J. Fetal Med. (September 2020) 7:243–252
https://doi.org/10.1007/s40556-020-00259-2

REVIEW ARTICLE

Diagnosis of Infections in Fetus: Ultrasound and Invasive Techniques

Anubhuti Rana[1] · **K. Aparna Sharma**[1]

Received: 15 August 2019 / Accepted: 7 July 2020 / Published online: 1 August 2020

Abstract Infections in pregnancy may lead to fetal morbidity and mortality. The major infections which can be transmitted from the mother to the fetus and can probably result in adverse consequences in the prenatal period include toxoplasmosis, rubella, cytomegalovirus (CMV), syphilis, parvovirus, and varicella. Factors determining the risks of transplacental transmission and associated fetal adverse events include the period of gestation at which transmission occurs and the immunity status of the mother. No single modality can diagnose all fetal infections and consequently prenatal diagnosis of fetal manifestations of infections is frequently made by fetal sonography and serology to identify the specific infectious agent. Ultrasound is now considered the safest and least invasive primary tool for the detection and monitoring of antenatal infection. Major findings in cases of fetal infection are observed in the central nervous system and heart with parenchymal calcifications, pleural/pericardial effusions, ascites, fetal growth restriction, oligo-/polyhydramnios and placentomegaly being frequently seen. Various diagnostic interventions are also performed under ultrasound guidance such as amniocentesis to determine underlying etiology and cordocentesis to detect fetal anemia. Knowledge of the various diagnostic modalities is required for appropriate counseling of the couple and for optimal treatment.

Keywords Fetal infections · Prenatal diagnostic procedures · Amniocentesis · Ultrasound · Chorionic villus sampling · Cordocentesis

Introduction

Infections in pregnancy are a common cause of fetal morbidity and mortality. Maternal infections can be transmitted to the fetus; however, neither are all fetuses infected nor symptomatic. The various infections which can cause congenital defects include TORCH infections, parvovirus B19, varicella zoster and most recently Zika virus. With the increasing awareness of the impact of fetal infections, the need for updating the diagnostic techniques for in utero infections has also been realized. Prenatal manifestation of fetal infections can be seen on ultrasound. Diagnosis would require demonstrating maternal seroconversion and/or invasive fetal testing.

Role of Ultrasound in Diagnosis of Infections in Fetus

The detection of microorganisms by cultures, immunologic methods, and special molecular biology techniques has traditionally been the mainstay for diagnosis of fetal infections [1]. However, it is seen that apart from cases of fetal infections with subtle abnormalities, obvious fetal malformation and/or fetal death can occur by the time infection is confirmed by these techniques. Ultrasound can be a useful modality in the detection of the majority of serious anomalies as well as minor manifestations that are characteristic of fetal infection. Therefore, certain non-specific findings or occasionally discovered few

✉ K. Aparna Sharma
kaparnasharma@gmail.com

[1] Division of Fetal Medicine, Department of Obstetrics and Gynecology, All India Institute of Medical Sciences, New Delhi 110029, India

specific findings (discussed separately below) can act as markers for manifestations of fetal infection on prenatal ultrasound.

Non-specific findings common to various viral infections:

- Cranium: ventriculomegaly/hydrocephalus, microcephaly, intracranial calcifications, hydranencephaly,
- Cardiac: atrial/ventricular septal defects, patent ductus arteriosus (PDA), cardiomegaly, pericardial effusion,
- Abdomen: hepatosplenomegaly, ascites, hyperechogenic bowel, intra-abdominal calcification,
- Fetal growth restriction (FGR),
- Amniotic fluid changes: oligohydramnios, polyhydramnios (especially with FGR is associated with congenital infections),
- Non-immune hydrops,
- Placentomegaly and shrunken placentas,
- Early pregnancy loss.

Ultrasound is not only used to point to certain non-specific/specific findings of congenital infections which may warrant further investigation for confirmation but can also be used for serial follow-up after serologic evidence of maternal infection for diagnosis of late manifestations of fetal infection which may not be apparent on the initial ultrasound scan. Doppler ultrasound is a very useful tool and is most commonly used to detect and monitor fetal anemia by serial measurement of peak systolic velocity in the middle cerebral artery (MCA-PSV) in case of parvovirus infection. It can also be used to analyse the nature of malformation, especially congenital heart defects and the subsequent evolution of these congenital heart defects can be judged (such as pulmonary artery stenosis [PS]) to decide timing of delivery and need for prenatal treatment like in utero balloon valvuloplasty. Therefore, ultrasound is now considered the safest, least invasive and effective way for detection and monitoring of antenatal infection.

