LR: 43.831 (95%CI 19.745 to 97.298) • Diagnostic odds ratio: 1085.71 (95%CI 229.94 to 5126.46) Subgroup analysis of studies with limit of detection (LOD) data available revealed that those with high LOD ≥1500 copies/ml had better sensitivity (4 studies; sensitivity 0.853, 95%CI 0.773 to 0.914, specificity 0.983, 95%CI 0.960 to 0.994) than those with low LOD <1500 copies/ml (5 studies; sensitivity 0.612, 95%CI 0.534 to 0.658, specificity 1.000, 95%CI 0.999 to 1.000). Subgroup analysis of studies with DBS area data available revealed that those with large area >25mm^2 had better sensitivity (5 studies; sensitivity 0.861, 95%CI 0.792 to 0.914, specificity 0.999, 95%CI 0.997 to 1.000) than those with small area ≤25mm^2 (5 studies; sensitivity 0.632, 95%CI 0.557 to 0.702, specificity 1.000, 95%CI 0.999 to 1.000). No significant difference in assay detection rates between asymptomatic and symptomatic infants (5 studies, 95.9% vs. 96.7%, p=0.579).
Comments Various inconsistencies in study design and inclusion criteria, area of DBS, length of DBS storage before testing, DNA extraction and PCR technique, and reference standard. Subgroup analysis revealed study design, LOD and area of DBS to all have significant effect on assay performance. LOD and area data wasn't available for all studies. Patient selection bias was higher in retrospective studies as they frequently selected based on confirmed or suspected infection. Knowledge of diagnostic confirmation in 2 of the 5 studies could have biased screen test interpretation.	

Overall unclear relationship between detection rate and presence or absence of clinical features.

Study sample size also varied widely and could have affected performance due to low prevalence CMV.

No UK based studies, unclear what diagnostic method or technique for DNA extraction and PCR amplification would be most often used here.

Summary performance statistics given only; no data on FPR or FNR

Question	**Assessment (Y, N, unclear)**	**Risk of Bias (low, high, unclear)**	**Supporting info**
Domain I: Patient selection			
Consecutive or random sample of population enrolled?	N	High	The included studies varied in their design from prospective universal screening studies to retrospective based on those with confirmed/suspected infection.
Case-control design avoided?	N	High	Some of the included studies were retrospective and so would have included a higher prevalence of cases which could influence performance.
Inappropriate exclusions avoided?	Unclear	Unclear	The systematic review sets out appropriate inclusion/exclusion criteria. Explicit inclusion/exclusion criteria of the individual studies are unclear.
Domain II: Index Test			
Index test results interpreted without knowledge of reference standard results?	Unclear	Unclear	Not explicitly reported for most studies – though for 2/15 it is reported that reference standard results were known.
Threshold pre-specified?	Unclear	Unclear	The LOD for the assay is reported as high or low for 9 studies; not reported for 6
Domain II: Reference standard			
Reference standard likely to correctly classify condition?	Y	Low	There is reported to be low risk of bias and low applicability concerns for the reference standard across all studies.
Reference standard results interpreted without knowledge of index test results?	Unclear	Unclear	Blinding of diagnostic tests not reported.
Domain IV: Test strategy flow and timing			
Appropriate interval between index test and	Unclear	High	Variation in prospective and retrospective design and highly variable duration of

reference standard?			storage of DBS before testing (days to years).
Did all participants receive same reference standard?	N	High	Variation in reference standard used
All patients included in analysis?	Unclear	Unclear	Not reported.
Applicability			
Applicable to UK screening population of interest?	Unclear	Unclear	No UK studies, variable countries for 15 studies. Unclear whether prevalence may vary.
Applicable to UK screening test of interest?	Y	Unclear	DBS within the first week of life is applicable to the UK though unclear what DNA extraction method and PCR amplification would be used in the UK.
Target condition measured by reference test applicable to UK screening condition of interest?	Y	Low	CMV

Appendix number	**2**
Relevant criteria	4
Publication details	Boppana SB, Ross SA, Shimamura M, et al. Saliva polymerase-chain-reaction assay for cytomegalovirus screening in newborns. New England Journal of Medicine. 2011;364(22):2111-8.[22]
Study details	Prospective cohort 7 US centres; study period June 2008 to November 2009
Study objectives	To determine the usefulness of real-time PCR assay of saliva specimens collected from newborns for the purpose of CMV screening.
Inclusions	All children born in the study period assumed eligible; no inclusion/exclusion criteria reported.
Exclusions	As above, none reported.
Population	n=34,989 infants 98% from well-baby nurseries, 2% intensive care
Intervention/test	Real-time PCR assay of liquid/dry saliva (mean age collection: 1.0+/-1.2 days)

<table>
<tr><td></td><td>Positive test if ≥5 CMV DNA copies per reaction were detected.

Saliva collected by swabbing insider of newborn's mouth using a sterile polyester-fibre-tipped applicator and transported for analysis within one week of collection.

Phase 1 (June 08 to March 09) tested liquid saliva. Phase 2 (March to Nov 09) tested air-dried saliva sample. A sample of infants born June 08 to Feb 09 was tested by all three methods: liquid and dry saliva PCR assay and the standard of rapid culture.</td></tr>
<tr><td>Comparator</td><td>Rapid culture assay using monoclonal antibody to detect early CMV antigen in saliva specimen.

Diagnostic confirmation: Infants positive by either PCR assay, rapid culture or both were reassessed by testing both saliva and urine samples with both PCR assay and rapid culture (mean age follow-up 3.6 weeks, range 2.6 to 6.6).</td></tr>
<tr><td>Results/outcomes</td><td>177 CMV-positive by PCR assay, rapid culture or both.

• Phase 1: 17,662 tested by liquid saliva real-time PCR and rapid culture; 93 infants (0.5%) positive by either test: 85 by culture and PCR, 8 by PCR only

• Phase 2: 17,327 tested by dried saliva real-time PCR and rapid culture; 84 (0.5%) positive by either test: 76 by culture, only 74 of which were positive by PCR along with an additional 8

o Above includes 5276 tested by all 3 methods (dried and liquid saliva PCR and culture): 42 infants positive by all methods, 1 additional by PCR only

Performance of real-time PCR assays on dried/liquid saliva vs. rapid culture:

<table>
<tr><th>PCR assay</th><th>Sensitivity (95%CI)</th><th>Specificity (95%CI)</th><th>NPV (95%CI)</th><th>PPV (95%CI)</th><th>LR + (95%CI)</th><th>LR - (95%CI)</th></tr>
<tr><td>Liquid saliva</td><td>100 (95.8 to 100)</td><td>99.9 (99.9 to 100)</td><td>100 (99.9 to 100)</td><td>91.4 (83.8 to 96.2)</td><td>2197 (1099 to 4393)</td><td>0 (0.0 to 0.01)</td></tr>
<tr><td>Dried saliva</td><td>97.4 (90.8 to 99.7)</td><td>99.9 (99.9 to 100)</td><td>99.9 (99.9 to 100)</td><td>90.2 (81.7 to 95.7)</td><td>2100 (1049 to 4202)</td><td>0.03 (0.0 to 0.1)</td></tr>
</table>

• Phase 1: 79/93 screen-positive infants (85%) were followed up of which:

o 72 were positive on both culture and PCR: 1/72 was negative on culture and PCR of both saliva and urine (FP)

o 7 were positive on PCR only: 6/7 were negative on culture and PCR of both saliva and urine (FP)</td></tr>
</table>

	○ Calculated PPV (TP/FP+TP) for PCR assay: 72/79 = 91.1% (based on all 79 being positive on PCR assay and total 7 FPs) ○ Calculated PPV for rapid culture: 71/72 = 98.6% (based on 72 positive on culture with 1 FP) • Phase 2: 74/84 screen-positive infants (88%) were followed up of which: ○ 66 were positive on both culture and PCR: all positive on culture and PCR of both saliva and urine (no FPs) ○ 2 positive on culture only (not PCR): both positive on culture and PCR of both saliva and urine (no FPs) ○ 6 positive on PCR only: 4/6 were negative on culture and PCR of both saliva and urine (FPs), 2/6 positive (TPs) ○ Calculated PPV (TP/FP+TP) for PCR assay: 68/72 = 94.4% (based on 72 being positive on PCR assay and 4 FPs) ○ Calculated PPV for rapid culture: 68/68 = 100% (based on 68 positive on culture with no FPs)

Comments

Test performance data is for PCR detection of CMV DNA vs. the standard viral culture from saliva – both of which may be considered as diagnostic methods (as the Wang SR).

