



# ISUOG Practice Guidelines: role of ultrasound in congenital infection

## Clinical Standards Committee

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## INTRODUCTION

Ultrasound examination is key in the diagnosis and management of congenital infection. In some cases, the initial finding of abnormal ultrasound features may trigger maternal serological testing for congenital infection; in others, either infection screening or the mother's symptomatology may lead to targeted ultrasound scans with the aim of detecting fetal sequelae. Once congenital infection is diagnosed, ultrasound can be used to help determine the fetal prognosis and to guide further investigation and management.

In this Guideline we examine the role of ultrasound in the diagnosis and management of congenital infection, discussing the ultrasound signs and the prognostic value of ultrasound findings. We look in detail at six types of infection and their causative agents: cytomegalovirus (CMV), toxoplasma, parvovirus B19, rubella virus, varicella-zoster virus (VZV, which causes chickenpox)

and Zika virus (ZIKV). For each, we discuss the ultrasound signs, timing of infection in relation to gestational age and diagnosis of maternal and fetal infection, and give a brief outline of appropriate management. This Guideline does not address prevention or routine screening for congenital infections, as this can differ between countries. Clinicians should follow local guidelines regarding whether to offer screening, gestational age at screening, method of screening, interpretation of the test and follow-up of those who are screen-positive or screen-negative.

Despite the fact that case reports of intrauterine herpes simplex virus (HSV) infection have been published, this infection is not included herein, as the majority of neonatal HSV infections are acquired at birth as a consequence of direct fetal contact with the infected birth canal or through an ascending infection after premature rupture of the amniotic membranes. Intrauterine transmission of HSV infection from mother to fetus is rare, having been estimated to occur in only 5% of cases, secondary to hematogenous transplacental dissemination<sup>1</sup>.

## Identification and assessment of the evidence

The Cochrane Library and Cochrane Register of Controlled Trials were searched for relevant randomized controlled trials, systematic reviews and meta-analyses. We also carried out a search of MEDLINE for the period 1966 to 2019. The date of the last search was 15<sup>th</sup> May 2019. Relevant conference proceedings and abstracts were also searched. Databases were searched using the relevant MeSH terms including all subheadings. This was combined with a keyword search, using 'congenital', 'infection', 'pregnancy', 'ultrasound', 'cytomegalovirus', 'zika', 'toxoplasma', 'rubella', 'varicella-zoster virus', 'parvovirus' and 'abnormalities'. The National Library for Health and the National Guidelines Clearing House were also searched for relevant guidelines and reviews. Gray (unpublished) literature was identified through searching the websites of health technology assessment and health technology assessment-related agencies, clinical practice guideline collections and clinical trial registries. The search was limited to the English language. Whenever possible, recommendations are based on, and explicitly linked to,

the evidence that supports them. Details of the grades of recommendation used in these Guidelines are given in Appendix 1. Reporting of levels of evidence is not applicable to these Guidelines. Recommendations lacking evidence are annotated as 'good practice points.'

## SIGNS SUGGESTIVE OF CONGENITAL INFECTION

Table 1 summarizes ultrasound signs suggestive of congenital infection. The presence of any of these ultrasound signs is not diagnostic, but merely suggestive, of congenital infection such as CMV, toxoplasma, rubella, VZV or ZIKV, and should trigger testing for these infections. In cases with fetal hydrops or anemia, testing for parvovirus should also be performed.

Pregnant women who present with a non-vesicular rash and/or other signs or symptoms suggestive of systemic viral infection should be offered testing for rubella and parvovirus B19. Women who present with a facial rash (suggestive of 'slapped cheek' syndrome) should be offered testing for parvovirus B19. Those with a history of potential exposure to toxoplasma and presenting with general malaise suggestive of systemic infection should be offered testing for this infection. Travel history of the woman or her partner to countries known to have ZIKV transmission should trigger testing for ZIKV.

### Diagnosing maternal infection

The most common tests utilized for diagnosing maternal infection are enzyme-linked immunosorbent assay (ELISA) tests. Paired serology testing for virus-specific immunoglobulin M (IgM) and immunoglobulin G (IgG) antibodies, ideally with one test from before the primary infection, is often helpful to diagnose and determine the timing of infection in relation to the gestational age.

Antibody avidity testing can also be helpful in this regard; the longer the time since the initial infection, the greater the level of avidity.

### Diagnosing fetal infection

If a fetus is infected, the earlier in gestation this happens, the more likely it is to be affected. Once a maternal infection has been diagnosed by serological testing (whether this is triggered by maternal symptoms, contact with another infected individual or the discovery of an ultrasound abnormality), the possibility of fetal infection should be contemplated. Definitive diagnosis of fetal infection is possible only by invasive testing, usually by obtaining amniotic fluid by amniocentesis, or occasionally by fetal cord blood sampling. As a rule, amniotic fluid polymerase chain reaction (PCR) analysis will not be positive until 6–8 weeks after the initial maternal infection. Moreover, fetal urination is not well established until at least 18–20 weeks' gestation, so amniocentesis is likely to be negative before then as the virus will not be being passed in urine in sufficient concentrations. This means that amniocentesis should be delayed until after 18–20 weeks' gestation and, ideally, until more than 8 weeks after the initial maternal infection. There is retrospective evidence showing that the most significant risk factors for false-negative results are time interval between maternal infection and amniocentesis < 8 weeks and gestational age at amniocentesis < 18 weeks<sup>2</sup>.

It is important to note that confirmation of fetal infection does not necessarily mean that the fetus will be affected by the pathogen. An infected fetus may never develop any structural abnormalities identifiable on ultrasound or postnatally on imaging such as magnetic resonance imaging (MRI). It is also important, however, to recognize that even fetuses that do not exhibit any imaging abnormalities may still suffer long-term sequelae, which may be difficult to predict. This should be considered in the long-term follow-up of such cases.

**Table 1** Ultrasound signs suggestive of congenital infection

<i>Cranial abnormalities</i>	<i>Extracranial abnormalities</i>	<i>Placental/amniotic fluid abnormalities</i>
Ventriculomegaly	Small-for-gestational age	Placentomegaly
Calcifications	Hyperechogenic bowel	Placental calcifications
Intraventricular synechiae	Hepatomegaly	Oligohydramnios/anhydramnios
Cerebellar abnormalities	Splenomegaly	Polyhydramnios
Vermian hypoplasia	Liver calcifications	
Cerebellar hemorrhage	Ascites	
Calcifications	Pericardial effusion	
Cysts	Skin edema	
Periventricular pseudocysts	Hydrops or fetal anemia	
Malformations of cortical development	(MCA-PSV > 1.5 MoM) in absence	
Lissencephaly-pachygyria	of maternal atypical antibodies	
Oligo-/pachygyria		
Polymicrogyria		
Schizencephaly		
Microcephaly		

Signs are listed in approximate order of frequency. Not all ultrasound signs are present in all infections; some tend to be more common in certain infections, depending on pathogen. MCA, middle cerebral artery; MoM, multiples of the median; PSV, peak systolic velocity.

## CYTOMEGALOVIRUS (CMV)

CMV, a member of the human herpesvirus family, is the most common viral cause of congenital infection, affecting 0.2–2.2% of all live births<sup>3–5</sup>. It is the leading non-genetic cause of sensorineural hearing loss (SNHL) and a major cause of neurological disability. Around 10–15% of neonates with congenital CMV will be symptomatic at birth, and up to 25% of infected children have long-term impairments<sup>6,7</sup>.

CMV infection may be acquired for the first time during pregnancy (primary infection) or it may result from reactivation of prior infection or reinfection with a different strain of the virus (non-primary infection). Antenatally, transmission of the virus to the fetus occurs via the placenta. Transmission is more likely following primary maternal infection during pregnancy than following non-primary infection<sup>8</sup>. Infants born to mothers with primary CMV infection during pregnancy have, on average, a risk of congenital infection in the order of 30–40%<sup>9</sup>, while those born following non-primary maternal infection have a risk in the order of 1–2%<sup>5</sup>. The risk of congenital infection appears to vary according to the point in gestation at which primary infection occurs, increasing from around 30% in the first trimester to 47% in the third trimester<sup>10,11</sup>. While the risk of viral transmission is lower in early pregnancy, the proportion of cases with a prenatal diagnosis of severe fetal infection is higher when infection occurs in the first compared with the third trimester of pregnancy<sup>12,13</sup>. Correspondingly, the long-term consequences for the fetus are more severe, with recent evidence suggesting that long-term sequelae occur only after first-trimester infection<sup>13,14</sup>.

The majority of women who acquire CMV infection during pregnancy for the first time (primary infection) will remain asymptomatic<sup>15</sup>. However, a minority experience symptoms similar to those of infectious mononucleosis (glandular fever), including fever, malaise, myalgia, cervical lymphadenopathy and, less commonly, hepatitis and pneumonia, though few suffer long-term sequelae. Just as with other herpes viruses, CMV can remain dormant lifelong at particular sites, primarily in the salivary glands, but can be reactivated at any time, including during pregnancy.

### Diagnosis of maternal CMV infection

#### Recommendations

- The diagnosis of primary CMV infection in pregnancy can be made following either: (i) the appearance of CMV-specific IgG in a woman who was previously seronegative; or (ii) detection of CMV IgM antibody and low IgG avidity (**GRADE OF RECOMMENDATION: B**).
- Non-primary maternal infection cannot be excluded using serological tests (**GRADE OF RECOMMENDATION: C**).

Since routine antenatal CMV screening does not meet several of the criteria for an effective screening test, not least the fact that until now there has been no effective treatment in pregnancy, routine prenatal screening is not recommended in most countries<sup>16,17</sup>. Consequently, serological testing for CMV is offered only to women who have developed influenza-like symptoms or symptoms of glandular fever (with negative test results for Epstein-Barr virus) or symptoms of hepatitis (with negative test results for hepatitis A, B and C) during pregnancy, or in whom routine ultrasound detects fetal abnormalities suggestive of possible CMV infection, such as ventriculomegaly, microcephaly, calcifications, intraventricular synechia, intracranial hemorrhage, periventricular cysts, cerebellar hypoplasia, cortical abnormalities, hyperechogenic bowel, fetal growth restriction (FGR), pericardial effusion, ascites or fetal hydrops<sup>18,19</sup>. The frequency of fetal ultrasound abnormalities in cases with congenital CMV infection is shown in Table S1<sup>18</sup>.

While for other viral infections, such as rubella, the presence of IgM is often diagnostic of recent primary infection, this is not the case for CMV<sup>20</sup>. There are several reasons for this: IgM may persist for many months after primary CMV infection; IgM may be detected during a secondary infection; there may be cross-reactivity with IgM due to another viral infection, e.g. Epstein-Barr virus; IgM may be detected as a result of non-specific polyclonal stimulation of the immune system. Therefore, CMV-specific IgG testing should be performed in parallel with IgM testing, along with IgG avidity testing for seropositive women, to indicate the timing of the infection (i.e. before or during pregnancy). In general, a low avidity index (< 30%) is strongly suggestive of a recent primary infection (i.e. within the past 3 months), while a high avidity index (> 60%) is strongly suggestive of past (i.e. more than 3 months previously) or secondary infection<sup>21</sup>.

Diagnosis of non-primary CMV infection can be difficult. A rise in IgG levels does not confirm secondary infection, as this may be due to non-specific polyclonal stimulation of the immune system. In practice, therefore, the only way of confirming secondary CMV infection and transmission to the infant is by CMV-PCR analysis of amniotic fluid.

### Diagnosis of fetal CMV infection

#### Recommendations

- Fetal infection should be diagnosed by detection of CMV DNA on PCR analysis of the amniotic fluid. Amniocentesis should be delayed until at least 8 weeks after estimated time of maternal infection and after 20 gestational weeks (**GRADE OF RECOMMENDATION: B**).
- The most significant risk factors for false-negative results are time interval between infection and amniocentesis of < 8 weeks and gestational age at amniocentesis of < 18 weeks (**GRADE OF RECOMMENDATION: C**).