Various diagnostic interventions are also performed under ultrasound guidance and include amniocentesis to determine underlying etiology and cordocentesis to detect fetal anemia. Therapeutic interventions such as intrauterine transfusion can also be performed simultaneously with cordocentesis if fetal anemia is detected. Apart from this, response to therapy can also be judged by ultrasound in certain infections such as the resolution of hydrops following parvovirus infection. 3-D ultrasound is a newer modality which can be used in the diagnosis of fetal anomalies like cleft lip, limb malformations and structural heart defects associated with fetal infections. The use of ultrasound to detect markers of infection may help the clinicians to accurately identify the causative agent, correlate with antepartum and postpartum syndromes associated with it and aid in reducing the problem of adverse outcomes which can happen with an unsuspected or undiagnosed fetal infection [2].

There are, however, limitations in the use of ultrasound in diagnosing fetal infections and a normal ultrasound examination can neither rule out fetal infection nor predict a normal postnatal result. It is essential to highlight that even though alterations on ultrasound may not be detected on the initial evaluation, they may develop over time and can be detected on subsequent ultrasound examinations. Also, the findings may be non-specific and overlapping and hence, may not always lead to an exact diagnosis of a particular infectious agent or a non infectious etiology.

Therefore, the role of ultrasound is clearly defined for the following purposes:

- Incidental findings during a systeatic examination that suggest a fetal infection.
- Follow-up for detection of fetal infection after documented maternal seroconversion.
- Ultrasound guided procedures to obtain samples for the detection of RNA by polymerase chain reaction (PCR) or IgM antibodies in the fetus.

Role of Invasive Procedures for Prenatal Diagnosis of Infections in Fetus

The traditional method for diagnosis of intrauterine infection is based on three pronged approach of detection of maternal infection which includes detection of specific histopathologic features in fetal/placental tissues, detection of immunologic response, and, use of culture methods designed for likely detection of microorganisms. Identification of organism, specific antigens or specific immunoglobulin (Ig) in amniotic fluid or fetal blood is the basis of evidence of infection.

The ability of the fetus to produce specific IgM antibodies is dependent on the maturity of the fetal immune system. Consequently, identification of specific IgM antibodies strongly favours diagnosis of fetal infection but the absence does not rule it out [3].

Prenatal invasive techniques such as chorionic villous sampling (CVS), amniocentesis and cordocentesis can be used to diagnose infections in the fetus. CVS can be used only when there is serological evidence of maternal infection in early pregnancy. Amniocentesis is the most common method used for the diagnosis of underlying etiology. PCR and nucleic acid hybridization techniques are some of the recent advances for the detection of microorganisms and diagnose fetal infections with greater accuracy. Fetal blood obtained by cordocentesis can be used for culture, serology and PCR. With the promising results of amniotic fluid PCR, the current role of cordocentesis is limited to the diagnosis of

fetal anemia in Parvovirus B19 infection and simultaneous therapy with intrauterine transfusion. The role of prenatal invasive procedures in the diagnosis of specific fetal infections is described under the specific causative organisms.

Common Fetal Infections

Rubella

Congenital rubella infection results in inhibition of cell division and cytopathic damage to blood vessels leading to ischemic changes in various organs which may result in miscarriage, stillbirth or congenital rubella syndrome. The congenital rubella syndrome includes a variable pattern of birth defects including cataracts, congenital glaucoma, patent ductus arteriousus (PDA), peripheral pulomnary stenosis (PS), sensorineural deafness, microcephaly, delay in development, mental retardation, meningoencephalitis, pigmentary retinopathy, purpura, organomegaly and radiolucent bone disease. Risk of maternal-to-fetal transmission depends upon the timing of maternal infection, with the highest risk of up to 80% in the first trimester, decreasing to 25% in the late second trimester and increasing again in the third trimester from 35% at 27–30 weeks gestation to almost 100% beyond 36 weeks gestation [4].

When to Suspect Fetal Infection

a. Maternal Clinical History

Although 20–50% of infections may be asymptomatic, the most common clinical feature in adults is a mild, febrile illness with a generalized maculopapular rash, typically beginning from the face and spreading to trunk and limbs. The rash usually resolves within 3 days in the same order as it appeared [4]. Other symptoms may include arthralgias or arthritis, lymphadenopathy, conjunctivitis and upper respiratory infection.

b. Prenatal Sonographic Findings

Certain features detected on prenatal ultrasound which can be a marker of rubella infection include septal defects (Fig. 1), PS, microcephaly, ventriculomegaly (Fig. 2), periventricular calcification, microphthalmia, cataract, FGR and amniotic fluid abnormalities.