Performance is not given against follow-up diagnostic confirmation of re-testing by both urine and saliva culture and PCR – which was incomplete for screen-positives and not performed for those screening negatives by both PCR and culture. For re-testing PPV only could be calculated.

Large study, non-selective inclusion should be applicable to UK screening, though uncertain what method of screening or diagnostic confirmation would be used here.

Question	**Assessment (Y, N, unclear)**	**Risk of Bias (low, high, unclear)**	**Supporting info**
Domain I: Patient selection			
Consecutive or random sample of population enrolled?	Y	Low	No apparent exclusions
Case-control design avoided?	Y	Low	Not a case control study.
Inappropriate exclusions avoided?	Y	Low	No apparent exclusions

Domain II: Index Test			
Index test results interpreted without knowledge of reference standard results?	Y	Low	Reports personnel interpreting PCR assay were unaware of rapid culture results.
Threshold pre-specified?	Y	Low	Five or more copies per reaction for PCR detection.
Domain II: Reference standard			
Reference standard likely to correctly classify condition?	Unclear	Unclear	Culture of saliva is reported as the reference standard, though confirmatory testing with both saliva and urine did reveal one FP and was not performed for all screen-positives. Follow-up of screen-negatives by index and reference standard was not performed.
Reference standard results interpreted without knowledge of index test results?	Y	Low	Reports personnel interpreting rapid culture were unaware of PCR assay results.
Domain IV: Test strategy flow and timing			
Appropriate interval between index test and reference standard?	Y	Low	No identified issues.
Did all participants receive same reference standard?	Y	Unclear	All received saliva culture, though as above, confirmatory re-testing was only performed for screen positives (and incomplete).
All patients included in analysis?	Y	Unclear	All included in performance analysis for PCR vs. culture, though further confirmatory testing was only performed for a proportion of screen positives. Not possible to calculate Sn, Sp or NPV against further confirmation as screen negatives by both methods were not followed.
Applicability			
Applicable to UK screening population of interest?	Y	Low	Non-selective infant sample from US which may be expected to have similar CMV prevalence.
Applicable to UK screening test of interest?	Unclear	Unclear	Unclear what screening test and confirmatory reference standard would be used.
Target condition measured by reference	Y	Low	CMV

test applicable to UK screening condition of interest?			

Appendix number	3
Relevant criteria	4
Publication details	Pinninti SG, Ross SA, Shimamura M, et al. Comparison of saliva PCR assay versus rapid culture for detection of congenital cytomegalovirus infection. Pediatric Infectious Disease Journal. 2015;34(5):536-7.[23]
Study details	Prospective cohort – brief report on continuation of study described by Boppana et al.[22] 7 US centres; study period June 2008 to March 2012
Study objectives	To determine whether the real-time PCR assay identified more newborns with CMV than the standard of rapid culture of saliva specimens.
Inclusions	All children born in the study period assumed eligible; no inclusion/exclusion criteria reported.
Exclusions	As above, none reported.
Population	n=35,334 infants to Dec 09 (to Nov 09 covered above) n=36,905 infants in the continuation Jan 10 to Mar 12 total n=72,239
Intervention/test	Real-time PCR assay of liquid saliva June 08 to Dec 09 Real-time PCR assay of dry saliva Jan 10 to Mar 12
Comparator	Rapid culture of saliva specimen. Diagnostic confirmation: Infants positive by either PCR assay, rapid culture or both were reassessed by testing both saliva and urine samples with both PCR assay and rapid culture.

Results/outcomes	284 CMV-positive by PCR assay, rapid culture or both enrolled for confirmation by PCR and rapid culture of saliva and urine.
	• 266 TPs and 18 FPs
	• 252/266 TPs were positive by PCR and culture, plus:
	  o 13 TPs by PCR and FN by rapid culture
	  o 1 FN by PCR and TP by rapid culture
	• Calculated PPV for PCR (TP/TP+FP)=265/284=93.3%*
	• Calculated PPV for culture (TP/TP+FP)=253/284=89.1%*
	Reportedly suggests that 900 to 1400 infants with congenital CMV in the US could

	be missed by using the standard of rapid culture only.

Comments

Follow-up diagnostic confirmation with culture and PCR of both urine and saliva has not been performed for screen negatives by PCR and culture (though neither was culture performed for PCR screen-negatives in the latter stages of the study). As such Sn, Sp and NPV cannot be calculated because of lack of certainty over the number of TNs and FNs.

*The method doesn't explain whether the 18 FPs were positive by PCR and/or culture. However, the end of the discussion does say that there were 18 FPs by PCR and that "during the latter part of the study rapid culture was only performed on samples that tested positive by PCR" suggesting that the 18 would have been FPs by both methods.

The study highlights the lack of clarity about the reference standard given the lower PPV, and the need for diagnostic confirmation of FNs by PCR only – and by both methods.

Large study, non-selective inclusion should be applicable to UK screening, though uncertain what method of screening or diagnostic confirmation would be used here.

Question	**Assessment (Y, N, unclear)**	**Risk of Bias (low, high, unclear)**	**Supporting info**
Domain I: Patient selection			
Consecutive or random sample of population enrolled?	Y	Low	No apparent exclusions
Case-control design avoided?	Y	Low	Not a case control study
Inappropriate exclusions avoided?	Y	Low	No apparent exclusions
Domain II: Index Test			
Index test results interpreted without knowledge of reference standard results?	Y	Low	Boppana reports personnel interpreting PCR assay were unaware of rapid culture results.
Threshold pre-specified?	Y	Low	Boppana reports five or more copies per reaction for PCR detection.
Domain II: Reference standard			
Reference standard likely to correctly classify condition?	N	High	Culture of saliva is reported as the reference standard, though confirmatory testing with both saliva and urine revealed culture to

			have a lower PPV than PCR. Also confirmatory testing was only performed for screen-positives by PCR and culture.
Reference standard results interpreted without knowledge of index test results?	Y	Low	Reports personnel interpreting rapid culture were unaware of PCR assay results.
Domain IV: Test strategy flow and timing			
Appropriate interval between index test and reference standard?	Y	Low	No identified issues.
Did all participants receive same reference standard?	N	High	In Boppana it reports that all received saliva culture, though in the latter stages of the study it says that the reference standard was only given to screen positives by PCR.
All patients included in analysis?	N	High	Not possible to calculate Sn, Sp or NPV against further confirmation as screen negatives by PCR (not all tested by culture) were not followed.
Applicability			
Applicable to UK screening population of interest?	Y	Low	Non-selective infant sample from US which may be expected to have similar CMV prevalence.
Applicable to UK screening test of interest?	Unclear	Unclear	Unclear what screening test and confirmatory reference standard would be used.
Target condition measured by reference test applicable to UK screening condition of interest?	Y	Low	CMV