The diagnosis of fetal infection is made by identification of the virus or viral genome (DNA) in the amniotic fluid following amniocentesis and PCR. The timing of amniocentesis is very important; the appearance of the virus in the amniotic fluid is dependent on the time taken for the virus to cross the placenta and on the excretion of the virus in fetal urine. It should be performed, therefore, at least 8 weeks after maternal infection and after 20 weeks of gestation<sup>2,14,22,23</sup>, when fetal urination is well established. There are retrospective data showing that the sensitivity of amniotic fluid PCR may be similar at 17 and 20 weeks, provided that there is a time interval of at least 8 weeks between maternal infection and amniocentesis. The two most significant risk factors for a false-negative result are time interval < 8 weeks and amniocentesis before 18 weeks<sup>2</sup>.

### Prenatal prognostic indicators in congenital CMV infection

The clinical features of congenital CMV at birth include small-for-gestational age (SGA) at delivery, microcephaly, jaundice, petechiae or purpura, 'blueberry muffin' rash, indicating extramedullary hematopoiesis, and hepatosplenomegaly. Following prenatal diagnosis of fetal CMV infection, the main aim is to predict the risk of symptomatic neonatal infection. The risk of a poor outcome as assessed prenatally is probably overestimated in the medical literature. This is because 'poor outcome' is usually defined based on both terminated fetuses in which postmortem examination confirms significant signs of CMV infection as well as infants born with symptomatic congenital CMV infection. However, features of CMV identified at postmortem examination, such as cytomegalic inclusions in the kidneys and isolated periventricular calcifications, are probably not associated with a high risk of symptomatic neonatal infection. Therefore, the estimates of poor outcome as defined in the literature should be viewed with a degree of caution. It is also important to point out that, often, what is being predicted is an infant that is symptomatic at birth or has abnormalities on brain MRI. It should be borne in mind that late-onset SNHL or less severe adverse neurodevelopmental sequelae may become evident only later, thus highlighting the importance of pediatric and hearing follow-up of all infected infants.

Accurate prediction prenatally of poor prognosis for affected infants has proved challenging. Estimates are based largely on three factors: (i) the timing of infection; (ii) the presence and type of fetal abnormalities; and (iii) laboratory parameters.

#### (i) Gestational age at maternal infection

#### Recommendations

- Women should be informed that an indicative likelihood of vertical transmission after primary maternal infection is around 30–40% on average, with

the rate increasing as gestation progresses, being around 0–10% in the preconception period, 25–45% in the periconceptional period and first trimester, 45% in the second trimester and 47–78% in the third trimester (GRADE OF RECOMMENDATION: C).

- Women should be informed that, based on limited data, an indicative likelihood of severe symptoms at birth in an infected fetus after primary maternal infection in the periconceptional period is 70%, in the first trimester is 20%, in the second trimester is 5% and in the preconception period or third trimester is probably negligible (GRADE OF RECOMMENDATION: C).

It appears that, in common with other viral infections, the risk of vertical transmission increases with increasing gestational age at the time of maternal infection. The association between the timing of infection and the severity of fetal/neonatal outcome is less well defined.

Although in 1986 Stagno *et al.*<sup>12</sup> did not find any difference in vertical transmission rates according to gestational age at infection, more recent studies strongly suggest a higher rate of transmission with advancing gestational age. For example, Gindes *et al.*<sup>24</sup> found a 75% rate of transmission following primary infection after 25 weeks' gestation. Bodéus *et al.*<sup>25</sup> followed 123 women who developed a primary CMV infection during pregnancy. The overall rate of transmission in the study population was 57.5% and they found a statistically significant difference in the rate of vertical transmission between cases with maternal seroconversion in the first trimester *vs* in the third trimester (36% *vs* 77.6%;  $P < 0.001$ ); the risk of transmission when seroconversion occurred during the second trimester was 44.9%. Another study<sup>26</sup> assessed the risk of vertical transmission following primary maternal infection at the preconception stage (between 8 and 2 weeks before the start of the last menstrual period (LMP)), periconception (between 1 week before and 5 weeks after the LMP) and later in pregnancy (between 6 and 20 weeks' gestation, and between 20 and 38 weeks' gestation). They found no cases of congenital infection in the preconception group, while congenital infection occurred in 45% of cases in the periconception group. When the primary infection occurred between 6 and 20 weeks' gestation, the transmission rate was 30%, and, when it occurred between 20 and 38 weeks' gestation, it was 58%. Revello and Gerna<sup>22</sup> found that 9% of neonates were CMV-infected following preconceptional maternal infection, but none of them had clinical features at birth, while 31% of the neonates for whom virological outcome was known were infected following periconceptional maternal infection. In another study, Revello *et al.*<sup>27</sup> found that, following preconceptional infection (2–18 weeks before LMP), 8% of the neonates examined at birth were CMV-infected; again, none of them showed clinical features at birth. More recently, Hadar *et al.*<sup>28</sup> examined periconceptional primary CMV infection and found a vertical transmission rate of 25%.

The association between the gestational age at primary maternal CMV infection and neonatal outcome is less well defined, mainly because, in the absence of a systematic antenatal serological screening program and given that 90% of primary infection is asymptomatic, the timing of maternal infection is often imprecise. Nevertheless, there is growing evidence that, in common with other viral infections in pregnancy, infection earlier in pregnancy is associated with a greater risk of more severe harm to the fetus/neonate, while maternal infection preconception appears to carry very little risk. Pass *et al.*<sup>13</sup> identified neonates with congenital CMV infection, then tested retrospectively the stored maternal serum collected during pregnancy. They tested these samples for IgG and IgM antibody levels and used the results to classify primary maternal infection as first-trimester (< 13 weeks' gestation) or later. They found SNHL in 24% of the children in the first-trimester group, compared with 2.5% in the later group (relative risk (RR), 9.6). They found any central nervous system (CNS) disability (SNHL, mental impairment, cerebral palsy, seizures or chorioretinitis) in 32% of first-trimester cases compared with 15% in the later group (RR, 2.2). None of the late-infection group had more than one disability, whereas 12% of the first-trimester group did ( $P=0.04$ ). Liesnard *et al.*<sup>14</sup> had similar findings. They dated fetal infection based on amniotic-fluid or fetal-blood testing in 55 cases of congenital CMV from 237 pregnancies that underwent prenatal evaluation, and found that 26% of fetuses infected before 20 weeks of gestation had severe disease compared with only 6% of fetuses infected after 20 weeks. Recent evidence from more than 350 pregnancies with maternal CMV seroconversion suggests that first-trimester infection is more likely to be associated with severe congenital CMV infection<sup>29,30</sup>.

#### (ii) Presence of fetal abnormalities

##### Recommendations

- Women should be informed that normal brain findings on ultrasound and fetal MRI are associated with low risk of infant disability. However, this is not indicative of hearing outcome (**GRADE OF RECOMMENDATION: C**).
- Women should be informed that ultrasound abnormalities can appear 12 weeks or more after maternal infection; therefore, detailed ultrasound follow-up (every 2–4 weeks) for the remainder of the pregnancy is indicated (**GRADE OF RECOMMENDATION: C**).

In the absence of a routine antenatal screening program for CMV, the most common circumstance in which CMV is diagnosed prenatally is following the discovery during a routine scan of an abnormal ultrasonographic finding suggestive of possible CMV infection. This should lead to serological testing of the mother and/or amniocentesis with PCR. As a result of this non-systematic mode of

discovery, severe ultrasound abnormalities are described more often than are subtle findings. Nevertheless, a retrospective study of women with maternal primary infection in the index pregnancy found that, once the diagnosis of fetal infection had been made by PCR confirmation of CMV in the amniotic fluid, ultrasound was found to be more sensitive for the detection of subtle abnormalities associated with the fetal infection<sup>18</sup>.

Ultrasound findings can be categorized as fetal cranial<sup>31</sup> (Figure 1), fetal extracranial (Figure 2) and placental/amniotic fluid abnormality (Figure 3).

It is important to be aware of the lag time between maternal infection and fetal infection, and then between fetal infection and the appearance of sonographically identifiable fetal abnormalities. The placenta appears to act as a reservoir for, and a barrier to, infection, explaining why not all maternal primary infection (and maternal viremia) results in fetal infection. Some studies have observed a thickened placenta with a heterogeneous appearance and calcification, suggesting placentitis, before the appearance of fetal infection<sup>32</sup>. The time interval between primary maternal infection and the appearance of fetal ultrasonographic abnormalities varies considerably in the cases reported in the literature. In a series of 189 cases of primary infection with known outcome, this interval appeared to be around 12 weeks (following maternal infection at 14 weeks' gestation)<sup>23</sup>. However, longer intervals have been reported; Nigro *et al.*<sup>33</sup> described a case in which primary maternal infection occurred at 6 weeks, but the ultrasound abnormalities (intraventricular hemorrhage) did not appear until 20 weeks' gestation. Another case report of infection in an HIV-positive woman at 6 weeks' gestation found that the ultrasound abnormalities were not apparent until as late as 36 weeks' gestation. The implications of these findings for clinical practice are that, even if fetal infection occurs early in pregnancy, detailed ultrasound follow-up for the remainder of the pregnancy is indicated<sup>34</sup>.

It appears that the main sonographic prognostic indicator for symptomatic fetal CMV infection is fetal cerebral abnormality. In a small retrospective study, Farkas *et al.*<sup>35</sup> found that if antenatal ultrasound examination of the fetal brain was normal, then normal early neuropsychological outcome was likely<sup>35</sup>. Such conclusions have led to the evaluation of fetal cerebral MRI for further assessment of the fetal brain. MRI using both T1 and T2 sequences can be used to help to define the timing and consequences of fetal infection.

Ultrasound and MRI should be considered as complementary imaging modalities for investigation of the fetal brain<sup>36</sup>; when both are performed in the third trimester in a fetus known to be infected with CMV, they have a 95% sensitivity for the identification of related CNS lesions. When both ultrasound and MRI of the fetal brain are normal prenatally, the neonatal outcome is generally good, and the same may be true for a normal ultrasound examination with only subtle findings on MRI<sup>37</sup>. Cannie *et al.*<sup>38</sup> recently found that subtle findings on prenatal MRI were associated with a favorable prognosis;

MRI had a high negative predictive value for SNHL and neurological impairment, and this was equally predictive at 27 weeks and at 33 weeks of gestation.

The combined predictive value of normal ultrasound and MRI evaluations after 30 weeks' gestation for an asymptomatic neonate, in fetuses known to be CMV-infected following amniocentesis, is at best 95%<sup>37</sup>. Fetal laboratory findings may bridge this 5% gap. It is important to point out that this is not indicative of hearing

outcome, i.e. normal antenatal ultrasound and MRI findings do not rule out the risk of SNHL in these fetuses.