Work Up for Fetal Infection

a. Maternal Seroconversion and Avidity Testing

Serological confirmation is required for diagnosis in pregnant women who have had contact with rubella or with clinical features suggestive of disease [5]. Enzyme linked immunoassays (ELISA) which measures rubella-specific IgG and IgM is sensitive, easy to perform, convenient and an accurate test. Specific IgM antibody can be detected using ELISA from 4 to 5 days after symptom onset and can persist for 6 weeks. Peak IgG antibody titres are seen 1–2 weeks after the onset of the rash in cases of primary exposure.

In the absence of a history of fever with rash and incidental detection of rubella IgM in a pregnant lady, further test using rubella specific avidity assay is required as the reactive IgM in such individuals with no or low exposure could be due to rheumatoid factor or cross-reactivity with other antibodies leading to a false positive result. Hence, screening in pregnancy using rubella IgM is discouraged because of the chances of false positive results.

b. Invasive Testing

Antenatal testing is recommended at least 6 weeks after known maternal infection and is best performed post 20 weeks of gestation. Reverse transcriptase PCR detecting rubella viral nucleic acid in samples obtained from chorionic villous sampling (CVS) and amniocentesis is useful for prenatal diagnosis of fetal infection. Fetal IgM can be detected in a fetal cord blood sample and it may be a better sample for diagnosis in the second trimester as the amniotic fluid sample may be negative [6]. Viral culture of chorionic villi, amniotic fluid or fetal blood may also aid in confirmation.

Cytomegalovirus

It is the commonest cause of intrauterine infection in the developed world, occurring in about 0.64% of all live births. It is the commonest and most important cause of non-genetic sensorineural hearing loss, apart from being a common cause of mental retardation [7]. The risk of vertical transmission post primary maternal infection increases progressively with advancing POG with the risk being highest in the 3rd trimester, although the risk of symptomatic neonatal disease is low. However, infection during the early half of pregnancy carries the maximum risk of adverse sequelae in the fetus [8].

When to Suspect Fetal Infection

a. Maternal Clinical History

Most infections are asymptomatic or present as a mild febrile illness, but about 15% of adults may present with infectious mononucleosis-like-syndrome. Following primary infection, the virus becomes latent. Recurrence, which may be due to re-activation or exogenous re-infection with a different strain, is not prevented by maternal immunity to the virus, nor does it prevent congenital infection.

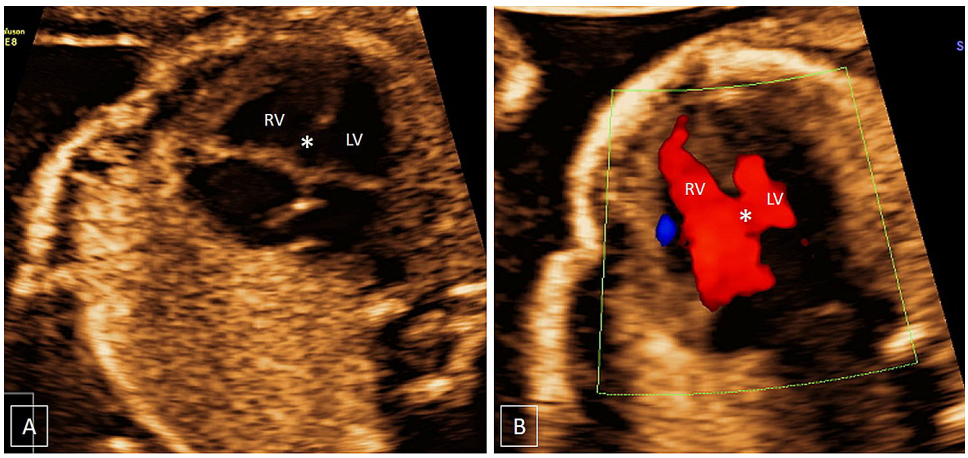

Fig. 1 B-mode ultrasound (**a**) and color Doppler (**b**) image in the four chamber view shows the presence of a ventricular septal defect (*) with flow across it. *RV* right ventricle, *LV* left ventricle

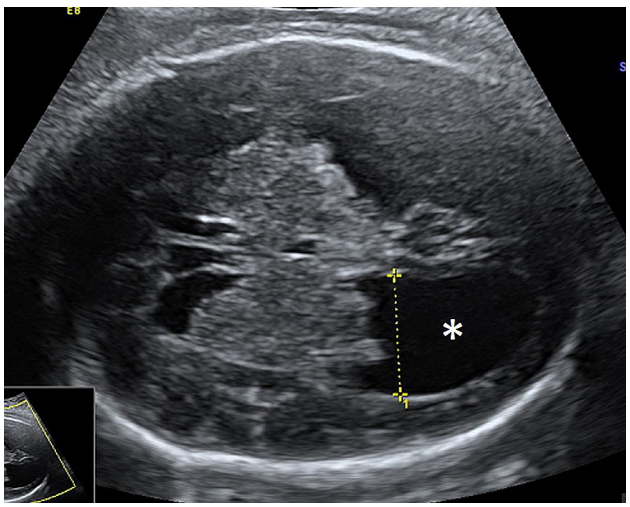

Fig. 2 Axial ultrasound image of the fetal brain shows dilated lateral ventricles (*)