Appendix number	4
Relevant criteria	4
Publication details	Waters A, Jennings K, Fitzpatrick E, et al. Incidence of congenital cytomegalovirus infection in Ireland: Implications for screening and diagnosis. Journal of Clinical Virology. 2014;59(3):156-60.[24]
Study details	Prospective cohort Single centre, Dublin, Ireland (reported coverage of 12.5 to 13.5% of the annual birth cohort for Ireland). Study period June 2011 to May 2012.
Study objectives	To determine the incidence of congenital CMV in Ireland and the feasibility of an optimal sample method for large-scale universal screening.
Inclusions	Asymptomatic infants in the first week of life and born at gestational age ≥35 weeks
Exclusions	Non-consenting mothers, symptomatic infants and those born <35 weeks gestation
Population	n=1044 asymptomatic infants
Intervention/test	Real-time PCR assay of saliva or urine sample taken within first week of life (analysis within 3 weeks). Started off as urine collection reported change to saliva collection as standard (timing unclear) due to difficulties in collection of urine.
Comparator	Confirmation of screen-positives by re-testing of urine and saliva, and blood samples taken for CMV viral load and serum IgM levels.
Results/outcomes	4/1044 infants screened positive – 2 by saliva, 2 by urine sample. 2 confirmed positive on re-testing – 2 classed as false positives • Sensitivity: 100% (95% CI 54.07 to 100.00) • Specificity: 99.74% (95% CI 99.23 to 99.93) • Calculated PPV (TP/TP+FP)= 2/4 = 50% Incidence of CMV in asymptomatic sample 2/1044: 0.19%, 95% CI 0.02 to 0.69 Annual birth cohort of hospital (outside of screen sample) n=9163 • 9068 asymptomatic (not tested)

	• 95 symptomatic infants – 4 tested positive by PCR, and all four confirmed positive • Overall incidence of congenital CMV in annual birth cohort: 21.4 per 9163 infants: 0.23%, 95% CI 0.14 to 0.35

Comments

Sample selection was selective including asymptomatic infants only.

Test performance data on sensitivity and specificity seems based on the assumption that there were no FNs among the screen-negatives; no follow-up testing of screen negatives was performed therefore the actual number of TNs and FNs cannot be known. NPV cannot be calculated and there may be some inaccuracy in sensitivity and specificity.

May have been more reliability if all had been able to be tested by PCR of both saliva and urine – unclear whether performance of the two may have differed, or how many were tested by urine before feasibility need to switch to saliva.

Irish study with expected similar CMV prevalence and tests used to UK. However, single site may have different prevalence from the rest of Ireland. Relatively small sample and low prevalence condition means only 2 cases detected which may affect reliability of test performance data.

Question	**Assessment (Y, N, unclear)**	**Risk of Bias (low, high, unclear)**	**Supporting info**
Domain I: Patient selection			
Consecutive or random sample of population enrolled?	N	High	All asymptomatic term infants born in the study period were included – but exclusion of symptomatic and preterm.
Case-control design avoided?	Y	Low	Not a case control study.
Inappropriate exclusions avoided?	N	High	Only included asymptomatic term infants which may affect applicability to universal screening
Domain II: Index Test			
Index test results interpreted without knowledge of reference standard results?	Y	Low	PCR assay interpreted before any further confirmatory testing.
Threshold pre-specified?	Unclear	Unclear	Doesn't report detection threshold.
Domain II: Reference standard			

Reference standard likely to correctly classify condition?	Y	Low	Confirmatory testing with both saliva and urine and blood samples is likely to reliably confirm the condition (though as below, follow-up of screen-negatives was not performed)
Reference standard results interpreted without knowledge of index test results?	Unclear	Unclear	Doesn't report whether personnel interpreting re-test results were aware of positive PCR assay.
Domain IV: Test strategy flow and timing			
Appropriate interval between index test and reference standard?	Y	Low	No identified issues.
Did all participants receive same reference standard?	N	High	Confirmatory re-testing was only performed for screen positives.
All patients included in analysis?	N	High	All included in performance analysis – but screen negatives were assumed to include no FNs and not followed up. Therefore uncertain whether there could be inaccuracy on sensitivity, specificity and negative predictive value could not be calculated.
Applicability			
Applicable to UK screening population of interest?	Unclear	Unclear	Prevalence from single Irish centre could be expected to be similar to UK, but selective screening of asymptomatic infants only
Applicable to UK screening test of interest?	Unclear	Unclear	Unclear what screening test and confirmatory reference standard would be used.
Target condition measured by reference test applicable to UK screening condition of interest?	Y	Low	CMV, and as above prevalence would be expected to be similar.

Appendix number	5
Criterion number	4
Publication details	Kimberlin DW, Jester PM, Sánchez PJ, et al. Valganciclovir for Symptomatic Congenital Cytomegalovirus Disease. Obstetrical and Gynecological Survey. 2015;70(8):489-90.[26]
Study details	Double-blind placebo-controlled randomised controlled trial 31 centres in the US, recruitment June 2008 to May 2011.
Study objectives	To assess whether 6 months treatment with oral valganciclovir improves hearing outcomes compared with 6 weeks treatment.
Inclusions	Newborns with symptomatic CCMV, with or without CNS involvement. CMV diagnosed by viral culture or PCR assay of urine or throat-swab sample. Symptomatic disease defined as one or more of: thrombocytopenia, petechiae, hepatomegaly, splenomegaly, intrauterine growth restriction, hepatitis, or CNS involvement such as microcephaly, intracranial calcifications, abnormal cerebrospinal fluid indexes, chorioretinitis, sensorineural hearing loss, or CMV DNA in cerebrospinal fluid.
Exclusions	Very preterm (gestational age <32 weeks), current weight <1800g, postnatal age >30 days.
Population	n=96 randomised 86/96 (90%) with compete auditory assessments for 6 month primary outcome. 68/96 (71%) with complete auditory assessments to 24 months; 83 (86%) with developmental assessments to 24 months. No significant difference in baseline characteristics between groups.
Intervention	All newborns received 6 weeks treatment with oral valganciclovir (16 mg per kilogram body weight, twice daily). Intervention group (n=47): continued oral valganciclovir for a further 4.5 months (total 6 months treatment)
Comparator	Comparison group (n=49): placebo for a further 4.5 months (total 6 weeks treatment)
Results/outcomes	Primary outcome: change in hearing in the better ear (“best-ear” hearing) from baseline to 6-month follow-up • Intervention: 2/43 improved, 5/43 worsened, 36/43 no change • Placebo: 3/43 improved, 3/43 worsened, 37/43 no change • No significant difference in change between groups after adjustment for

	baseline neurological involvment: adjusted odds ratio (aOR) 1.75, 95% CI 0.69 to 4.43 (p=0.24)
	
	Secondary outcomes:
	• change in best-ear hearing from baseline to 12 and 24 months
	  ○ 12 Months
	  ▪ Intervention: 2/41 improved, 3/41 worsened, 36/41 no change
	  ▪ Placebo: 2/40 improved, 5/40 worsened, 33/40 no change
	  ▪ aOR 2.81, 95% CI 0.99 to 7.99 (p=0.05)
	  ○ 24 months
	  ▪ Intervention: 2/37 improved, 3/37 worsened, 32/37 no change
	  ▪ Placebo: 2/31 improved, 2/31 worsened, 27/31 no change
	  ▪ aOR 3.28, 95% CI 0.91 to 11.9 (p=0.07)
	• total-ear hearing (i.e. hearing in one or both ears that could be evaluated) from baseline to follow-up at 6, 12, and 24 months (n=ears):
	  ○ 6 Months
	  ▪ Intervention: 6/82 improved, 11/82 worsened, 65/82 no change
	  ▪ Placebo: 7/84 improved, 9/84 worsened, 68/84 no change
	  ▪ aOR 1.69, 95% CI 0.76 to 3.73 (p=0.20)
	  ○ 12 Months
	  ▪ Intervention: 6/79 improved, 6/79 worsened, 6/79 no change
	  ▪ Placebo: 4/77 improved, 10/77 worsened, 63/77 no change
	  ▪ (73% vs. 57% improvement) aOR 3.04, 95% CI 1.26 to 7.35 (p=0.01)
	  ○ 24 months
	  ▪ Intervention: 6/70 improved, 8/70 worsened, 56/70 no change
	  ▪ Placebo: 2/58 improved, 5/58 worsened, 51/58 no change
	  ▪ (77% vs. 64% improvement) aOR 2.61, 95% CI 1.05 to 6.43 (p=0.04)
	• neurologic impairment at 12 and 24 months (assessed on Bayley-III Scales of Infant and Toddler Development)
	  ○ treatment significantly improved language-composite scores at 24 months (p=0.005) and receptive-communication scale scores at 24 months (p=0.003)
	  ○ no other components significant
	• adverse events leading to permanent discontinuation of therapy: none
	