(iii) Laboratory parameters

Recommendations

- Although the median viral load in the amniotic fluid may be higher in symptomatic than in asymptomatic

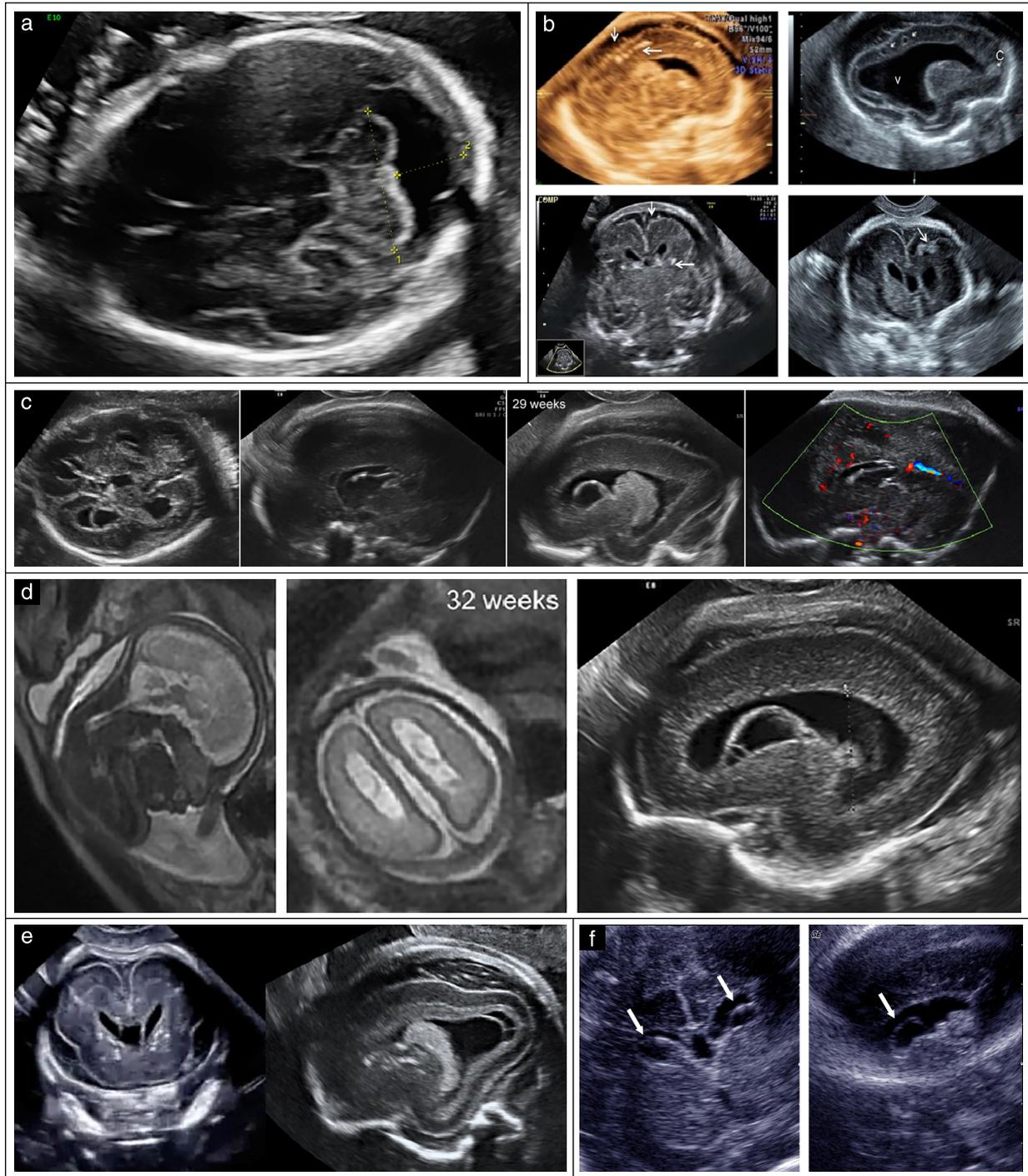
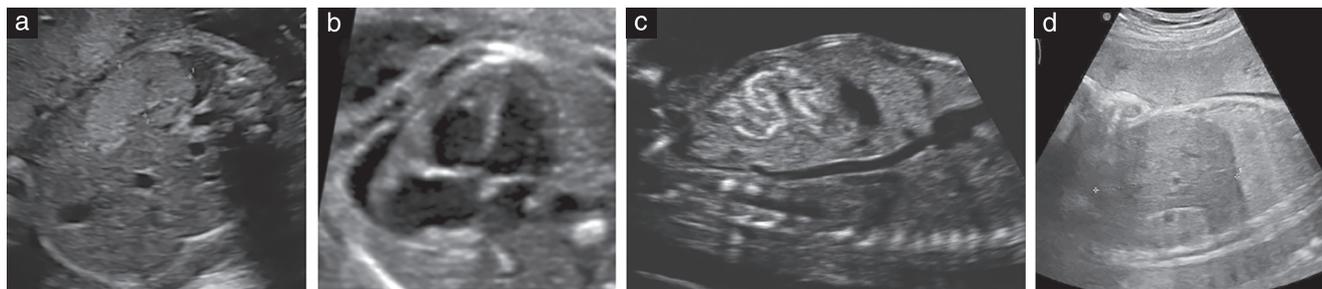
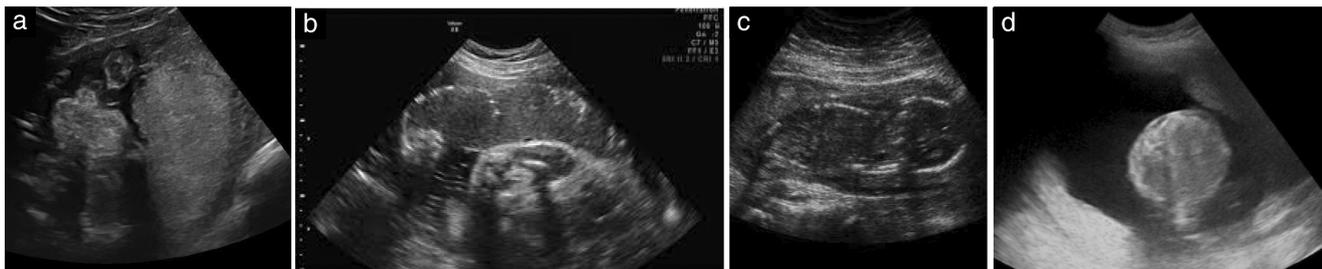


Figure 1 Ultrasonographic and MRI cranial features typical of CMV infection vary, and include: megacisterna magna (a), intracranial calcifications (b), ventriculomegaly, germinolytic cysts, agenesis of corpus callosum and intraventricular adhesions (c,d), periventricular cystic changes (c,f), lissencephaly (d), cerebral calcifications and periventricular cysts (e) and subependymal cysts (f).



**Figure 2** Ultrasonographic extracranial features typical of CMV infection vary, and include: splenomegaly (a), cardiomegaly and pericardial effusion (b), echogenic bowel (c) and hepatomegaly (d).



**Figure 3** Ultrasonographic placental/amniotic fluid abnormalities typical of CMV infection vary, and include: placentomegaly (a), placental calcifications (b), oligohydramnios (c) and polyhydramnios (d).

fetuses, the overlap between these two groups, and its dependence on technical and temporal factors, reduce its prognostic value (**GRADE OF RECOMMENDATION: B**).

- Although fetal blood markers such as platelet count, beta-2 microglobulin and CMV IgM have been associated with prognosis, the added value of fetal blood sampling in the prognostic workup of these women is not certain (**GOOD PRACTICE POINT**).

Several studies<sup>39–41</sup> have examined the relationship between the viral load in the amniotic fluid and the likelihood of the fetus being symptomatic. All of these studies found that the median viral load was higher in the amniotic fluid of symptomatic fetuses than in that of asymptomatic fetuses; however, this difference was statistically significant in only one study<sup>39</sup>. Furthermore, some fetuses with high amniotic-fluid viral loads were born asymptomatic, while others with low amniotic fluid viral load had severe ultrasound abnormalities<sup>40</sup>. Some of these differences between studies may be explained by differences in the methodology used or in the time interval between seroconversion and amniocentesis, as there is evidence that the amniotic fluid viral load changes with time lapsed since seroconversion<sup>39,41</sup>.

Studies of CMV genotypes have not found a good correlation with the fetal outcome<sup>40,42,43</sup>.

Fetal blood sampling has also been investigated, looking at both virus-specific markers as well as non-specific fetal blood parameters as possible prognostic indicators. It has been shown that the mean viral load in the blood of infected neonates is significantly higher in symptomatic

neonates compared with asymptomatic ones ( $P=0.02$ ), and this difference was more marked when considering only infants with severe symptomatic congenital CMV infection<sup>44</sup>. However, there is significant overlap in viral load between symptomatic and asymptomatic neonates, so setting a discriminatory cut-off is not feasible<sup>45</sup>. Revello *et al.*<sup>46</sup> found that antigenemia, viremia and DNA load were higher in the blood of neonates with ultrasound abnormalities compared to those without, but the difference was statistically significant only for antigenemia.

Several authors have proposed various non-specific neonatal blood parameters, including thrombocytopenia (platelet count  $< 100\,000/\text{mm}^3$ ), alanine aminotransferase level ( $> 80\text{ IU/mL}$ ) and direct bilirubin level ( $> 4\text{ mg/dL}$ ), as potential prognostic indicators. Rivera *et al.*<sup>47</sup> found that all of these were associated with symptoms at birth, with odds ratios of 2.4, 7.1 and 2.8, respectively. Another study pointed to the importance of thrombocytopenia, finding that, among symptomatic CMV-infected neonates with normal cranial computed tomography (CT) scan, 56% had thrombocytopenia, compared with 86% of those with abnormal cranial CT findings<sup>48</sup>. It has, therefore, been suggested that the platelet count in a fetal blood sample is an independent prognostic indicator of neonatal outcome and that certain circumstances could justify the risk of fetal loss (in the order of 1–2%<sup>49</sup>) associated with fetal blood sampling. However, this view has proved controversial among clinicians, with some arguing that a 1–2% risk of fetal loss does not justify fetal blood sampling to obtain platelet count, which does not give sufficiently certain information upon which to base decisions.

In general, fetal blood sampling may be considered to be of greatest value in the 'intermediate' prognostic group, i.e. in a fetus that has non-cerebral ultrasound abnormalities, or in a pregnant woman who requires as much information as possible regarding prognosis in order to decide between her management options. At the time of prenatal diagnosis of congenital CMV infection, the negative predictive value of ultrasound findings for symptomatic infection at birth or termination of pregnancy is estimated to be 93%<sup>50</sup>. The combined negative predictive values of ultrasound and viral load in the amniotic fluid and that of ultrasound and fetal blood parameters are 95% and 100%, respectively. In fetuses presenting with non-severe ultrasound features, the positive predictive values of ultrasound alone and in combination with amniotic fluid viral load or with fetal blood parameters are 60%, 78% and 79%, respectively<sup>50</sup>. This questions the additional value of the fetal blood markers obtained via cordocentesis over the amniotic fluid markers already obtained at the time of amniocentesis for prenatal diagnosis<sup>50</sup>.

### Overview of prognostic categorization and challenges

Overall, CMV-infected fetuses may be classified into one of three prognostic categories<sup>51</sup>: (i) asymptomatic fetuses; (ii) mild or moderately symptomatic fetuses; and (iii) severely symptomatic fetuses.

- (i) Asymptomatic fetuses are defined as those with no ultrasound abnormalities, normal cerebral MRI and normal biological parameters, fetal-blood platelet count in particular. The prognosis is generally good for these fetuses but there is a residual risk of SNHL.
- (ii) Mild or moderately symptomatic fetuses are defined as those with isolated biological abnormalities (on fetal blood sampling), either without brain abnormality on ultrasound or with isolated ultrasound abnormality, such as hyperechogenic bowel, mild ventriculomegaly or isolated calcifications. In this group, the prognosis is uncertain, and further follow-up (with ultrasound and possibly MRI) may help to refine the prognosis. Therapeutic options, such as antiviral therapy, are currently being evaluated, but their use is still limited to the research setting. The option of termination of pregnancy should also be discussed.
- (iii) Severely symptomatic fetuses are defined as those with severe cerebral ultrasound abnormality (e.g. microcephaly, ventriculomegaly, white-matter abnormalities and cavitations, intracerebral hemorrhage, delayed cortical development) associated with thrombocytopenia. The prognosis for this group is poor, and counseling regarding the option of termination of pregnancy should be performed.

The accurate prenatal prognosis of fetal CMV infection is challenging. There is a need for new and better prognostic tests for fetuses with congenital CMV. A recent study<sup>52</sup> performed peptidome analysis in the amniotic fluid of 13 symptomatic and 13 asymptomatic

neonates (discovery cohort) and, in their validation cohort, found that a panel of 34 peptides had a sensitivity of 89%, specificity of 75% and area under the receiver-operating-characteristics curve of 0.90 to differentiate the nine severely symptomatic from the 12 asymptomatic neonates. This analysis may represent a useful prognostic indicator for the future<sup>52</sup>.