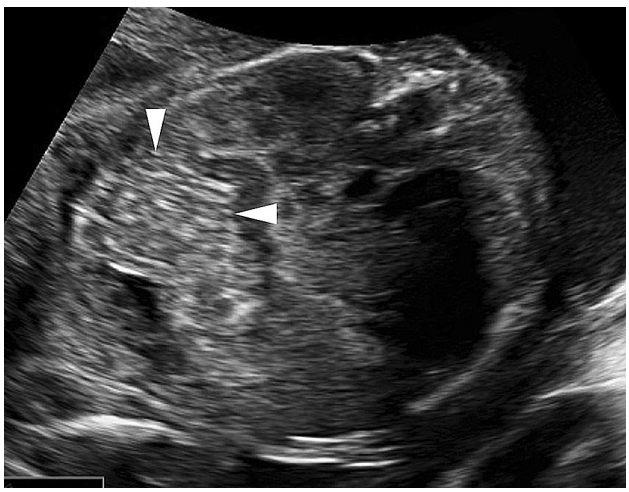

Fig. 3 Axial ultrasound image of the fetal abdomen shows hyperechogenic bowel (arrowheads)

Recurrent maternal infection is a milder disease process as it results in less severe fetal infection and even vertical transmission is seen in only 0.15–2% cases as compared to a 30–40% rate of transmission in case of primary CMV infection [8]. Reactivation of CMV infection presents with the mildest clinical picture with most infants being asymptomatic at birth [8].

b. Prenatal Sonographic Findings

Prenatal sonographic findings of CMV infection include ventriculomegaly, porencephaly, intracranial calcifications, FGR, oligohydramnios, hyperechogenic bowel (Fig. 3), ascites, hepatosplenomegaly, placentomegaly, pulmonary hypoplasia and rarely pericardial or pleural effusion [9]. The presence of fetal cerebral abnormalities has been shown to be the major prognostic indicator on ultrasound [10].

Work Up for Fetal Infection

a. Maternal Seroconversion and Avidity Testing

Maternal seroconversion and avidity testing is offered as a part of the evaluation of mononucleosis-like illnesses or when a fetal anomaly suggestive of congenital CMV infection is detected on prenatal ultrasound examination [11].

Diagnosis is made by documenting:

1. Seroconversion: Appearance of de novo CMV-specific IgG antibodies, when paired acute and convalescent serum samples are tested 3–4 weeks apart or pre-pregnancy serological status is known.
2. In case, the maternal immune status of the patient is unknown or pre-pregnancy serum sample is not available, as is often the case, diagnosis is based on documenting CMV IgM antibody in the maternal serum. However, the use of specific IgM antibody is limited for serological diagnosis as IgM may persist for

months after primary infection and there may be cross-reactivity with other antibodies [10]. IgG avidity testing better detects the timing of infection and distinguishes primary from secondary infection. Avidity, described as the strength with which the IgG antibody attaches to the antigen, matures with the amount of time following primary infection. In a person with a recent primary CMV infection, the body produces low-avidity IgG (< 30%) indicating a primary infection in the past 3 months [12]. After 3 months, high-avidity (> 60%) CMV IgG are produced indicating CMV infection that is more than 3 months old.

b. Invasive Testing

Detection of CMV DNA using PCR or viral culture in the amniotic fluid is definitive for diagnosis. However, to avoid false negative results, amniocentesis is best performed after 21 weeks of gestation or 7 weeks after maternal infection as the viral transmission from mother to fetus does not occur immediately [13].

Varicella

Varicella infection in the fetus can present as either devastating embryopathy due to transplacental infection which manifests as congenital varicella syndrome or a less severe disease process in the form of neonatal varicella.