	Other safety outcomes:

	• 19% of participants had grade 3 or 4 neutropenia during the first 6 weeks open treatment • 21% of the intervention group vs. 27% of placebo had grade 3 or 4 neutropenia from 6 weeks to 6 months (p=0.64) • Aminotransferase levels increased slightly more between months 4 and 5 in the treatment group, but no significantly more than placebo (p>0.59).
Comments Double blind: participants unaware of treatment and hearing assessed by an independent audiologist who was unaware of treatment assignment. Adequately powered: calculated that 37 per group would be needed to have 85% power to detect on effect size of 0.169. Over-enrolment allowed for drop-out. Findings not applicable to asymptomatic newborns. Assesses outcomes of neutropenia, but uncertain whether valganciclovir may be associated with other carcinogenic and gonad toxicity effects observed with ganciclovir.	

Appendix number	6
Relevant criteria	10
Publication details	Alarcon A, Martinez-Biarge M, Cabañas F, et al. Clinical, biochemical, and neuroimaging findings predict long-term neurodevelopmental outcome in symptomatic congenital cytomegalovirus infection. Journal of Pediatrics. 2013;163(3):828-34.e1.[28]
Study details	Cohort (prospective from 2000; 15 infants) Single centre, Spain, 1993 to 2009
Study objectives	To assess clinical and CSF and neuroimaging findings in the newborn as predictors of long-term neurodevelopmental outcomes in symptomatic congenital CMV infection.
Inclusions	All newborns with symptomatic CMV admitted to the study hospital. CMV diagnosed through isolation of CMV DNA in blood or urine or detection of CMV IgM or viral antigen in blood during the first 2 weeks of life. Symptomatic defined as: IUGR, petechiae, hepatomegaly, splenomegaly, microcephaly, SNHL, chorioretinitis, thrombocytopaenia, intracranial calcifications, serology indicating hepatitis or cholestasis.
Exclusions	Those with major malformations, genetic and chromosomal syndrome, other congenital infection or significant CNS disease unrelated to CMV infection.
Population	N=26
Assessments/tests	At birth: • CSF evaluation (performed for n=21), median age of analysis 8.5 days (ranged 1-30 days) • Neuroimaging studies (n=9 US and CT; n=8 US and MRI; n=1 US, CT and MRI; n=1 CT only; n=7 US only) Neurodevelopmental assessments: • Bayley Scales of Infant and Toddler Development, Third Edition (BSID-III) up to 3 years. • Wechsler Preschool and Primary Scale of Intelligence, Third Edition at 4-6 years. • Wechsler Intelligence Scale for Children, Fourth Edition at 6-17 years. • Child Behaviour Checklist at 1.5-5 and 6-18 years.

Comparator

NA

Results/outcomes

Follow-Up: mean 8.7 years (+/- 5.3); 3/26 had died.

Neonatal findings with a significant association to adverse long-term outcomes: microcephaly (adjusted for birthweight), elevated CSF protein and β2-microglobulin (β2-m) concentration, and grade 2-3 neuroimaging abnormalities:

Disability	**Adjusted microcephaly**			**CSF β2-m >7.9mg/l**			**Neuroimaging score 2-3**		
	Yes (%)	**No (%)**	**p**	**Yes (%)**	**No (%)**	**p**	**Yes (%)**	**No (%)**	**p**
SNHL	4/6 (66)	6/17 (35)	0.314	6/7 (85)	2/11 (18)	0.013	6/8 (75)	4/15 (26)	0.039
Visual deficit	3/6 (50)	1/17 (5)	0.04	2/7 (28)	1/11 (9)	0.528	4/8 (50)	0/15 (0)	0.008
Seizures	5/6 (83)	3/17 (17)	0.009	3/7 (42)	3/11 (27)	0.627	6/8 (75)	2/15 (13)	0.006
Cerebral palsy	6/6 (100)	4/17 (23)	0.002	4/7 (57)	3/11 (27)	0.332	8/8 (100)	2/15 (13)	<0.001
Cognitive deficit	6/6 (100)	6/17 (35)	0.014	6/7 (85)	3/11 (27)	0.050	8/8 (100)	4/15 (26)	0.001
Behavioural disorder	1/3 (33)	3/17 (17)	0.509	1/5 (20)	1/11 (9)	1.000	1/5 (20)	3/15 (20)	1.000
Death	2/8 (25)	1/17 (5)	0.215	2/9 (22)	0/11 (0)	0.189	3/11 (27)	0/15 (0)	0.063
Moderate/severe disability	8/8 (100)	10/18 (55)	0.031	9/9 (100)	4/11 (36)	0.005	11/11 (100)	7/15 (46)	0.007

Predictive value of prognostic factors for poor outcome:

	Sensitivity %	**Specificity %**	**PPV %**	**NPV %**	**OR (95%CI)**	**AUC**
Adjusted microcephaly	44	100	100	44	1.80 (1.19 to 2.72)	0.72(+/-0.09)
CSF β2-m >7.9mg/L	69	100	100	63	3.25 (1.43 to 7.34)	0.84(+/-0.08)
Neuroimaging score 2-3	61	100	100	53	2.57 (1.44 to 4.58)	0.80 (+/-0.08)
Microcephaly or high β2-m	82	100	100	70	5.66 (2.02 to 15.82)	0.91 (+/-0.06)
Microcephaly or neuro 2-3	66	100	100	57	3.00 (1.56 to 5.76)	0.83 (+/-0.07)
high β2-m or neuro 2-3	87	100	100	77	8.00 (2.18 to 29.24)	0.92 (+/-0.06)

Comments

Excludes newborns with asymptomatic CMV. Very small sample size – outcomes available for only 23 surviving children. Wide confidence intervals decrease reliability of findings. Partially retrospective study covering long time period. Inconsistency in availability of CSF analysis and neuroimaging across the sample. Age at CSF analysis ranged from birth to 4 weeks of age. Possible confounding from ganciclovir treatment given to 17/26 (65%). Assessed for prediction of global adverse outcome rather than specific outcome such as hearing loss.