### Management of maternal and fetal CMV infection

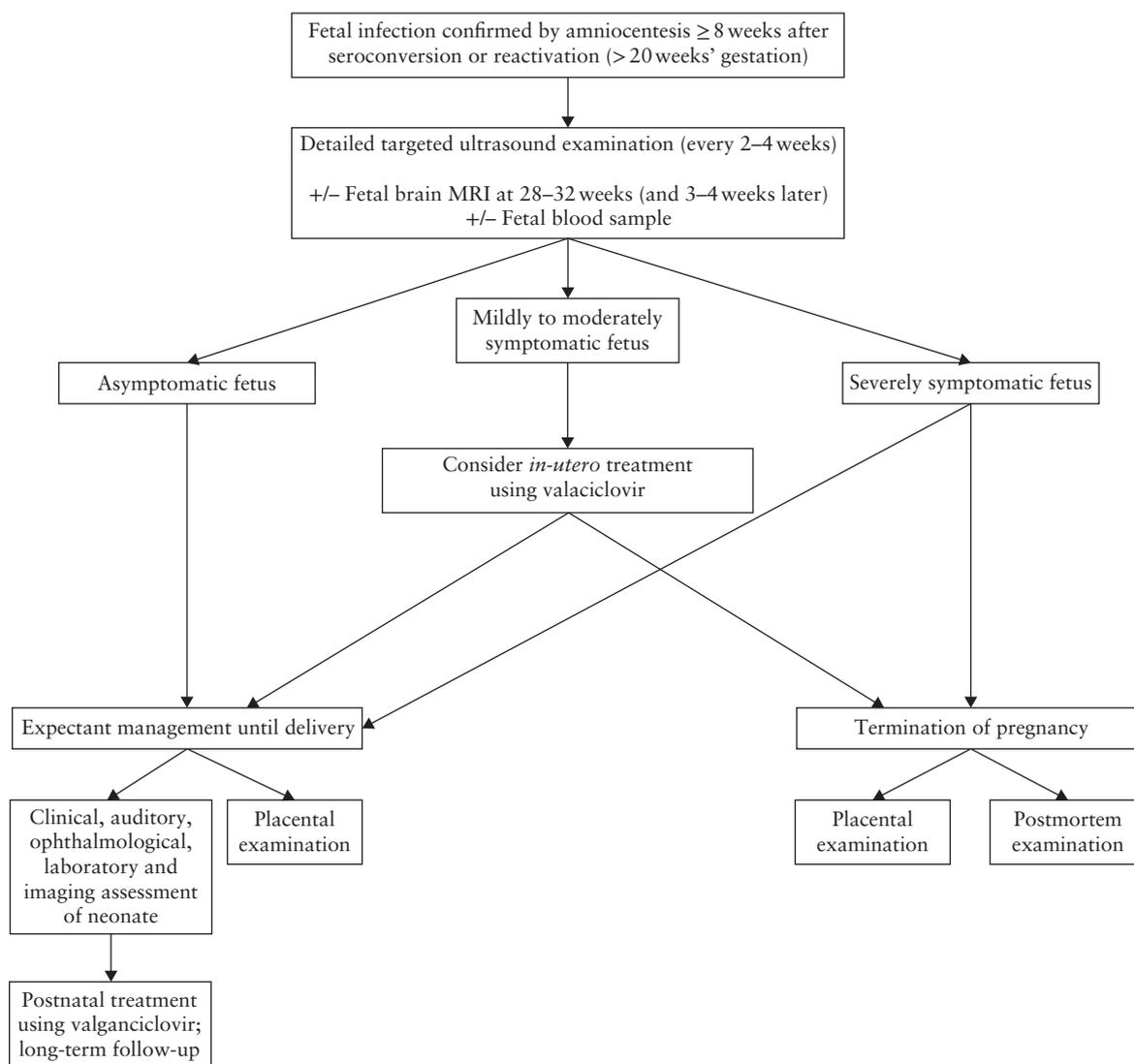
#### Recommendations

- Due to the lack of randomized controlled trials, high-dose valaciclovir for congenital CMV infection should only be given in the context of research (**GOOD PRACTICE POINT**).
- Based on the results of one randomized controlled trial, administration of CMV-specific hyperimmune globulin (HIG) for congenital CMV infection is not recommended as part of clinical care and should only be given in the context of research (**GRADE OF RECOMMENDATION: B**).

A proposal for the management of CMV fetal infection is presented in Figure 4<sup>59</sup>. The antenatal diagnosis of CMV infection is challenging, and options for prevention and treatment are limited. In general, options involve either conservative management, i.e. continuation of the pregnancy with regular monitoring, or termination of pregnancy. More recently, medical therapies aimed at reducing the risk of transmission, and the likelihood and/or severity of neonatal infection, have been investigated, including antiviral drugs and CMV HIG<sup>53–55</sup>.

Two studies have shown promise for the use of valaciclovir in pregnancies with CMV-infected fetuses, but a randomized controlled trial is needed to confirm whether this antiviral should be recommended routinely to reduce the risk of symptomatic congenital CMV disease<sup>53,54</sup>. High-dose valaciclovir was given for a median of 89 days to pregnant women carrying a moderately infected fetus presenting with non-severe ultrasound features (extracerebral ultrasound abnormalities and/or mild ultrasound brain abnormalities (Table S2)<sup>54</sup>). Valaciclovir administration was associated with a significantly greater proportion of neonates born asymptomatic (82%) compared with a historical cohort (43%). This study also provided reassuring safety data for the use of valaciclovir in pregnancy: maternal clinical and laboratory tolerances to this high-dose regimen were excellent, and no adverse neonatal effects were observed.

Nigro *et al.*<sup>55</sup> reported that CMV HIG therapy was associated with a significantly lower risk of congenital CMV infection, especially symptomatic infection. Recently, a prospective observational study reported that, after a primary maternal CMV infection in the first trimester, biweekly HIG administration at a dose of 200 IU/kg prevented maternal–fetal transmission up to 20 weeks' gestation<sup>56</sup>. Unfortunately, the potential efficacy of HIG was not borne out in a phase-II randomized,



**Figure 4** Proposed management of congenital cytomegalovirus (CMV) infection (adapted from Benoist *et al.*<sup>59</sup>). +/-, with or without.

placebo-controlled, double-blind study<sup>57</sup>, which found no significant improvement in the risk of transmission, levels of virus-specific antibodies, T-cell mediated immune response, viral DNA in the blood or clinical outcome at birth. Given these conflicting findings, HIG is currently not recommended routinely for the treatment of women with primary CMV infection in pregnancy. A trial assessing HIG in pregnancy was expected to finish in 2018<sup>58</sup>, but this study was stopped for futility before completion.

There is currently no licensed vaccine for CMV. An alternative strategy to reduce the risk of infection is behavior modification aimed at minimizing direct contact with saliva or urine of young children who may be excreting CMV in these fluids. Simple hygiene-based measures to reduce the risk of CMV acquisition include avoiding sharing utensils, drinks or food with young children, not kissing young children directly on the lips and handwashing after contact with their urine or saliva.

Congenital CMV should be confirmed at birth following an antenatal diagnosis of maternal infection, even when a diagnosis of fetal infection has been made by invasive sampling. Infants should have a urine sample or saliva swab for CMV-PCR as soon as possible after birth and it is important to collect samples within 3 weeks of birth to confirm congenital rather than postnatal acquisition of CMV.

## TOXOPLASMA

It is currently estimated that in the USA about 170 infants each year are born with congenital toxoplasmosis; this figure has fallen significantly from levels reported before 1999<sup>60</sup>. Similarly, the incidence in Europe has fallen in recent years, due to improvements in levels of hygiene, increased knowledge and avoidance of cat litter and undercooked meat, particularly during pregnancy. Over the 5 years from 2008 to 2012, there were 33 cases

of congenital toxoplasmosis identified through enhanced surveillance in England and Wales<sup>61</sup>.

*Toxoplasma gondii* is a parasite acquired by the ingestion of toxoplasma tissue cysts<sup>62</sup>. These cysts may be found in meat, so pregnant women should ensure that any meat they eat is cooked well and they should avoid processed meat. The infectious oocysts are secreted by cats and can contaminate soil, so pregnant women should ensure that salads/vegetables are thoroughly washed and should take care to wash their hands, particularly before they eat, when they have handled cats<sup>63</sup>.

In the UK, only 10% of women of childbearing age are immune to toxoplasma and the incidence of maternal infection is around 2–5 per 1000<sup>64,65</sup>. Primary maternal infection is asymptomatic in around two-thirds of women; the remainder have a mild coryzal illness, with malaise, low-grade fever, headache and lymphadenopathy.

The overall risk of congenital toxoplasmosis following maternal infection ranges from 20% to 50% without treatment<sup>66,67</sup>. As with most viral infections in pregnancy, the risk of fetal infection increases with gestational age at maternal infection (being < 1% before 4 weeks, 4–15% at 13 weeks and > 60% at 36 weeks)<sup>66,68</sup>. However, the earlier the gestational age at infection, the greater the risk that the fetus will be affected (Table S3)<sup>66</sup>.

The main sequelae of congenital toxoplasma infection involve the CNS and eyes, and typically include microcephaly, hydrocephalus, ventriculomegaly and chorioretinitis<sup>69,70</sup>. These may lead to developmental delay, epilepsy and blindness. Hepatosplenomegaly, anaemia, rash, jaundice and pneumonitis may also occur<sup>69,70</sup>. Even though most infected infants do not have clinical signs of infection at birth, up to 90% will develop sequelae later in life<sup>71–73</sup>.

## Diagnosis of maternal toxoplasmosis infection

### Recommendations

- Diagnosis of maternal toxoplasmosis infections can be made by testing maternal serum, including toxoplasma IgM and IgG. In case of positive or equivocal IgM with negative IgG results, a new specimen for IgM and IgG antibody testing should be obtained within 2 weeks. If the results remain unchanged, the IgM result is probably false positive (**GRADE OF RECOMMENDATION: C**).
- In the case of equivocal results for either IgM or IgG antibody and positive results for the other, a new specimen should be obtained within 2 weeks. If the results remain unchanged, both specimens should be sent to a reference toxoplasmosis laboratory (**GOOD PRACTICE POINT**).
- Women should be informed that a high IgG avidity result within the first 12–16 weeks of pregnancy (depending on the kit used) essentially rules out maternal infection during the index pregnancy (**GRADE OF RECOMMENDATION: C**).

- Physicians should be aware that spiramycin treatment can delay maturation of IgG antibodies and therefore result in lower avidity titers than in untreated women (**GRADE OF RECOMMENDATION: B**).
- An experienced reference laboratory should be consulted in any case of inconclusive serology results (**GOOD PRACTICE POINT**).

The interpretation of toxoplasma test results may be challenging, and a specialist microbiologist should be consulted. As with most infections, diagnosis is based on testing of maternal serum for IgG and IgM antibodies, and avidity testing can be useful to help determine the timing of infection (Health Protection Agency, 2006<sup>74</sup>). IgM is the first antibody to increase, reaching its highest level about 1 month after infection and remaining relatively stable for about 1 more month, before starting to decrease, while IgG reaches its maximum level about 3 months after infection and, in the absence of treatment, shows only a mild decrease after this point<sup>75</sup>. IgM testing may not be particularly helpful for timing the infection; it usually appears within 2 weeks of exposure but can persist for years<sup>72,76</sup>. IgG will also normally be detectable 2 weeks after exposure; a change in level on repeat testing (usually after 2 weeks) may help to determine the timing of infection. It is also notable that serologic assays for toxoplasmosis are not well-standardized and have high rates of false-positive and false-negative test results<sup>76,77</sup>. Therefore, the tests should be performed in an experienced reference toxoplasmosis laboratory, in which specific confirmatory tests, such as the Sabin–Feldman dye test or indirect fluorescent antibody test, are performed<sup>72,76–78</sup>. This is the case particularly for pregnant women with positive or equivocal IgM test results<sup>78,79</sup>.