When to Suspect Fetal Infection?

a. Maternal Clinical History

The typical maculopapular and vesicular rash is accompanied by fever and constitutional symptoms for 3–5 days. Secondary streptococcal or staphylococcal skin infection is the most common complication. 10–20% of pregnant women infected with Varicella may develop pneumonia with the incidence and associated mortality of pneumonia being greater compared to non-pregnant women [14]. Post primary infection, the virus may remain dormant in sensory nerve root ganglia. Reactivation in the form of herpes zoster or shingles is not associated with viremia and does not appear to cause fetal adverse effects.

b. Prenatal Sonographic Findings

Ultrasonographic abnormalities may be detected after documented acute maternal infection. There should be a minimum interval of 5 weeks between the appearance of maternal rash and a detailed ultrasound for the diagnosis of congenital varicella syndrome [15]. Various findings on prenatal ultrasound include cerebral abnormalities such as ventriculomegaly, hydrocephalus, microcephaly with polymicrogyria, and porencephaly, asymmetric limb shortening or malformations, chest wall malformations, echogenic foci in the liver and intestine, FGR, polyhydramnios, fetal hydrops and fetal demise [15].

Work Up for Fetal Infection

a. Maternal Testing

The diagnosis of varicella infection is clinical. In case of doubt, a sample taken from an unroofed skin lesion or vesicular fluid can be tested using a qualitative varicella PCR assay or through the detection of VZV antigen by immunofluorescence. Culture of VZV can also be done from the vesicular fluid, although it is less sensitive. Serologic testing is usually not necessary for the diagnosis of maternal varicella and may be potentially confusing since the assays vary in sensitivity and specificity. Only the immune status of the mother can be assessed in early pregnancy by documenting the history of either a previous infection or varicella vaccination. In patients with no such history, IgG serology can help determine the immune status.

b. Invasive Testing

Amniotic fluid PCR for Varicella zoster virus DNA is now the method of choice for prenatal diagnosis of congenital varicella syndrome.

Herpes Virus

Vertical transmission of herpes simplex virus (HSV) usually occurs secondary to direct contact with the virus following membrane rupture or at delivery where it is being shed from infected sites (cervix, vagina, vulva, perineum) and is thus most commonly transmitted in peripartum period (85%); intrauterine (5%) or postnatal (10%) period being less common timing of acquiring the infection [16]. Transmission risk is greatest in cases of primary maternal infection especially in the third trimester or within 6 weeks of delivery because the shedding of the virus can continue in this period and birth can occur prior to the development of protective maternal antibodies.

When to Suspect Fetal Infection?

a. Maternal Clinical History

The manifestations of maternal infections are categorized into three groups:

1. *First-episode Primary Infection (Type-1)* It is described when HSV-1 or 2 is isolated from genital secretions in the absence of their antibodies. The typical incubation period is 2–10 days. Itchy papular eruptions appear

which later become painful and vesicular with multiple coalescing lesions seen on the vulva and perineum. A transient flu-like syndrome is common and presumably caused by viremia. Occasionally, hepatitis, encephalitis, or pneumonia may develop. In 2–4 weeks, all signs and symptoms of infection disappear.

2. *Non-primary First Episode (Type-2)* It is diagnosed when HSV is isolated in women who have antibodies to the other HSV. It is characterized by fewer lesions, lesser systemic manifestations and pain, and shorter duration of viral shedding which is probably secondary to cross-immunity to previously acquired type 1 infection.
3. *Reactivation Disease* It is described when HSV-1 or 2 is isolated from the genital tract in the presence of the same serotype antibodies. The viral particles reside in nerve ganglia during the latent period. Again, lesions associated with reactivation disease are fewer, less painful, and have a shorter duration of viral shedding (2–5 days) compared to primary infection.

b. Prenatal Sonographic Findings

Prenatal ultrasound may demonstrate brain abnormalities like ventriculomegaly, intracranial calcifications, microcephaly, hydranencephaly, cerebellar hypoplasia, microphthalmia, cataracts, non-immune hydrops, myocardial calcification, hepatosplenomegaly, echogenicity in the esophagus and bowel, absent fetal movements, flexion deformities, FGR and polyhydramnios.

Work Up for Fetal Infection

a. Confirmation of Maternal Infection

Diagnosis of genital herpes should be confirmed by laboratory testing. Clinical infection, as well as subclinical recurrences, can be confirmed by isolating the virus by culture. Prior to crusting of the lesions, the sensitivity of culture is as high as 95%. Cytological examination has a maximum sensitivity of 70%. PCR assay is the most sensitive and rapid test [17]. Type-specific serologic assays (ELISA) are available to detect antibody to HSV glycoproteins.

b. Zika Virus

Infection with Zika virus is caused by the bite of an infected Aedes mosquito and the virus can be passed from a pregnant lady to her fetus.