Appendix number	7
Relevant criteria	10
Publication details	Forner G, Abate D, Mengoli C, et al. High cytomegalovirus (CMV) DNAemia predicts CMV sequelae in asymptomatic congenitally infected newborns born to women with primary infection during pregnancy. Journal of Infectious Diseases. 2015;212(1):67-71.[27]
Study details	Prospective cohort Single centre, Italy, 2004 to 2007
Study objectives	To investigate the relationship between the viral load in blood at birth and the development of late-onset sequelae in asymptomatic congenital CMV infection.
Inclusions	All newborns with asymptomatic CMV born to mothers with primary infection. Primary infection defined as seroconversion in previously seronegative mothers or specific IgG, IgM, and low IgG avidity (<25%). CMV diagnosed through isolation of CMV in urine during the first week of life.
Exclusions	Symptomatic and treated newborns. Symptoms defied as: ≥1 of microcephaly (head circumference below the fifth percentile), hypotonia, hemiparesis and seizures, SNHL and deafness, chorioretinitis, cholestasis, and hepatosplenomegaly (with elevated ALT).
Population	N=33
Assessments/tests	At birth: • Serology: CMV IgM and IgG, PCR analysis of CMV DNA (sensitivity 100 copies per ml blood) • Cerebral ultrasound • Hearing and visual assessment Follow-up assessments at 1, 3, 6, 12, 18, 24, 30, and 36 months, then annually to 6 years: • clinical, virological, hearing, visual and neurodevelopmental (Bayley III scale up to 36 months and NEPSY II scale from 4-6 years) • Late-onset sequelae defined as: ≥1 of hemiparesis, hypertonia or hypotonia, psychomotor retardation, and unilateral or bilateral SNHL.
Comparator	NA

Results/outcomes	10/33 (30%) developed late-onset sequelae at 3 months to 5 years: 8 with unilateral or bilateral SNHL, 2 with psychomotor retardation, 1 with progressive right-side hemiparesis. Remaining 23/33 (70%) had no symptoms up to 6 years. Mean viral load at birth: 1770 DNA copies/ml (95% CI 960 to 3262) in symptom-free children vs. 17,045 copies/ml (95% CI 6164 to 47,133) in those who developed late-onset disease (p=0 .0002). Risk of clinical disease crossed the 50% threshold with a DNAemia at birth of ≥12,000 copies/ml (p=0.0002). Risk of hearing deficit crossed the 50% threshold with a DNAemia of ≥17,000 copies/ml (p=0.0001). No significant difference in time to CMV clearance in blood and urine of symptom-free children vs. those who developed late onset disease (p=0.96 and p=0.88) No significant association with predictive factors of maternal age or gestation at time of primary infection.
Comments Excludes newborns with symptomatic CMV. Very small sample size with only 10/33 developing late-onset sequelae. Timing of blood sample could have significant effect (performed in this sample during first days of life).	

Appendix number	**8**
Relevant criteria	10
Publication details	Bilavsky E, Schwarz M, Pardo J, et al. Lenticulostriated vasculopathy is a high-risk marker for hearing loss in congenital cytomegalovirus infections. Acta Paediatrica, International Journal of Paediatrics. 2015;104(9):e388-e94.[29]
Study details	Retrospective cohort study. Schneider Children's Medical Center, Israel, January 2005 to December 2012.
Study objectives	To report the experiences of one centre when treating a large group of infants with CCMV and to determine the relationship between lenticulostriated vasculopathy (LSV) and hearing loss.
Inclusions	All newborns with CCMV (diagnosed by urine culture or PCR assay during the first 2 weeks of life). Symptomatic infection defined by any one of the following: (i) microcephaly, head circumference <3%; (ii) hearing impairment detected by the ABR; (iii)

	chorioretinitis; and (iv) abnormal findings on brain ultrasound including calcifications, periventricular hyperechosity, ventricular dilatation and pseudocysts. Symptomatic infants treated with either 6 weeks intravenous ganciclovir then 6 weeks oral valganciclovir, or with 12 weeks oral valganciclovir, both followed by one daily dose to one year of age. LSV was considered a sign of CNS involvement from mid-2009 onwards and a basis for starting treatment.
Exclusions	None reported.
Population	N=210 newborns – 158 symptomatic and 52 asymptomatic – with follow-up to over one year. 139/158 symptomatic newborns with abnormal cerebral US. LSV most common in 114/158 (82%) – 25 (22%) of whom had other abnormalities and for 89 (78%) this was the only abnormality. N=89 symptomatic newborns with isolated LSV: • Group 1 – normal ABR at birth and not treated (n=13) (pre-2009) • Group 2 – normal ABR at birth and treated (n=51) (post-2009) • Group 3 – abnormal ABR at birth and treated (n=25)
Assessments/tests	Ultrasound over the anterior and posterior fontanelle and asterion performed after birth. Auditory thresholds of all infants diagnosed with CCMV studied using audiometry brainstem response (ABR) within 4 weeks of birth and repeated every 4-6 months to age 4.
Comparator	Comparison: Group 4 asymptomatic newborns (n=52)
Results/outcomes	Hearing deterioration defined as an increase of $\geq$10 dB in the auditory threshold in one or two ears during two consecutive assessments or two behavioural tests resulting in a change in hearing category, such as from normal-to-mild, mild-to-moderate or moderate-to-severe hearing loss. Hearing deterioration occurred in 16/116 with normal hearing at birth: • Group 1: 11/13 (84.6%) • Group 2: 0/51 (0%) • Group 4: 5/52 (9.6%) Group 1 experienced significantly higher hearing loss than group 2 ($p<0.001$) and group 4 ($p<0.001$), and in 2 more than 4 ($p=0.008$).

Comments

Newborns with normal hearing and isolated LSV on ultrasound are assumed otherwise asymptomatic but unclear if newborns may have had other organ-involvement that may have been an indication for treatment. However, as organ involvement was not assessed for the asymptomatic group either, assume this would have balanced out both groups.

Very small sample size in the untreated group due to change in policy to start treating those with LSV as the isolated finding.

92% of the full sample was born to mothers with primary infection which the researchers consider may account for the high rate of LSV – may not be representative of all newborns with CCMV.

As researchers say, incidence of LSV in healthy newborns without CCMV isn't known so don't know how specific a marker/indicator it is. Their literature search suggested about 5% of infants scanned for other indications had LSV, but none observed in a healthy population – estimated around 1% of all births.

Better follow-up is needed of untreated infants with LSV.

Appendix B: studies of antenatal screening

Appendix number	i
Publication details	Ebina Y, Minematsu T, Sonoyama A, et al. The IgG avidity value for the prediction of congenital cytomegalovirus infection in a prospective cohort study. Journal of Perinatal Medicine. 2014;42(6):755-9.[12]
Study details	Prospective cohort Single centre, Japan. Study period April 2009 to Jan 2013.
Study objectives	To assess IgG avidity for predicting congenital CMV infection.
Inclusions	Pregnant women suspected of having CMV infection and referred to the university hospital (indications for suspicion not given)
Exclusions	None reported.
Population	913 referred pregnant women and their newborns.
Test	Serum CMV IgG measured at 16-18 weeks gestation or at referral. Positive test: IgG avidity measured. All newborns received PCR analysis for CMV DNA in urine collected during the first year of life.
Comparator	IgG avidity index of CMV-positive newborns compared with those CMV-negative.
Results/outcomes	759/913 women (83.1%) IgG positive 14/759 newborns (1.8%) had congenital CMV (745, 98.2% negative) CMV IgG avidity indices in the congenital CMV group were significantly lower than in the non- CMV group: median 35.1% (range 2.3 to 77.8%) vs. 70.4% (range 7.6 to 97.3%), p<0.0001. Infection rates by IgG avidity index: • <20%: 6/12 (50%) • <30%: 6/20 (30%) • <40%: 9/38 (23.7%) • >40%: 5/721 (0.7%) • >70%: 3/389 (0.8%) ROC area under the curve 0.802 for IgG avidity index for predicting congenital infection. IgG avidity index 40% was the optimal cut-off: 64.3% sensitivity and 96.1%

DRAFT

	specificity for infection. Best performance by gestational age: <28 weeks: sensitivity 88.9%, specificity 96.2%, PPV 27.6%, NPV 99.8%
Comments Not fit to criterion 5 because of a selective population of women suspected of having infection. IgG avidity index <40% assessed for predicting congenital infection, but does not correspond with certainty with primary maternal infection. Serological tests and interpretation of IgG avidity index may vary between laboratories. Broad range in the timing of measurements. Small sample with only 14 cases.	