The combination of negative IgM and negative IgG test results indicates either the absence of infection or a recent acute infection without enough time for seroconversion. The combination of negative IgM and positive IgG test results indicates remote infection and no risk of fetal transmission in an immunocompetent woman<sup>76–78</sup>. Interpretation of these results in the third trimester is more difficult. In case of positive or equivocal IgM with negative IgG results, a new specimen for IgM and IgG antibody testing should be obtained within 2 weeks for confirmatory testing at a reference laboratory. If the results remain unchanged, the IgM result is likely to be false positive. The combination of positive IgM and positive IgG test results either indicates that the pregnant mother has had a recent infection or is a false-positive IgM result. If acute infection is a possibility, serum testing should be repeated in 2–3 weeks to determine if there has been an increase in IgG antibodies consistent with recent infection<sup>76–78</sup>. Table S4 makes recommendations regarding the interpretation of serum test results for toxoplasmosis performed at clinical (non-reference) laboratories<sup>79</sup>.

As with other viral infections, IgG avidity testing may be helpful<sup>80</sup>; in general, high avidity is associated with primary infection occurring more than 4–5 months

previously (depending on the test method used), while low avidity usually indicates infection within the previous 4–5 months<sup>77,81–83</sup>. However, in the case of toxoplasma, spiramycin treatment can delay the maturation of IgG antibodies<sup>84</sup>, and avidity tends to be lower than expected in treated women<sup>85,86</sup>.

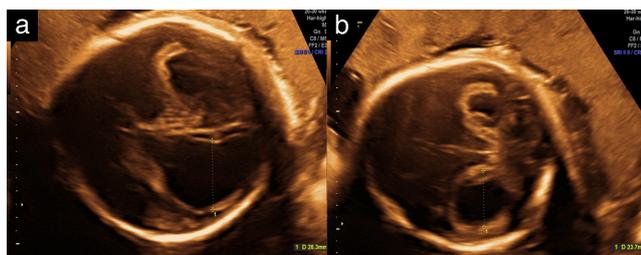
### Diagnosis of fetal toxoplasmosis infection

#### Recommendations

- Fetal infection should be diagnosed by detection of toxoplasma DNA in the amniotic fluid. Amniocentesis should be delayed at least 4 weeks after maternal infection and performed after 18 gestational weeks (GRADE OF RECOMMENDATION: B).
- Women should be informed that the sensitivity of current molecular methods in detecting toxoplasma DNA in the amniotic fluid is  $\leq 90\%$ ; false-negative results can occur when the DNA concentration is low (GRADE OF RECOMMENDATION: B).

Fetal infection can be diagnosed by identification of toxoplasma DNA following PCR analysis of the amniotic fluid (obtained by amniocentesis)<sup>87</sup>. This should be delayed until at least 4 weeks after maternal infection and should be performed after 18 weeks' gestation, when fetal urine production is well established<sup>79,88,89</sup>. The sensitivity of current PCR assays following amniocentesis is  $\leq 90\%$ <sup>90</sup>. False-negative results can be attributed to low levels of toxoplasma DNA in the amniotic fluid<sup>90,91</sup>. However, these cases may have a better prognosis, as low titers of DNA have been associated with less severe manifestation in the infant<sup>90,91</sup>.

Ultrasound signs suggestive of fetal infection are commonly non-specific and include ventriculomegaly, intracranial bleeding, intracranial calcifications, microcephaly, ascites, hepatosplenomegaly, FGR and hydrops; Figure 5 shows ultrasound images of affected fetuses. Limited evidence indicates that the combination of cerebral echogenic lesions and ventriculomegaly is associated with adverse prognosis (chorioretinitis with or without developmental delay)<sup>92</sup>, whereas the prognosis of echogenic lesions with normal ventricles appears better (normal neurodevelopment in four of five cases)<sup>93</sup>.



**Figure 5** Typical ultrasound findings in fetuses with congenital toxoplasma infection: (a) severe bilateral ventriculomegaly; (b) echogenic thickened ventricular walls.

### Management of maternal and fetal toxoplasmosis infection

#### Recommendations

- Spiramycin (1-g tablet taken orally three times daily until the end of the pregnancy, in the absence of confirmed vertical transmission) should be used to prevent vertical transmission after maternal toxoplasma infection during pregnancy (GRADE OF RECOMMENDATION: C).
- Spiramycin treatment should be initiated without delay (within 3 weeks) following maternal seroconversion (GRADE OF RECOMMENDATION: B).
- If vertical transmission is confirmed, fetal infection should be treated by spiramycin only for 1 week (1-g tablet three times daily), followed by pyrimethamine (50 mg once daily) plus sulfadiazine (1g three times daily) plus folinic acid (50 mg weekly) throughout the pregnancy and the infant treated for 1 further year (GRADE OF RECOMMENDATION: C).
- The combination of pyrimethamine, sulfadiazine and folinic acid may be more effective than is spiramycin in preventing vertical transmission, but more data are needed before this can be adopted in clinical practice (GRADE OF RECOMMENDATION: C).
- Ultrasound follow-up should include 4-weekly examinations of the fetus, focusing on brain, ocular and growth assessment (GOOD PRACTICE POINT).
- Women should be informed that, even when fetal imaging studies are normal, there is a risk of approximately 30% for long-term sequelae, especially chorioretinitis, which occasionally causes vision loss (GRADE OF RECOMMENDATION: B).

When maternal toxoplasma infection occurs before 18 weeks' gestation, treatment with spiramycin should be commenced without delay until amniocentesis after 18 weeks' gestation. The most common dosing regimen is 1-g spiramycin orally three times a day. The sooner after primary maternal infection that spiramycin is started, the more effective it is likely to be in reducing the risk of fetal infection. Nonetheless, there is no evidence that prenatal treatment can decrease significantly the risk of clinical manifestation (adjusted odds ratio, 1.11; 95% CI, 0.61–2.02)<sup>94</sup>. It is noteworthy that, while increased gestational age at seroconversion was associated with decreased risk of cerebral lesions, it did not affect the rate of ocular lesions<sup>94</sup>. Recently, the combination of pyrimethamine (50 mg/day, orally) plus sulfadiazine (1g three times daily, orally) plus folinic acid (50 mg weekly) was compared with spiramycin (1g three times daily, orally) in a randomized controlled trial in the context of prevention of vertical transmission. It was found that the transmission rate was 18.5% in the combined pyrimethamine + sulfadiazine + folinic acid group *vs* 30% in the spiramycin group. The rate of cerebral anomalies was 0/73 in the combined group *vs* 6/70 (8.5%) in the spiramycin group. Moreover, there appeared to be a 3-week window of opportunity for initiation of treatment

after maternal seroconversion. Two women in the pyrimethamine + sulfadiazine group developed a severe rash that required hospitalization<sup>95</sup>. Since sulfadiazine can precipitate a hemolytic crisis in individuals with glucose 6-phosphate dehydrogenase (G6PDH) deficiency, G6PDH testing should be considered before initiation of treatment.

After 18 weeks' gestation, it is still worth performing amniocentesis in case of known maternal infection, in order to confirm or exclude fetal infection. This is because, if fetal infection is confirmed, the treatment regimen will be changed to spiramycin only for 1 week (1-g tablet three times daily), followed by pyrimethamine (50 mg once daily) plus sulfadiazine (1 g three times daily) plus folic acid (50 mg weekly) throughout the pregnancy and the infant treated for 1 further year<sup>88,89</sup>. If fetal infection is not confirmed, treatment with spiramycin (1-g tablet taken orally three times daily) should be continued until the end of the pregnancy.

Postnatal treatment of newborns with symptomatic congenital toxoplasmosis consists of pyrimethamine, sulfadiazine and folic acid administration for 1 year<sup>60</sup>.

It should be borne in mind that there is a risk of a negative amniocentesis being a false-negative result, so serial ultrasound follow-up of the fetus is indicated regardless of the test results. Ultrasound may identify the following features suggestive of congenital toxoplasma: microcephaly, hydrocephalus, ventriculomegaly, cerebral calcifications, intracranial bleeding, hepatosplenomegaly, FGR, hydrops, cataracts or ascites. When ultrasound of the brain is normal, consideration should be given to fetal MRI as this has greater sensitivity for detecting subtle brain abnormalities. When the fetal ultrasound is normal, and particularly when the fetal MRI is also normal, the risk of significant neonatal sequelae is low, but parents should be counseled that, even in this situation, there remains a residual risk (approximately 30%) of significant sequelae, especially ocular<sup>71-73</sup>.

## HUMAN PARVOVIRUS B19

Parvovirus B19 is a single-stranded DNA, non-enveloped virus from the family *Parvoviridae*, and the only member of the family that can cause human disease. Also known as fifth disease, it is such a common childhood viral infection that approximately 60–75% of pregnant women are immune<sup>96,97</sup>. Infected children exhibit a characteristic facial rash and fever, which is known as 'slapped-cheek syndrome'. This will often run in epidemics through schools, especially during late winter and spring. It is spread by respiratory droplets from infected people, by blood or blood-product transfusion or by transplacental passage<sup>98</sup>. The incidence of acute parvovirus B19 infection in pregnancy is 1–2%<sup>97</sup>. Cases are often asymptomatic, although prodromal symptoms may be present after the incubation period. In some cases, the more definable symptoms of rash (erythema infectiosum) and arthralgia are present 7 days after the prodromal illness. The incubation period is 4–14 days following exposure; women remain infectious for 3–10 days post-exposure or until

the rash appears. The most common trigger for maternal testing for parvovirus B19 in pregnancy is a report of recent exposure; it can also result from an incidental finding, generally of fetal hydrops (Figure 6), on ultrasound. Table S5 lists the reported ultrasound abnormalities in fetuses infected by parvovirus B19<sup>99-102</sup>.

When a mother contracts infection, the risk of vertical transmission to the fetus ranges from 25% to 32%<sup>103,104</sup>. The main receptor for parvovirus B19 is globoside, a blood-group-P antigen, which is found primarily in erythroid precursors<sup>105</sup>, but also in other tissues, including the myocardium and the first-trimester placenta<sup>106</sup>. Parvovirus B19 causes fetal anemia by inhibiting erythropoiesis, thus leading to aplastic crisis. In healthy adults, this crisis is well-tolerated, with minimal anemia. However, compared with adults, the fetus has a greater relative demand for red blood cells and a larger red-cell mass, with associated rapid cell turnover. This renders the fetus particularly vulnerable to any insult to erythropoiesis, and profound anemia may result from infection with parvovirus B19. In the fetus, the virus affects mainly the bone marrow, but it can also affect areas of extramedullary hematopoiesis, such as the liver or spleen. The fetal anemia, as well as the associated hepatitis, hypoalbuminemia and myocarditis, can lead to cardiac failure and subsequent hydrops fetalis<sup>107</sup>. Intrauterine red-blood-cell transfusion can be used to treat fetal hydrops caused by parvovirus B19.

### Recommendation

- Given the potential for long-term neurodevelopmental sequelae of parvovirus infection, cerebral imaging should be considered for fetuses with hydrops or severe anemia (GRADE OF RECOMMENDATION: C).

When a fetus is infected, there is no evidence that parvovirus is teratogenic, but, as mentioned above, it can lead to fetal anemia. The risk of fetal hydrops is low (4–13%), but, when it occurs, it carries a 50% risk of intrauterine fetal death<sup>99,104,108</sup>. Hydrops occurs at a median of 3 weeks after the primary maternal infection, and 95% of cases develop by the 8<sup>th</sup> week after maternal infection<sup>108</sup>. Spontaneous resolution has been reported between 1 to 7 weeks after the diagnosis<sup>109</sup>. It is worth noting that thrombocytopenia has been reported in more than 95% of hydropic transfused fetuses, with an incidence of severe thrombocytopenia ( $< 50 \times 10^9$  platelets/L) of up to 46%<sup>99,110,111</sup>. This should be taken into account when performing cordocentesis or intrauterine transfusion. Case reports of neonatal liver insufficiency<sup>112-114</sup>, myocarditis<sup>115-117</sup>, transfusion-dependent anemia<sup>118,119</sup> and CNS abnormalities<sup>112,114,115</sup> have been reported. The general consensus is that parvovirus B19 itself, in the absence of hydrops or significant fetal anemia, does not cause long-term neurological disability, but severe anemia and fetal hydrops may be independent risk factors for long-term neurological sequelae<sup>101,110,120</sup>. Therefore, fetal-medicine specialists should consider cerebral imaging in fetuses or neonates who have had

hydrops or severe anemia. Furthermore, the myocarditis caused by parvovirus B19 can lead to severe dilated cardiomyopathy<sup>112,115,116</sup> and may even require heart transplantation<sup>121</sup>.