When to Suspect Fetal Infection

a. Maternal Clinical History

The pregnant lady can present with a mild disease initially with acute onset low-grade fever. It can be followed by a pruritic rash with erythematous macules and papules which can be associated with arthralgia and non-purulent conjunctivitis.

b. Prenatal Sonographic Findings

Fetal infection with Zika virus shows some similarities with CMV infection but it is more destructive. Optimal timing between exposure and initial sonographic screening and follow-up screening are unknown however, the minimum time may be as short as 2 weeks in some cases usually detected in the late second and early third trimesters of pregnancy. The prenatal ultrasound features include microcephaly, intracranial calcifications, absence of corpus callosum, vermian dysgenesis, enlarged cisterna magna and periventricular hyperechogenecities, microphthalmia, fetal growth restriction.

Work Up for Fetal Infection

a. Maternal Seroconversion

In the acute phase (3–5 days), the viral genome can be detected by RT-PCR in maternal serum. In the convalescent phase ($\geq$ 5 days), diagnosis can be made by testing IgM antibodies in the blood. However, this is not the mainstay of diagnosis as cross reactivity with other flaviviruses is very high.

b. Invasive Testing

Amniocentesis should be offered to women who present with ultrasound features indicating congenital zika virus syndrome and/or positive/inconclusive maternal laboratory findings. Although a positive Zika virus RT–PCR on amniocentesis is diagnostic of fetal viral exposure, the sensitivity and specificity are not well established. It is also dependent on the time interval between amniocentesis after the onset of maternal infection with the sensitivity being higher for testing done at $\geq$ 21 weeks or likely 6–8 weeks after maternal infection. Importantly, as the Zika virus RNA may be detected transiently, a negative amniocentesis does not conclusively negate congenital Zika virus infection.

Parvovirus B 19

Maternal parvovirus infection can lead to fetal infection in about 1/3rd cases [18]. As a result of suppression of erythropoiesis, severe anemia with consequent high-output congestive heart failure can occur in the fetus. Other manifestations of the in utero infection include abortion, hydrops fetalis and even fetal death [19]. Fetal infection develops usually within 10 weeks of maternal infection

and, therefore, > 80% cases of hydrops are detected in the 2nd trimester on antenatal ultrasound [20].

When to Suspect Fetal Infection?

a. Maternal Clinical History

The virus causes only mild, self-limited infection in adults and manifests as fever, headache, and flu-like symptoms. After the initial viremic phase, the patient's face shows a bright-red erythrodermic rash which progresses to a lace-like appearance spreading to the trunk and limbs.

b. Prenatal Sonographic Findings

Congenital malformations are uncommon despite the high risk of fetal loss. The findings which can be detected on ultrasound include fetal anemia and non-immune hydrops (NIH) (Fig. 4). Only 1% of infected women develop NIH, and especially if the infection is acquired in the initial 20 weeks of gestation as it is the phase of maximum fetal hepatic hematopoiesis [21].

Work Up for Fetal Infection

a. Maternal Seroconversion

Post exposure, IgM antibodies can be detected within 10 days and just before the clinical presentation; they may persist for three months or longer. Several days after IgM antibodies, IgG antibodies can be detected which usually persist for years and are an indicator of past infection. Exposed pregnant women or those presenting with symptoms suggestive of parvovirus B19 infection should undergo assessment for levels of IgG/IgM antibodies by radioimmunoassay and/or ELISA to assess susceptibility to infection (non-immune) or diagnosis of current infection [21]. Absence of IgG and IgM antibodies indicates susceptibility to infection and warrants repeat serologic testing in 4 weeks. Presence of IgG antibodies in the absence of IgM antibodies indicates immunity and the patient can be reassured. Presence of IgM antibodies in the absence of IgG antibodies indicates acute infection while the presence of both IgG and IgM antibodies indicates subacute infection. In both scenarios, monitoring for fetal infection with Doppler ultrasound for MCA-PSV and for signs of hydrops on ultrasound.

b. Invasive Testing

Cordocentesis can be performed to detect parvovirus-specific IgM in fetal blood using ELISA, immunofluorescence and radioimmunoassay. Fetal infection can also be diagnosed by the detection of viral DNA on cordocentesis or amniocentesis by PCR [21].

Toxoplasmosis

Congenital infection can result from transplacental transmission of parasites after a primary maternal infection in pregnancy. High parasite load and maternal infection at an advanced gestational age are important risk factors for fetal infection with the risk of fetal infection increasing from 6% at 13 weeks of gestation to 72% at 36 weeks of gestation. However, the frequency of severe sequelae is greater when infection occurs early in pregnancy [22].