<table>
<tr><th>Appendix number</th><th>ii</th></tr>
<tr><td>Publication details</td><td>Yamamoto AY, Mussi-Pinhata MM, Isaac MDL, et al. Congenital cytomegalovirus infection as a cause of sensorineural hearing loss in a highly immune population. Pediatric Infectious Disease Journal. 2011;30(12):1043-6.[13]</td></tr>
<tr><td>Study details</td><td>Prospective cohort
Two hospital centres, Brazil.
Study period March 2003 to May 2009.</td></tr>
<tr><td>Study objectives</td><td>To assess the rate, associated factors and predictors of CMV-associated sensorineural hearing loss (SNHL) in a highly seropositive maternal population.</td></tr>
<tr><td>Inclusions</td><td>Total eligible population 12,295 newborns screened for CMV during the study period – those with congenital CMV included.</td></tr>
<tr><td>Exclusions</td><td>NA</td></tr>
<tr><td>Population</td><td>121 newborns with congenital CMV diagnosed by PCR detection of CMV DNA in saliva or urine collected in the first two weeks of life, and confirmed by virus isolation in tissue culture.</td></tr>
<tr><td>Test</td><td>Primary maternal infection: IgG seroconversion during pregnancy or initial low IgG avidity index with increase at time of delivery.
Non-primary infection: IgG before pregnancy and high IgG avidity without IgM.
Newborn assessments: Affected infants underwent clinical examination, opthalmology and CT of the brain. Auditory brainstem evoked response (ABR) within the first year and up to 3 years of age. SNHL defined as 2 assessments with ABR >30dB.</td></tr>
<tr><td>Comparator</td><td>NA</td></tr>
<tr><td>Results/outcomes</td><td>10% (12/121) infants had symptomatic congenital infection.
11.8% (10/85 with 2 ABR assessments) had SNHL. No children had progressive deficit as assessed up to median 56 months.
Maternal infection status known for 50% of mothers of infected newborns with 2 ABR assessments (43/85).
<table>
<tr><th rowspan="2">Hearing status</th><th colspan="3">Maternal CMV infection (n=85)</th></tr>
<tr><th>Primary (n=3)</th><th>Non-primary (n=40)</th><th>Uncertain (n=42)</th></tr>
<tr><td>Normal</td><td>2</td><td>34</td><td>39</td></tr>
<tr><td>Moderate/ severe unilateral</td><td>0</td><td>4</td><td>1</td></tr>
<tr><td>Moderate/ profound bilateral</td><td>1</td><td>2</td><td>2</td></tr>
</table></td></tr>
</table>

Hearing loss by infection	33.3% (1/3)	15% (6/40)	7.1% (3/42)
Proportions of hearing loss	10% (1/10)	60% (6/10)	30% (3/10)

Birth characteristics of 85 infected newborns with 2 ABR assessments with hearing loss

	Hearing loss (n=10)	**Normal hearing (n=75)**	**Adjusted OR (95% CI)**
Asymptomatic newborn (n=75)	4 (5.3%)	71 (94.7%)	1.0
Symptomatic newborn (n=10)	6 (60.0%)	4 (40.0%)	38.1 (1.6 to 916.7)
Normal for gestational age (n=63)	4 (6.4%)	59 (93.6%)	1.0
Small for gestational age (n=22)	6 (27.3%)	16 (72.7%)	7.3 (0.7 to 72.9)
Term ≥37 wks (n=60)	8 (13.3%)	52 (86.7%)	1.0
Preterm <37 wks (n=25)	2 (8.0%)	23 (92.0%)	7.2 (0.5 to 106.2)
Male (n=49)	3 (6.1%)	46 (93.9%)	1.0
Female (n=36)	7 (19.4%)	29 (80.1%)	12.4 (0.9 to 163.9)

Comments

Hearing assessments only performed for 70% of those with congenital CMV.

Maternal infection status only known for half of these.

Small sample size when divided by characteristics greatly reduces confidence in associations.

Appendix number	iii
Publication details	Townsend CL, Forsgren M, Ahlfors K, et al. Long-term outcomes of congenital cytomegalovirus infection in Sweden and the United Kingdom. Clinical Infectious Diseases. 2013;56(9):1232-9.[14]
Study details	Re-analysis of data from two prospective cohorts: 1 centre in Sweden (1977 to 1985), and 3 hospital centres in London (1979 to 1986)
Study objectives	To better understand the natural history of congenital CMV.
Inclusions	Sweden: pregnant women with serology at weeks 24 to 32, and newborn cases and matched controls followed up to 10 years. UK: pregnant women with serology at first antenatal visit and weeks 27 to 35, and newborn cases and matched controls followed up to 5 years
Exclusions	NA
Population	Total of around 50,000 infants screened and 176 with congenital CMV Sweden: 76 infants (incidence 4.6 per 1000 births) and 62 controls UK: 100 infants (incidence of 3.2 per 1000 births) and 152 controls
Test	<u>Maternal infection</u> Confirmed primary: seronegative at first antenatal blood sample and subsequent sample IgG positive (n=48); or IgG negative on single antenatal blood sample and infant born with confirmed congenital infection (n=11) Presumed primary: first antenatal blood sample IgM positive (n=23) Confirmed non-primary: IgG positive on blood sample before conception (n=21) Presumed non-primary: IgG positive/IgM negative on 1st trimester blood sample (n=23); or ≥4-fold rise in IgG without the appearance of IgM (n=1) Unclassified: Tests not fulfilling criteria, or no maternal sample available (n=49) <u>Newborn</u> Defined as symptomatic: ≥ 1 of petechiae, tachypnea, hepatomegaly, splenomegaly, thrombocytopenia, seizures, microcephaly, and hypotonia Child classed as normal development, mild (unilateral SNHL, mild bilateral SNHL, mild motor impairment with minimal implications, or clinically recognized developmental or language delay in the absence of hearing loss or other problems), moderate (moderate or severe bilateral SNHL, mild bilateral SNHL and mild cerebral palsy, or moderate learning difficulties) or severe (severe disability or multiple problems) impairment.

<table>
<tr><td>Comparator</td><td>NA</td></tr>
<tr><td>Results/outcomes</td><td>19/176 (11%) symptomatic at birth – more from Sweden (18%) than UK (5%)

157/176 (89%) asymptomatic

154 (87%) with known outcome at 5 years.

• 127/154 (82%) normal development – 11 symptomatic at birth
• 11/154 (7%) mild development – 2 symptomatic at birth
• 7/154 (5%) moderate development – 1 symptomatic at birth
• 9/154 (6%) severe development – 5 symptomatic at birth
• 14/154 (9%) had SNHL

<table>
<tr><th>Maternal infection</th><th>Number (n=176)</th><th>Symptomatic at birth</th><th>Normal/mild outcome</th><th>Moderate/severe outcome</th></tr>
<tr><td>Confirmed primary</td><td>59 (33.5%)</td><td>4 (6.8%)</td><td>52 (98.1%)</td><td>1 (1.9%)</td></tr>
<tr><td>Presumed primary</td><td>23 (13.1%)</td><td>4 (17.4%)</td><td>16 (80.0%)</td><td>4 (20.0%)</td></tr>
<tr><td>Overall primary</td><td>82 (46.6%)</td><td>8 (9.8%)</td><td>68 (82.9%)</td><td>5 (6.1%)</td></tr>
<tr><td>Confirmed non-primary</td><td>21 (11.9%)</td><td>2 (9.5%)</td><td>15 (88.2%)</td><td>2 (11.8%)</td></tr>
<tr><td>Presumed non-primary</td><td>24 (13.6%)</td><td>4 (16.7%)</td><td>15 (68.2%)</td><td>7 (31.8%)</td></tr>
<tr><td>Overall non-primary</td><td>45 (25.6%)</td><td>6 (13.3%)</td><td>30 (66.7%)</td><td>9 (20%)</td></tr>
<tr><td>Unknown</td><td>49 (27.8%)</td><td>5 (10.2%)</td><td>40 (95.2%)</td><td>2 (4.8%)</td></tr>
</table>

SNHL reported for 15% of children following non-primary maternal infection (6/39) and 5% following primary infection (4/73) (p=0.09).