### Diagnosis of maternal parvovirus B19 infection

#### Recommendations

- Pregnant women who have had contact with an infected individual, who present with the suggestive rash or who have a hydropic fetus should be tested for parvovirus B19-specific IgM and IgG antibodies (**GRADE OF RECOMMENDATION: B**).
- As IgM can be false negative, especially in asymptomatic patients, an IgM-negative result in a woman with strong suspicion of parvovirus B19 infection should be complemented with molecular methods (**GRADE OF RECOMMENDATION: C**).

Pregnant women presenting with a rash suggestive of parvovirus B19 infection or who have had contact with an infected individual should be tested for parvovirus B19-specific IgM and IgG antibodies<sup>122,123</sup> (Figure S1). If serology is positive (both IgM and IgG), it is useful if another serum sample from before the apparent infection (e.g. from the pregnancy booking blood sample) can be tested; if this tests negative, the diagnosis can be confirmed and the timing of the infection estimated. Women with positive IgM, regardless of IgG status, should be monitored for potential fetal infection. Negative IgM with positive IgG indicates previous exposure and immunity, and these women are not at risk of transplacental transmission. Those in whom both IgM and IgG are negative are susceptible and serologic testing should be repeated in 4 weeks. If repeat testing shows positive IgM or IgG, these pregnancies should be monitored for potential fetal infection.

High (20–40%) rates of false-negative IgM results have been reported, especially in the early asymptomatic stages, when the viral load is high and the viral particles form complexes with parvovirus B19-specific antibodies<sup>124</sup>. The clinical implications of relying on IgM alone are that some hydropic fetuses with false-negative IgM may receive a delayed intrauterine blood transfusion, or not

have one at all. Therefore, in case of strong suspicion of parvovirus B19 infection with negative IgM results, the assessment should be complemented by DNA-detection methods such as PCR, by determination of IgG avidity<sup>124</sup> or by amniocentesis for detection of viral DNA<sup>110</sup>.

### Diagnosis of fetal parvovirus B19 infection

#### Recommendation

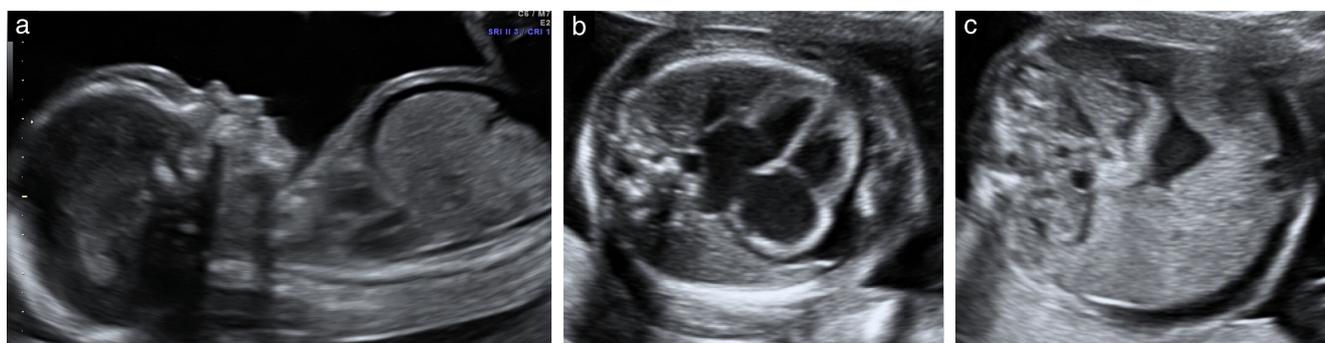
- Although the viral DNA can be detected in the amniotic fluid and blood of infected fetuses, invasive testing is not indicated unless cordocentesis is being performed anyway for severe fetal anemia (**GOOD PRACTICE POINT**).

Fetal infection can be diagnosed only by invasive testing, usually amniocentesis and occasionally cordocentesis to obtain fetal blood. The amniotic fluid or fetal blood can be analyzed for the presence of parvovirus DNA using PCR. The qualitative PCR analysis is reported to have a sensitivity as high as 100%<sup>122</sup>. However, as a general rule, invasive testing is not indicated unless severe fetal anemia is diagnosed by ultrasound<sup>125–127</sup>, given the likelihood of coexisting thrombocytopenia<sup>99,110,111</sup>.

### Management of maternal and fetal parvovirus B19 infection

#### Recommendations

- Serial ultrasound monitoring should start 4 weeks after infection or seroconversion and be performed every 1–2 weeks thereafter until 12 weeks after infection (**GRADE OF RECOMMENDATION: B**).
- Serial ultrasound examinations, looking for evidence of ascites, cardiomegaly, hydrops fetalis and raised MCA-PSV, should be performed every 1–2 weeks for 8–12 weeks after exposure (**GRADE OF RECOMMENDATION: C**).
- MCA Doppler readings should not be made during or immediately after a period of fetal activity (**GRADE OF RECOMMENDATION: C**).
- Fetal blood sampling, with preparation for intrauterine blood transfusion, is indicated when



**Figure 6** Typical hydropic ultrasound findings in fetuses with congenital parvovirus B19: skin edema and ascites (a); cardiomegaly, pericardial effusion and skin edema (b) and ascites (c).

MCA-PSV > 1.5 MoM, or when there is fetal ascites or hydrops (GRADE OF RECOMMENDATION: B).

- Regarding prognosis, parents should be informed that the risk of perinatal death is approximately 30% for infected fetuses presenting with hydrops *vs* 6% for non-hydrops fetuses. The long-term outcome in surviving fetuses is generally good, with a 10% risk for neurodevelopmental abnormalities for hydropic fetuses (GRADE OF RECOMMENDATION: C).

The presence of fetal hydrops is a clear indication of fetal anemia in the context of parvovirus B19 infection. Fetal hydrops in this context resolves spontaneously in around a third of cases<sup>109</sup>. An alternative method commonly used for diagnosing moderate or severe fetal anemia is measurement of middle cerebral artery (MCA) peak systolic velocity (PSV); it has been shown that MCA-PSV > 1.5 multiples of the median (MoM) can predict severe fetal anemia in confirmed parvovirus B19 infection with a sensitivity of 94% and specificity of 93%<sup>128</sup>. Serial ultrasound monitoring, looking for evidence of ascites, cardiomegaly, hydrops fetalis or raised MCA-PSV, should start 4 weeks after maternal exposure/infection and should be performed every 1–2 weeks for 8–12 weeks after exposure/infection. If severe fetal anemia is detected, cordocentesis can be performed to confirm fetal infection by PCR analysis of fetal blood, as described above.

In the absence of ultrasound evidence of fetal sequelae by 8–12 weeks after exposure, adverse outcome related to parvovirus B19 infection is highly unlikely<sup>99,123</sup>. While ultrasound monitoring focuses on fetal anemia and hydrops, fetal death can occur without evidence of hydrops fetalis<sup>126,129</sup>.

The first large series investigating the performance of MCA-PSV in the prediction of moderate or severe fetal anemia reported an extremely high sensitivity and specificity, both in the setting of alloimmunization (100% and 88%, respectively)<sup>130</sup> and in the setting of parvovirus infection (94% and 93%, respectively)<sup>128</sup>. Several publications followed, reporting similar performance<sup>131,132</sup>, and a 2019 meta-analysis reported a sensitivity of 79% and specificity of 73% for the prediction of moderate/severe fetal anemia (of any cause), when using 1.5 MoM as the cut-off for MCA-PSV<sup>133</sup>.

MCA-PSV is measured easily if the angle between the ultrasound beam and the direction of blood flow is close to 0°, thereby maintaining the accuracy of the velocity measurement. However, measurement of MCA-PSV does not detect all cases of fetal anemia: it may not change in cases of mild anemia; it may not increase further in cases of severe anemia in which the hemoglobin concentration falls below 3 g/dL; and the false-positive rate increases after 35 weeks' gestation<sup>134</sup>.

Figure S2 illustrates the steps necessary for MCA Doppler assessment to ensure that intra- and interobserver variation are as low as possible<sup>134</sup>. The operator should be aware of possible pitfalls, including normal variants of the MCA, such as double MCA and MCA collaterals (the lenticulostriate arteries). Doppler readings should

be obtained in the absence of fetal breathing and neither during nor immediately following a period of fetal movement/activity. In late gestation, MCA Doppler measurements may also be affected by fetal heart rate accelerations or decelerations, or following uterine contractions<sup>134,135</sup>.

Fetal blood sampling is indicated when MCA-PSV > 1.5 MoM, or when there is fetal ascites or hydrops. When fetal anemia is confirmed by testing of fetal blood, intrauterine blood transfusion may be indicated<sup>120,136–138</sup>. This reduces the risk of intrauterine fetal death (odds ratio, 0.14; 95% CI, 0.02–0.96)<sup>139</sup>. Fetal transfusion can return the fetal hemoglobin to a normal level, thus helping with the resolution of cardiac failure and hydrops. Moreover, mature erythrocytes are transfused, which are less susceptible to the influence of parvovirus, and therefore likely to persist for the normal erythrocyte half-life of 120 days. A meta-analysis of observational studies showed that intrauterine blood transfusion resulted in resolution of hydrops in 55% of affected fetuses, whereas resolution of anemia on follow-up scans was reported in all non-hydrops fetuses<sup>140</sup>. Finally, transfused blood, if taken from a donor seropositive for parvovirus IgG, can confer some passive immunity on the fetus.

A common site for transfusion is the placental insertion of the umbilical cord; other options include the intrahepatic umbilical veins or the cardiac ventricles. Non-hydrops fetuses usually need only one transfusion, whereas 36% of hydropic fetuses will need two or more transfusions<sup>140</sup>. The risk of fetal demise depends on the presence of hydrops (29% in hydropic *vs* 5.5% in non-hydrops fetuses) and the gestational age at transfusion (highest risk before 20 weeks)<sup>140</sup>. At later gestations, it may be preferable to deliver the baby early and transfuse the neonate. Fetal hydrops usually resolves within 6 weeks of intrauterine blood transfusion<sup>136,137</sup>. The ascites may persist for several weeks; this should not be regarded as failed treatment. Other congenital infections that can cause fetal anemia include CMV, syphilis and toxoplasmosis. However, in these cases, the anemia is generally not sufficiently severe to cause fetal hydrops.

Overall, the risk of perinatal death is 30% for fetuses presenting with hydrops *vs* 6% for non-hydrops fetuses. Evidence regarding long-term outcome of infected fetuses is limited, but it appears that the risk for abnormal neurodevelopment is low (approximately 10%) in hydropic and negligible in non-hydrops fetuses<sup>120,140</sup>.

## RUBELLA

The widespread implementation of rubella immunization led to the elimination in the World Health Organization (WHO)'s Region of the Americas in 2015 of rubella and congenital rubella syndrome, and 33 of 53 (62%) countries in the European Region have now eliminated them also. The uptake of vaccination continues to increase

internationally and, by December 2016, 152 of 194 (78%) countries worldwide were using the vaccine<sup>141</sup>. In the UK, routine rubella immunity testing at the pregnancy booking visit has recently been discontinued because the risk of rubella infection in pregnancy is now so low; the vaccination program has led to high levels of herd immunity in the community (98–99% of women of childbearing age are immune)<sup>123</sup>.

The incubation period for rubella is 14–21 days, and individuals are infectious from 7 days before until 10 days after onset of the rash. In adults, including pregnant women, rubella infection is generally mild; it may be asymptomatic or consist of mild general malaise, headache, cold-like symptoms and lymphadenopathy. This is usually followed by the rubella rash, which is diffuse, fine and maculopapular.