When to Suspect Fetal Infection

a. Maternal Clinical History

Acute maternal infection is asymptomatic in up to 80% of cases. In the remaining cases, it presents with non-specific mild symptoms such as fever, chills, headaches, myalgias,

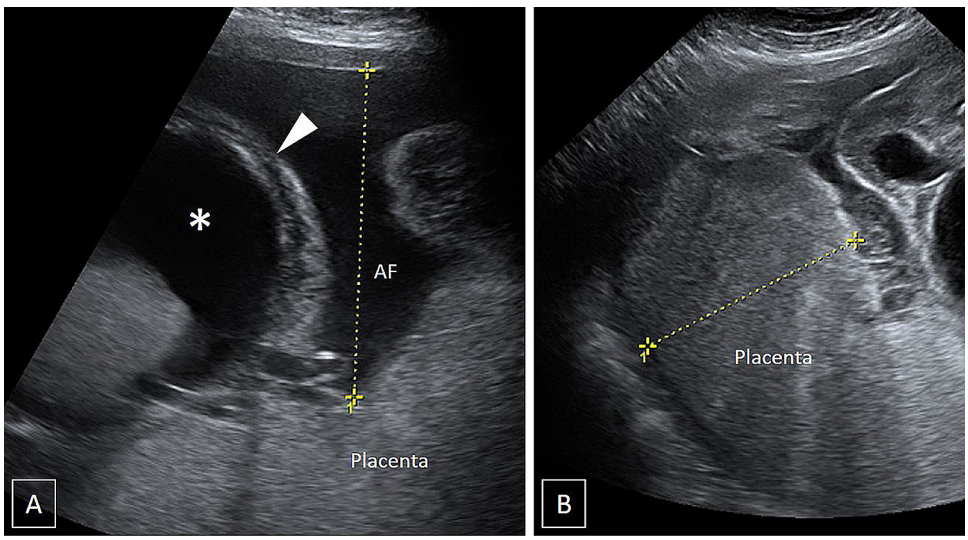

Fig. 4 Ultrasound images (**a**, **b**) show presence of fetal ascites (*), skin edema (arrowhead), placentomegaly and a single liquor pocket of > 8 cm suggestive of polyhydramnios. (*AF* amniotic fluid)

Table 1 Role of prenatal testing in diagnosis of specific fetal infections (ultrasound and invasive procedures)

S. no.	Infection	When to offer prenatal testing?		Which prenatal test to be offered and when?	
		Features on prenatal ultrasound	Maternal serology	Prenatal test offered	Timing of test
1.	Rubella virus	Structural heart defects (ASD, VSD, PS), Eye anomalies (microphthalmia, cataracts), microcephaly, hepatosplenomegaly, FGR	Offer when clinical suspicion of infection or findings on ultrasound. *Diagnosis of acute rubella infection made when there is*: IgG seroconversion Positive IgM Ab with low-avidity IgG Ab 4-times increase in IgG Ab titres between acute and convalescent samples	CVS or amniocentesis-RT-PCR will detect rubella viral nucleic acid and is the preferred test and considered superior to rubella specific IgM on fetal blood samples	Best for infections occurring between 12–16 weeks Not required for infections occurring before 12 weeks as termination of pregnancy is offered due to very high chance of fetal malformations Questionable value of testing after 16 weeks the risk of transmission is very low Best done 6 weeks after maternal infection for highest sensitivity
2.	Cytomegalovirus	Microcephaly, porencephaly, lissencephaly, cerebellar hemorrhage or calcifications, ventriculomegaly, periventricular echogenicities halo or leukomalacia, subependymal cysts, microphthalmia, anemia, NIH, cardiomegaly, pericardial calcifications, hepatosplenomegaly, echogenic bowel	Offer if findings on prenatal ultrasound *Acute infection diagnosed*: IgG seroconversion Positive IgM Ab with low-avidity IgG Ab 4-times increase in IgG Ab titres between acute and convalescent samples	Amniocentesis: PCR for viral DNA (preferred) or viral culture CMV DEAFF: IF test to detect CMV after limited growth in cell culture. (high specificity) Cordocentesis: CMV antigen or CMV specific IgM (least sensitive)	Best done 5–7 weeks after maternal seroconversion Ideal time is 21 weeks
3.	Toxoplasma Gondi	Ventriculomegaly, Calcifications in brain parenchyma and periventricular region, microcephaly, eye anomalies (microphthalmia, cataracts, retinal calcifications), anemia, NIH, myocardial calcifications, hepatomegaly, calcifications in liver, enlarged placenta	Offer when clinical suspicion of infection based on maternal signs/symptoms or findings on prenatal ultrasound Acute infection diagnosed by seroconversion of IgG and IgM antibodies or by a greater than fourfold rise in paired samples or positive IgM with low avidity	Amniocentesis: DNA detected Cordocentesis: fetal IgM (less sensitive)	Done best 6 weeks after maternal seroconversion Ideal time is 18–20 weeks as fetal diuresis is established by then
4.	Varicella-Zoster virus	Ventriculomegaly, cerebral calcifications, microcephaly, cerebral hypoplasia, microphthalmia, cataracts, musculoskeletal contractures, club foot, limb atrophy/hypoplasia, rudimentary digits, hypoplastic scapula/clavicle, NIH, transient ascites, calcifications in lung, liver, myocardium, FGR, Oligo- or polyhydramnios	Serological testing mostly not offered as clinical diagnosis is reliable. Offer if no history of varicella in the past and significant contact in the current pregnancy	Amniocentesis: PCR for viral DNA	–
5.	Parvovirus B 19	Anemia, NIH	Clinical suspicion of infection based on maternal signs/symptoms or findings on prenatal ultrasound	Amniocentesis/cordocentesis: nested PCR or RT-PCR for Parvovirus B19 DNA Cordocentesis: Parvovirus specific IgM (less sensitive and higher risk of fetal loss with cordocentesis, approximately 1%) The value of above tests in uncertain in absence of fetal anemia	–