42.1% of symptomatic newborns had any impairment vs. 14.1% of asymptomatic (p=0.006) – not specified by symptoms.</td></tr>
<tr><td colspan="2">Comments

Small number of symptomatic cases and uncertain association with maternal infections status.

Studies conducted 30 years ago. Diagnostic techniques may have changed.</td></tr>
</table>

Appendix number	iv
Publication details	De Vries JJC, Van Zwet EW, Dekker FW, et al. The apparent paradox of maternal seropositivity as a risk factor for congenital cytomegalovirus infection: A population-based prediction model. Reviews in Medical Virology. 2013;23(4):241-9.[15]
Study details	Predictive model incorporating a new systematic review and meta-analysis
Study objectives	To analyse the contribution of non-primary maternal CMV infection on congenital infection and CMV-related hearing loss as a function of population seroprevalence. A prediction model was developed, informed by data from previously published meta-analyses and a newly performed systematic review and meta-analysis on the risk of hearing loss after primary and non-primary maternal infection.
Inclusions	New SR on risk of hearing loss by infection: PubMed search for relevant English language articles using the search strategy: (congenital AND cytomegalovirus) AND (hearing loss OR hearing impairment OR deafness OR sequela OR sequelae OR CNS) AND (primary OR non-primary OR non-primary OR secondary OR recurrent OR immunity OR seropositive OR seronegative) in the title/abstract field. Included articles: prospective studies of at least 4 years duration, sample size >30, CMV detected by newborn screening, and reporting the proportion of hearing loss and both primary maternal and non-primary infections. The parameters in the population-based prediction model were developed from serosurvey data from previously published meta-analyses (method of identification not reported in main study).
Exclusions	None specified.
Population/studies	7 prospective cohorts – 2 reporting the same cohort but one containing primary data, the other non-primary. Specifics on method, total sample size and country of studies not reported.
Results/outcomes	The pooled proportions (by random effects model) for hearing loss: 13% (50/385, 95% CI 10 to 16%) after primary vs. 11% (28/253, 95% CI 7 to 15%) after non-primary. Prediction model estimates (development not reported here): Survey data indicated at all population seroprevalences, non-primary maternal infections were responsible for most congenital CMV infections, ranging from

	57% contribution (95%CI 24 to 85%) at seroprevalence of 30% to 96% (95% CI 88 to 99%) at seroprevalence of 95%. Including data from the new SR, the proportions of children with CMV-related hearing loss attributable to non-primary infection • 53% (95% CI 13 to 86%) at seroprevalence of 30% to 95% (95% CI 62 to 99%) at seroprevalence of 95% (66% at 50% seroprevalence); • 2 per 10,000 births (95% CI 0 to 6) in a population with seroprevalence 30%, to 22 per 10,000 births (95% CI 0 to 48) at 95% seroprevalence Comparative proportion from primary infection: • 1 per 10,000 births (95% CI 0 to 2) in populations with population seroprevalence of 30% to 95%
Comments Variability in design and methods of included studies: potential for selection bias (e.g. symptomatic cases or different socioeconomic groups), misclassification of primary or non-primary infection (e.g. some studies reported "presumed"), differences in laboratory diagnosis of congenital CMV, and different follow-up assessments and timing. Cannot assess the severity of hearing loss (e.g. unilateral or bilateral).	

Appendix number	v
Publication details	Goderis J, De Leenheer E, Smets K, et al. Hearing loss and congenital CMV infection: A systematic review. Pediatrics. 2014;134(5):972-82.[16]
Study details	Systematic review and meta-analysis
Study objectives	To provide an overview of the prevalence of congenital CMV-related hearing loss, to better define the nature of the condition, and to investigate the importance of congenital CMV infection in hearing-impaired children.
Inclusions	Medline search (2013) for articles about hearing loss and congenital CMV using the subject headings: congenital cytomegalovirus AND (hearing OR deafness OR auditory), combined with the results for perinatal cytomegalovirus AND (hearing OR deafness OR auditory) in all fields; plus manual searching. Inclusion criteria: studies including 3 or more symptoms at birth (petechiae, jaundice with conjugated hyperbilirubinemia, hepatosplenomegaly, thrombocytopenia, chorioretinitis, seizures, microcephaly, and intracranial calcifications), diagnostic confirmation by virus isolation or PCR analysis of urine or saliva in the first 3 weeks of life, and including 20 or more cases.
Exclusions	Duplicates, non-English articles, non-primary sources, studies of treated infants.
Population/studies	14 prospective cohorts were included in quantitative analysis which reports on primary/non-primary infection. Meta-analysis cites 8 studies, but total sample and further specifics not reported. Other analyses were quantitative (n=10) and retrospective (n=13).
Results/outcomes	Relevant results: • Hearing loss in primary infection: 12.1%, 95% CI 8.6 to 16, $I^2$18.8, p for heterogeneity 0.2814 • Hearing loss in non-primary infection: 11.8%, 95% CI 7.5 to16.8, I^2 21.7, p for heterogeneity 0.2568
Comments Included studies were only of symptomatic newborns. Other potential for variability in design and methods of included studies: potential selection bias, misclassification of primary or non-primary infection, differences follow-up assessments and timing. Cannot assess the severity of hearing loss (e.g. unilateral or bilateral).	

Appendix vi: Summary of cohorts reporting transmission rate and newborn outcomes by timing of primary infection

Study	Population	Confirmation maternal infection	Confirmation congenital infection	Transmission rate by trimester	Severity of newborn outcomes by timing of transmission
Enders et al. (2011)[10] Single centre, Germany Prospective cohort (1990 to 2010)	248 pregnant women with confirmed primary infection by trimester and known pregnancy outcome	IgG seroconversion; ≥four-fold IgG-rise in presence of high IgM; high IgM and IgG with low IgG avidity and clinical symptoms	CMV DNA in urine of newborns (n=230) or fetal tissue in TOP (n=18)	Overall transmission rate: 37.9% (94/248) Pre-conception (1-10 wks before LMP): 16.7% (4/24) Peri-conception (1 wk before LMP to 5wks): 34.5% (10/29) 1st trimester (5 to 14 wks): 30.1% (25/83) 2nd trimester: (14 to 26 wks): 38.2% (29/76) 3rd trimester: (26 wks to delivery): 72.2% (26/36) (p<0.0001)	Overall 22.8% (19/87) symptomatic CNS involvement in 10.3% (9/87) Not reported by timing of infection.
Feldman et al. (2011)[37] Single centre, Israel Prospective cohort (2000 to 2006)	508 pregnant women with confirmed primary infection by trimester and known pregnancy outcome	IgG seroconversion in a previously seronegative woman; or IgM and IgG with low IgG avidity	CMV DNA in amniotic fluid (485; 93%); urine of newborn (39; 7%); or both (379; 85%)	Overall transmission rate: 23.2% (118/508) Pre-conception (1 yr to 8wks before LMP): 0% (0/97) Peri-conception (8 wk before LMP to 6wks): 4.6% (6/130) 1st trimester (6 wks to 13 wks): 34.8% (53/152) 2nd trimester: (13 to 26 wks): 42.0% (42/100) 3rd trimester: (26 wks to delivery): 58.6% (17/29) (p=0.049)	Abnormal ultrasound: 14.4% (17/118) Pre-conception: 0% (no infections) Peri-conceptual: 33% (2/6) 1st trimester: 17% (9/53) 2nd trimester: 14% (6/42) 3rd trimester: 0% (0/17)
Picone et al. (2013)[11] Single centre, France Retrospective cohort (2004 to 2012)	238 pregnant women with confirmed primary infection with precise gestational dating and known fetal/newborn status	IgG seroconversion or positive IgM with low IgG avidity	CMV DNA in amniotic fluid (86, 36%) and or/ confirmation in newborn (not specified)	Overall transmission rate: 24.9% (60/241) Pre-conception (8 to 3wks before LMP): 8.8% (3/34) Peri-conception (3 wk before LMP to 3wks): 19% (15/79) 1st trimester (3 wks to 14 wks): 30.6% (22/72) 2nd trimester: (14 to 28 wks): 34.1% (14/41) 3rd trimester: (28 wks to delivery): 40% (6/15) (p=0.025)	Abnormal ultrasound: 38.3% (23/60) Pre-conception: 100% (3/3) Peri-conceptual: 60% (9/15) 1st trimester: 45.4% (10/22) 2nd trimester: 7.1% (1/14) 3rd trimester: 0% (0/6)