In contrast to most viral infections during pregnancy, the risk of fetal infection decreases with increasing gestational age at maternal infection; it is around 90% before 12 weeks' gestation, 55% from 12 to 16 weeks and 45% after 16 weeks. In common with other viral infections, however, the risk of an infected fetus being affected (i.e. risk of developing congenital defects) is greatest when infection occurs earlier in gestation: it is 97% when infection is before 12 weeks and 20% when it is from 12 to 16 weeks, while infection from 16 to 20 weeks is associated with a minimal risk of deafness only<sup>123,142–144</sup>. The risk of the fetus being affected as a result of primary maternal infection after 20 weeks' gestation is very small. Reinfection has been reported, but the risk to the fetus in this situation is small (< 5%)<sup>145</sup>.

### Diagnosis of maternal rubella infection

#### Recommendation

- Clinicians should be aware of the high (15–50%) false-positive rate of rubella IgM and interpret results within the clinical context (**GRADE OF RECOMMENDATION: C**).

Maternal rubella infection is diagnosed by testing for serum levels of IgM and IgG. Rubella-specific IgG is usually present within a week after the onset of the rash. IgM levels rise early but IgM assays have a 15–50% false-positive rate<sup>146</sup>, which may be related to cross-reactivity with other viruses, long-term persistence after vaccination or even the presence of autoantibodies<sup>147,148</sup>. Therefore, the diagnosis of acute rubella infection should not rely on a positive IgM test alone, but should also take into account the history of relevant exposure, development of a rash, history of vaccination and results of previous rubella testing<sup>146</sup>. Similar to other viral testing, rubella IgG avidity can help to determine the timing of infection; high avidity usually indicates infection occurring more than 3 months earlier<sup>149–151</sup>, while low-avidity antibodies are usually associated with infection within the previous 3 months.

### Diagnosis of fetal rubella infection

#### Recommendations

- When primary infection occurs before 12 weeks' gestation, given the risk of fetal infection and the risk of an infected fetus developing severe abnormalities, termination of pregnancy can be considered, even without invasive testing (**GOOD PRACTICE POINT**).
- Amniocentesis performed within 6 weeks of primary maternal infection carries a risk of being false-negative; therefore, a negative result in these circumstances may justify later repeat invasive testing (**GRADE OF RECOMMENDATION: D**).

Congenital rubella infection can have serious consequences for the fetus. Congenital rubella syndrome includes hearing loss, learning disability, heart malformations and eye defects. As mentioned previously, the risk of fetal abnormalities is greatest when infection occurs before 16 weeks' gestation. The fetus can also be affected by FGR, hepatomegaly, splenomegaly, jaundice, thrombocytopenic purpura, anemia and rash. Some sequelae may present late after birth; these include late-onset deafness, eye defects, neurodevelopmental delay, and endocrinopathies.

Infection of the fetus can be confirmed by amniocentesis. This is usually delayed until after 18–20 weeks' gestation, when fetal urination is established. When primary infection occurs before 12 weeks' gestation, given the risk of fetal infection and the risk of an infected fetus developing severe abnormalities, it is reasonable to consider termination of pregnancy when appropriate, even without invasive testing. As a result, invasive testing is usually performed for primary maternal infections occurring between 12 and 16 weeks of gestation, the risk to the fetus of infection after that time being small.

The viral nucleic acid can be detected in the amniotic fluid using PCR; this test has high sensitivity and specificity. Amniocentesis performed within 6 weeks of the primary maternal infection carries a risk of being false-negative<sup>152</sup>, so a negative result in these circumstances may justify repeat invasive testing later.

### VARICELLA-ZOSTER VIRUS (VZV)

VZV is a DNA virus of the herpesvirus family that is highly contagious. It is transmitted by respiratory droplets and by direct personal contact with vesicle fluid or indirectly via fomites. Over 90% of pregnant women are already immune to VZV, having previously had the infection, usually in childhood. This means that primary infection in pregnancy occurs in only 3 per 1000 pregnancies<sup>123</sup>. Varicella (chickenpox) has a characteristic rash that is initially maculopapular, then becomes vesicular; the vesicles subsequently crust over, then heal completely. The rash is usually accompanied by fever and malaise. The incubation period is 7–21 days, but patients are infectious from 48 h before the rash

appears until the vesicles have crusted over<sup>153</sup>. Maternal VZV infection during pregnancy can be serious, with significant morbidity, including varicella pneumonia and, potentially, maternal death. It is also associated with the risk of perinatal mortality and morbidity.

### Diagnosis of maternal VZV infection

#### Recommendations

- Non-immune pregnant women should be considered at high risk for contracting VZV if exposed to significant contact (face-to-face for 5 min or in the same room for 15 min or more) with an infectious patient (**GRADE OF RECOMMENDATION: D**).
- For the purposes of counseling, an estimate for the risk of congenital varicella syndrome is 0.5% if maternal infection was in the first 13 gestational weeks, and 2% for infections between weeks 13 and 20. The risk for congenital varicella syndrome is minimal after this point; however, there is a 25% risk of clinical neonatal varicella if infection occurs after 36 weeks (**GRADE OF RECOMMENDATION: D**).
- Pregnant women developing herpes zoster (shingles, caused by the same virus) during pregnancy should be reassured that it has not been associated with fetal or perinatal harm (**GRADE OF RECOMMENDATION: D**).

The diagnosis of VZV is based on the clinical findings of chickenpox of a classic pruritic, vesicular rash, so laboratory testing is not usually needed. Maternal serology testing for VZV in pregnancy is usually performed following contact with a known case of chickenpox. The risk of infection is associated with significant contact (face-to-face for 5 min or in the same room for 15 min or more), although, if a woman has had chickenpox previously, she can be assumed to be immune (because the rash is so characteristic) and serological testing is not essential. If she does not give such a history, testing for VZV IgG can demonstrate immunity or otherwise; a large proportion of women who do not have any known history of chickenpox infection will prove to be immune on testing. It may be possible to test the booking blood sample, which is often retained in the virology laboratory until the end of pregnancy.

The risk in case of congenital VZV infection is the development of fetal varicella syndrome. This does not occur at the time of initial fetal infection but when there is reactivation of the virus *in utero* at a later stage. Although the absolute numbers are quite small, the risk of fetal varicella syndrome may be approximately 0.5% for maternal infection before 13 weeks and 2% for infection between 13 and 20 weeks<sup>154,155</sup>. The risk of miscarriage does not appear to be increased if chickenpox occurs in the first trimester. If maternal infection occurs between 20 and 36 weeks of gestation, there does not appear to be any risk of fetal varicella syndrome. Maternal infection after 36 weeks is associated with a 50% fetal infection rate and a 25% clinical varicella rate in the neonate.

Maternal herpes zoster (shingles, caused by the same virus) does not carry any risk to the fetus<sup>154</sup>.

### Diagnosis of fetal VZV infection

Fetal varicella syndrome may include any of the following features: polyhydramnios (due to decreased movement or digestive-tract atresia), limb defects and dermatomal skin scarring (due to fetal herpes zoster), soft-tissue calcification and damage to the eyes and CNS<sup>156–160</sup>. Neurological defects include cortical atrophy, microcephaly, limb paresis, spinal cord atrophy, encephalitis, seizures and Horner's syndrome. In around half of fetuses/infants, the eyes will be affected by microphthalmia, chorioretinitis, cataracts or optic atrophy (Figure S3) and limb defects are present in around half of cases. FGR may be diagnosed on ultrasound imaging and developmental delay may occur<sup>156–159</sup>.

Fetal infection can be confirmed by amniocentesis; PCR can be used to detect VZV DNA. As ever, diagnosis of fetal infection (i.e. as confirmed by positive PCR analysis following amniocentesis) does not confirm that the fetus will be affected by varicella syndrome. One study<sup>161</sup> of nine women who suffered primary VZV infection before 24 weeks of gestation, and had a subsequent amniocentesis with positive results for the virus, found that, while four had fetuses that were affected, five babies were apparently unaffected. It is also worth noting that a negative result following amniocentesis does not completely rule out the possibility of fetal varicella syndrome developing.

### Management of maternal and fetal VZV infection

#### Recommendations

- Following maternal infection in the first 20 gestational weeks, serial ultrasound examinations should be performed from 5 weeks after the initial infection or from 16 gestational weeks, whichever is soonest (**GOOD PRACTICE POINT**).
- Following exposure to VZV, non-immune pregnant women should be offered varicella zoster immunoglobulin (VZIG) within 10 days after exposure. Oral acyclovir can also be considered as post-exposure prophylaxis from 7 days after exposure (**GRADE OF RECOMMENDATION: D**).
- Oral acyclovir should be offered to pregnant women with varicella within 24 h of the rash developing (**GRADE OF RECOMMENDATION: C**).
- The option of pregnancy termination should be considered in the event of prenatal diagnosis of fetal varicella syndrome after maternal infection in the first 20 gestational weeks (**GOOD PRACTICE POINT**).

Ultrasound features typical of varicella syndrome include microcephaly, hydrocephalus, limb defects, FGR and soft tissue calcification<sup>161</sup>. In the vast majority of fetuses that develop varicella syndrome, these

abnormalities can be diagnosed by  $\geq 5$  weeks after the initial maternal infection<sup>162</sup>. This suggests that serial ultrasound scans should be commenced from 5 weeks after the initial maternal infection or from 16 weeks of gestation, whichever is soonest.

VZIG and/or acyclovir have been given to the mother in an attempt to reduce the risk or severity of fetal varicella syndrome, although there is no conclusive evidence that they are of benefit<sup>163,164</sup>. VZIG should be commenced up to 10 days after exposure and oral acyclovir from 7 days after exposure. Oral acyclovir appears to be safe<sup>165</sup>. It should also be offered if maternal lesions develop<sup>166</sup> and has been shown to reduce both the duration of new lesion formation and the total number of new lesions, as well as improving constitutional symptoms, if started within 24 h of the rash developing<sup>167–169</sup>.

When maternal VZV infection is confirmed before 20 weeks of gestation, and fetal varicella syndrome is subsequently identified on ultrasound scan, there is a high chance of a severely affected baby; in this situation, the offer of termination of pregnancy should be considered where appropriate. When expert fetal-medicine ultrasound examination confirms that there are no apparent fetal abnormalities, the risk of neonatal sequelae is very small.

## ZIKA VIRUS (ZIKV)

ZIKV is a flavivirus that is usually transmitted by *Aedes* mosquitoes, but it can also be transmitted from human to human through sexual contact<sup>170–172</sup>. A safe and effective vaccine for ZIKV is not likely to be available for several years. During the 2015–2016 epidemic, the WHO advised that pregnant women avoid travel to ZIKV-affected areas, and that both men and women returning from these areas should practise safe sex or abstinence for 6 months after their return, regardless of whether or not they had symptoms<sup>173</sup>. Currently, the disease is considered endemic and travelling to countries where the virus is still present is permitted with some restrictions<sup>174</sup>.

### Diagnosis of maternal ZIKV infection

#### Recommendations

- Pregnant women should be asked routinely about their travel history (**GOOD PRACTICE POINT**).
- Pregnant women with suggestive symptoms and a history of recent travel to an area of high or moderate ZIKV risk, or of sexual contact with a person returning from an affected area, should be investigated for ZIKV (**GOOD PRACTICE POINT**).
- The primary test for ZIKV infection is real-time reverse transcription-PCR (rRT-PCR) of serum and urine (**GRADE OF RECOMMENDATION: C**).

Up to 80% of people infected with ZIKV may have minimal or no symptoms<sup>175,176</sup>. In the 20% that have symptoms, it is usually a mild self-limiting illness, with

mild fever, skin rash, conjunctivitis, muscle and joint pain, malaise and headache. ZIKV is also associated with the development of Guillain Barré syndrome<sup>177</sup>. ZIKV does not appear to affect pregnant women any differently from the general population<sup>173,178</sup>. The incubation period is thought to be between 3 and 12 days<sup>179</sup>. Any pregnant woman presenting with these symptoms and with a history of recent travel to an area of high or moderate ZIKV risk, or a history of sexual contact with a person returning from an affected area, should be investigated for ZIKV. Pregnant women should be asked routinely about their travel history.

The primary test for ZIKV infection is rRT-PCR of serum and urine. Antibody testing can be performed once more than a week has passed since symptom onset. Serology tests for ZIKV are prone to false-positive results, due to cross-reactivity from other flaviviruses such as dengue (which is transmitted by the same vector and to which many of the ZIKV-exposed population are also exposed). For those with negative results, before ZIKV infection can be excluded with confidence, repeat testing may be recommended a few weeks after the last possible exposure<sup>180</sup>.