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Revello et al. (2010)[38] Single centre, Italy Retrospective cohort (1990 to 2009) (Symptom outcomes reported by Zavattoni et al. 2014[39])	695 pregnant women with confirmed primary infection (aim to review role of diagnosis and counselling on pregnancy outcome)	IgG seroconversion in a previously seronegative woman; kinetics of IgM and IgG; low IgG avidity; CMV antigen or DNA in blood.	CMV DNA in amniotic fluid and/or viral isolation in newborn blood and urine or autopsy analysis (data reported for a total 555)	Overall transmission rate: 37.1% (206/555) Pre-conception (not defined): 5.7% (6/106) 1st trimester (to 12 wks): 42.2% (111/263) 2nd trimester: (13 to 22 wks): 43.5% (64/147) 3rd trimester: (23 wks to delivery): 64.1% (25/39) (p=0.035)	Symptomatic: 18.7% (28/150) Peri-conceptual: 33.3% (6/18) 1st trimester: 25.5% (12/47) 2nd trimester: 14.3% (8/56) 3rd trimester: 8% (2/25) NB. Unclear discrepancy in number of cases (150 rather than 206 in Revello) and peri-conceptual definition (not used in Revello)

Appendix number	vii
Publication details	Yoshida M, Matsuda H, Yoshinaga Y, et al. Can measurement of maternal anti-cytomegalovirus immunoglobulin-M antibody levels be used to screen for cytomegalovirus infection in embryos and fetuses? Journal of Obstetrics and Gynaecology Research. 2013;39(1):166-9.[17]
Study details	Prospective cohort study Single centre (National Defense Medical College Hospital), Japan. Study period January 2005 to December 2009.
Study objectives	To see whether measurement of maternal anti-CMV immunoglobulin M (IgM) antibody levels are useful as a screening tool for early detection of CMV infection.
Inclusions	All pregnant women attending the study hospital during their first trimester.
Exclusions	None reported.
Population	n=2865 women
Intervention/test	Serological testing for CMV IgM Titre ≥0.08 was screen positive.
Comparator	Screen positives received confirmation by real-time PCR assay for CMV DNA in amniotic fluid sample.
Results/outcomes	21/2865 (0.73%) screen positives 2844/2865 (99.27%) screen negatives 1/21 positive for CMV DNA in amniotic fluid (TP) 20/21 negative (FPs) PPV of IgM serology for predicting amniotic CMV DNA (TP/TP+FP) = 1/21 = 4.8% Follow-up conducted for the one TP – no symptoms or signs of CMV infection at birth and no CMV antigen in cord blood.
Comments Specifically evaluating 1st trimester testing. No follow-up of screen negatives, therefore cannot determine Sn, Sp or NPV due to uncertainty about TN and FN rates. Uncertain performance of reference standard - no signs or symptoms of congenital infection in the positive case, though analysis of newborn urine was not performed.	

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Non-selective sample though single centre and unsure if prevalence could differ from UK.

Question	Assessment (Y, N, unclear)	Risk of Bias (low, high, unclear)	Supporting info
Domain I: Patient selection			
Consecutive or random sample of population enrolled?	Y	Low	All women attending during the study period.
Case-control design avoided?	Y	Low	Not case control.
Inappropriate exclusions avoided?	Y	Low	No apparent exclusions.
Domain II: Index Test			
Index test results interpreted without knowledge of reference standard results?	Y	Low	IgM used as guide to further confirmation.
Threshold pre-specified?	Y	Low	Yes.
Domain II: Reference standard			
Reference standard likely to correctly classify condition?	Unclear	High	Reported performance of amniocentesis for detecting CMV DNA ranged 70-100%. Screen negatives not followed, and those positive by amniocentesis did not all show signs of congenital infection.
Reference standard results interpreted without knowledge of index test results?	N	High	Screen result used as guide to performing reference standard.
Domain IV: Test strategy flow and timing			
Appropriate interval between index test and reference standard?	Unclear	Unclear	No apparent issues.
Did all participants receive same reference standard?	N	High	Only screen positives were tested
All patients included in analysis?	N	High	As above only screen positives were tested.

Applicability			
Applicable to UK screening population of interest?	Unclear	Unclear	Unclear whether CMV prevalence may differ in the UK.
Applicable to UK screening test of interest?	Unclear	Unclear	Amniocentesis to check for CMV DNA may be used but no congenital testing such as newborn urine sample.
Target condition measured by reference test applicable to UK screening condition of interest?	Y	Low	CMV

Appendix number	viii
Publication details	Revello MG, Lazzarotto T, Guerra B, et al. A randomized trial of hyperimmune globulin to prevent congenital cytomegalovirus. New England Journal of Medicine. 2014;370(14):1316-26.[19]
Study details	Phase II, double-blind RCT 11 centres in Italy, June 2009 to March 2011.
Study objectives	To verify previous results from a non-randomised study finding that CMV-specific hyperimmune globulin was effecting in preventing fetal infection.
Inclusions	Pregnant women with primary infection at 5 to 26 weeks gestation and presumed onset within the past 6 weeks. Diagnosis based on seroconversion or presence of CMV-specific IgM antibodies and low IgG avidity in presence of symptoms. In asymptomatic cases, onset was placed midway between a negative sample and seroconversion.
Exclusions	None reported. 33/157 eligible women (21%) declined to participate.
Population	124 randomised: 61 to intervention, 63 to placebo. 1 drop-out in the placebo group; 123 included in ITT analysis.
Intervention	Hyperimmune globulin (50 U of anti-CMV IgG antibody per ml) infused once every 4 weeks until 36 weeks gestation, detection of CMV in amniotic fluid or miscarriage/stillbirth.
Comparator	Placebo (0.9% saline)
Results/outcomes	Primary outcome: transmission to fetus/newborn • Overall transmission rate 37% (45/123): diagnosed by amniocentesis in 18 and at birth in 27 • No significant difference in rate between intervention (18/61; 30%) and placebo group (27/62; 44%), p=0.13 Secondary outcomes: • No significant difference in viral load in amniotic fluid or urine or blood of newborn • No significant difference in fetal ultrasound • No significant difference in maternal serological measures of IgM, IgG, IgG avidity and neutralising antibodies, lymphocytes and viral load • No significant difference in characteristics of women who did and did not transmit infection Adverse events: 20 in 16 women: 11 serious in 10 women: 7 in the intervention group, 3 in the

	placebo group. Most frequent adverse event in intervention group was preterm delivery: 7/48 vs. 1/47 in placebo group (p=0.06).
Comments Double blind, ITT analysis. Power calculation estimated required enrolment of 60 per group. However, due to low outcome rate it was estimated that triple the participants would have been needed to have had the power to detect a 14% point difference in the primary outcome.	

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