### Diagnosis of fetal ZIKV infection

#### Recommendations

- A baseline fetal ultrasound examination should be performed after potential maternal exposure to ZIKV, with referral to an ultrasound or fetal-medicine specialist in case of concerning features (**GOOD PRACTICE POINT**).
- If the baseline scan is normal, a repeat scan in the third trimester can be considered (**GOOD PRACTICE POINT**).
- Fetuses of mothers with a third-trimester rash and which have a normal head circumference (HC) may still have underlying brain abnormalities and should be screened for the remainder of the pregnancy and after birth (**GRADE OF RECOMMENDATION: C**).
- Pregnant women with ZIKV infection should be informed that the risk of congenital disabilities is higher with earlier infection and may be independent of the presence or absence of maternal symptoms (**GRADE OF RECOMMENDATION: C**).

Baseline fetal ultrasound examination should be performed after potential maternal exposure to ZIKV, with referral to a fetal-medicine specialist if there are any concerning features<sup>181–183</sup>. In women with negative ZIKV testing but fetal abnormalities such as microcephaly and intracerebral calcifications on ultrasound, consideration should be given to other congenital infections, including CMV, toxoplasmosis and rubella, which can present with similar findings.

Potentially exposed pregnant women with no reported symptoms should have an ultrasound examination for fetal growth and anatomy. If this is abnormal, referral for specialist fetal-medicine review is advised. If the baseline scan is normal, a repeat scan in the third trimester can

be considered. No serological testing would be advised in these cases<sup>181</sup>.

A systematic review of 72 studies concluded that there is now sufficient evidence to confirm ZIKV as a cause of congenital brain abnormality<sup>177</sup>. Congenital ZIKV infection can lead to microcephaly, as well as craniofacial disproportion, specific brain abnormalities and neurological symptoms (Table S6)<sup>182,184–190</sup>. Recently, the term ‘congenital Zika Syndrome’ (CZS) has been used to describe the spectrum of abnormalities associated with maternal ZIKV infection in pregnancy<sup>182,184–191</sup>. Infants with confirmed ZIKV infection born with normal HC may still have underlying brain abnormalities<sup>192,193</sup>. This knowledge has important implications for counseling and neonatal screening.

The risk of CZS following infection during pregnancy, and whether this risk is related to the gestational age at infection, remain unclear. One retrospective study estimated the risk of ZIKV-associated microcephaly to be 95 per 10 000 women infected in the first trimester (compared with a background rate of two cases of microcephaly per 10 000 neonates); however, this was based on only eight cases<sup>194</sup>. A prospective cohort study with a short follow-up of pregnancies in the recent Brazilian outbreak found that, among liveborn infants of ZIKV-infected women, 55% of those infected in the first trimester had adverse outcome, 52% if in the second trimester and 29% if in the third trimester<sup>184</sup>. Preliminary analysis of data from the USA Zika Pregnancy Registry has shown that, among 442 completed pregnancies with laboratory evidence of possible recent ZIKV infection, 6% of the fetuses or infants had a ZIKV-associated congenital anomaly. This rate varied from 11% for infections during the first trimester or at the periconception period, to 0% for exposure exclusively in the second or third trimester. Interestingly, the rate of congenital anomalies was very similar in symptomatic (6%; 95% CI, 3–11%) and in asymptomatic (6%; 95% CI, 4–9%) women<sup>195</sup>. Comparable rates of 5% amongst symptomatic mothers and 4% amongst asymptomatic mothers were subsequently reported in a larger study from the USA territories<sup>196</sup>. In a large cohort study that followed 301 pregnant women with laboratory-proved ZIKV infections from French Guyana, fetal CNS involvement was higher in the infected group than in the control group (9.0% *vs* 4.3%; RR, 2.11 (95% CI, 1.18–4.13))<sup>197</sup>; in a follow-up paper, the authors found that, in cases of a known maternal ZIKV infection, approximately one-quarter of fetuses will become congenitally infected, of which a third will have severe complications at birth or undergo fetal loss<sup>198</sup>.

## Diagnosis of CZS

### Recommendations

- Microcephaly should be diagnosed when the HC measures  $\geq 2$  SD below the mean, although HC  $\geq 3$  SD below the mean is associated with a higher risk of brain abnormalities (**GRADE OF RECOMMENDATION: D**).

- HC should not be used to ascertain gestational age in pregnancies in which there has been exposure to ZIKV (**GRADE OF RECOMMENDATION: C**).
- Following maternal exposure to ZIKV, an assessment of fetal anatomy (including intracerebral abnormalities) and biometry should be undertaken (**GRADE OF RECOMMENDATION: C**).
- Any pregnancy with signs of CZS should be managed in a fetal-medicine center with experience in diagnosis of fetal infections (**GOOD PRACTICE POINT**).
- The abnormalities described in Table S6 are usually diagnosed on ultrasound; when there are doubts regarding ultrasound findings, clinicians may consider fetal MRI if available (**GOOD PRACTICE POINT**).
- Amniocentesis should not be performed for detection of ZIKV until after 20 weeks’ gestation (**GOOD PRACTICE POINT**).

ZIKV infection appears to cause a characteristic pattern of brain abnormalities, which is different from those observed in cases of severe CMV infection; however, there is still much that is unknown. The mainstay of diagnosis is fetal ultrasound examination. Microcephaly is defined as head size that is smaller than expected, but it will almost never be found with normal brain imaging. Relying only on the HC measurement, with differences in interpretation of this definition, led to over-reporting of cases in Brazil, particularly in the early stages of the epidemic. Microcephaly should be suspected when HC  $\geq 2$  SD below the mean, although correlation with brain abnormalities is greater when HC  $\geq 3$  SD below the mean<sup>173,199</sup>. HC should not be used to ascertain gestational age in pregnancies in which there has been exposure to ZIKV<sup>183</sup>. Microcephaly in itself is not a disease and has many different causes. However, its presence in the context of ZIKV infection should raise the suspicion of an underlying abnormality. As well as biometry, an assessment of fetal anatomy, including intracerebral abnormalities, should be undertaken. Any pregnancy with signs of CZS should be managed in a fetal-medicine center with experience in fetal infections. The abnormalities described in Table S6 are usually diagnosed on ultrasound; when there are doubts regarding the ultrasound findings, the clinicians may consider fetal MRI if available. Consideration should also be given to the risks and benefits of amniocentesis to test for ZIKV by rRT-PCR. The correlation of a positive amniocentesis PCR result with fetal abnormality remains unclear and expert virology advice should be sought first. Amniocentesis should not be performed for the detection of ZIKV until after 20 weeks’ gestation, as fetal urination is not well established before this stage and fetal urine is the source of ZIKV in the amniotic fluid<sup>173,181,183,200</sup>.

## Management of pregnancies with CZS

### Recommendations

- ZIKV-affected pregnancies should be managed in an ultrasound or fetal-medicine unit with serial ultrasound

scans and the availability of further laboratory testing (GOOD PRACTICE POINT).

- The option of termination of pregnancy should be discussed when appropriate.
- Clinicians should acknowledge the limitations of existing knowledge on the prognosis for CZS. Following a thorough follow-up imaging assessment with normal findings, the risk of developing CZS is apparently low (GOOD PRACTICE POINT).
- Women who continue a pregnancy with suspected CZS should have input from a multidisciplinary team, including fetal-medicine specialists, neonatologists and radiologists, as appropriate. After birth, follow-up until at least 12 months of age is recommended (GOOD PRACTICE POINT).

These pregnancies should be managed in an ultrasound or fetal-medicine unit with serial ultrasound scans and the availability of further laboratory testing. Other causes of microcephaly and brain abnormalities should be considered and excluded as appropriate. Women should be counseled on an individual basis and the option of termination of pregnancy should be discussed when appropriate. Clinicians should acknowledge the limitations of existing knowledge on the prognosis for CZS. Following a thorough follow-up imaging assessment with normal findings, the risk of developing CZS is apparently low<sup>198</sup>. In other congenital viral infections, such as CMV and toxoplasmosis, which can cause similar brain abnormalities, the presence of microcephaly would suggest a poor prognosis, whilst normal ultrasound findings would suggest a good prognosis<sup>35,92,173</sup>. However, this may not be entirely true of ZIKV in which brain abnormalities have been found in infants with a normal HC<sup>192,193</sup>. Women who continue a pregnancy with suspected CZS should have input from a multidisciplinary team, including fetal-medicine specialists, neonatologists and radiologists, as appropriate. After birth, follow-up until at least 12 months of age is recommended<sup>201</sup>.

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#### CITATION

This Guideline should be cited as: 'Khalil A, Sotiriadis A, Chaoui R, da Silva Costa F, D'Antonio F, Heath PT, Jones CE, Malinger G, Odibo A, Prefumo F, Salomon LJ, Wood S, Ville Y. ISUOG Practice Guidelines: role of ultrasound in congenital infection. *Ultrasound Obstet Gynecol* 2020; 56: 128–151.'

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## APPENDIX 1 Levels of evidence and grades of recommendation used in ISUOG Guidelines

### Classification of evidence levels

1++	High-quality meta-analyses, systematic reviews of randomized controlled trials or randomized controlled trials with very low risk of bias
1+	Well-conducted meta-analyses, systematic reviews of randomized controlled trials or randomized controlled trials with low risk of bias
1–	Meta-analyses, systematic reviews of randomized controlled trials or randomized controlled trials with high risk of bias
2++	High-quality systematic reviews of case–control or cohort studies or high-quality case–control or cohort studies with very low risk of confounding, bias or chance and high probability that the relationship is causal
2+	Well-conducted case–control or cohort studies with low risk of confounding, bias or chance and moderate probability that the relationship is causal
2–	Case–control or cohort studies with high risk of confounding, bias or chance and significant risk that the relationship is not causal
3	Non-analytical studies, e.g. case reports, case series
4	Expert opinion

### Grades of recommendation

A	At least one meta-analysis, systematic review or randomized controlled trial rated as 1++ and directly applicable to the target population; or systematic review of randomized controlled trials or body of evidence consisting principally of studies rated as 1+ applicable directly to the target population and demonstrating overall consistency of results
B	Body of evidence including studies rated as 2++ applicable directly to the target population and demonstrating overall consistency of results; or evidence extrapolated from studies rated as 1++ or 1+
C	Body of evidence including studies rated as 2+ applicable directly to the target population and demonstrating overall consistency of results; or evidence extrapolated from studies rated as 2++
D	Evidence of level 3 or 4; or evidence extrapolated from studies rated as 2+
Good practice point	Recommended best practice based on clinical experience of the Guideline Development Group

**SUPPORTING INFORMATION ON THE INTERNET**

The following supporting information may be found in the online version of this article:



**Figure S1** Investigation for parvovirus B19 in pregnant women presenting with rash or following exposure to viral rash (adapted from Public Health England<sup>153</sup>).

**Figure S2** Technique for measuring middle cerebral artery Doppler (adapted from Mari *et al.*<sup>134</sup>).

**Figure S3** Ultrasound findings in fetuses with fetal varicella syndrome.

**Table S1** Frequency of abnormal ultrasound findings in pregnancies complicated by congenital cytomegalovirus infection<sup>18</sup>

**Table S2** Inclusion criteria used by Leruez-Ville *et al.*<sup>54</sup> to define fetus moderately infected with cytomegalovirus (CMV)

**Table S3** Risk of *Toxoplasma gondii* congenital infection (transmission) and development of clinical signs in offspring < 3 years of age, according to gestational age (GA) at maternal seroconversion<sup>66,79</sup>

**Table S4** Interpretation of serological test results for toxoplasma infection performed at clinical (non-reference) laboratories<sup>79</sup>

**Table S5** Ultrasound abnormalities in fetuses infected by parvovirus B19<sup>202</sup>

**Table S6** Fetal and neonatal abnormalities reported in pregnancies with congenital Zika syndrome (CZS)<sup>184–190,203</sup>