Table 1 continued

S. no.	Infection	When to offer prenatal testing?		Which prenatal test to be offered and when?	
		Features on prenatal ultrasound	Maternal serology	Prenatal test offered	Timing of test
6.	Zika virus	Microcephaly, cerebellar hypoplasia, ventriculomegaly, intracranial calcifications, hypoplasia of corpus callosum, FGR, cardiac defects (ASD, VSD, PDA), NIH	Clinical suspicion of infection based on maternal signs/symptoms or findings on prenatal ultrasound	Amniocentesis: PCR for Zika virus	Best time is 6–8 weeks after maternal infection

ASD atrial septal defect, *VSD* ventricular septal defect, *PS* pulmonic stenosis, *FGR* fetal growth restriction, *Ab* antibody, *CVS* chorionic villous sampling, *RT-PCR* reverse transcriptase-polymerase chain reaction, *RNA* ribonucleic acid, *NIH* non-immune hydrops, *DNA* deoxyribonucleic acid, *CMV* cytomegalovirus, *DEAFF* detection of immediate-early antigen fluorescent foci, *IF* immunofluorescence, *PDA* patent ductus arteriosus

Table 2 Ultrasound findings in various intra-uterine bacterial and fungal infections

S. no.	Infection	Ultrasound findings
1.	Syphilis	Placentomegaly, FGR, bone deformities; cardiac, retinal, intracranial, hepatic, and placental calcifications, hydrops, intrauterine death
2.	Listeriosis	Hydrops, intracranial calcifications, intrauterine death
3.	Tuberculosis	Hepatomegaly, echogenic bowel, IUGR; hepatic, renal, placental, and intracranial calcifications, chronic hypoxemia, intrauterine death
4.	Gonococcal	IUGR, ophthalmic and intracranial calcifications
5.	Malaria	Placental calcifications, hydrops, chronic hypoxemia
6.	Fungal	Placental calcifications, umbilical cord anomalies, intrauterine death

pharyngitis, posterior cervical lymphadenopathy and/or a diffuse non-pruritic maculopapular rash.

b. Prenatal Sonographic Findings

Ultrasound findings are non-specific and thus, prenatal ultrasound may not be able to differentiate between congenital toxoplasmosis and other congenital infections. Chorioretinitis, intracranial calcifications, and hydrocephalus form the classic triad for congenital infection, of which the last two are the most common ultrasound features in fetal toxoplasmosis and also are poor prognostic signs. Other features seen on prenatal ultrasound are echogenic bowel, intrahepatic calcifications, intrauterine growth restriction, ascites, pericardial and/or pleural effusions, hydrops fetalis and placentomegaly [8].

Work Up for Fetal Infection

a. Maternal Seroconversion and Avidity Testing

Serological testing for toxoplasmosis should be performed if there is clinical suspicion of acute toxoplasmosis during pregnancy based upon maternal symptoms and/or abnormalities on prenatal ultrasound [23]. IgM antibodies can appear within 2 weeks of infection and can persist for years. IgG antibodies appear 6–8 weeks post-infection and then gradually reduce over the following 2 years; however, they can persist for years. Absence of IgG and IgM antibodies indicates the absence of infection or a very recent infection without sufficient time for seroconversion and warrants repeat serologic testing in 2–3 weeks. Presence of IgG antibodies in the absence of IgM antibodies indicates a past infection and the patient can be reassured. Presence of IgM antibodies in the absence of IgG antibodies indicates acute infection while presence of both IgG and IgM antibodies indicates subacute infection. Avidity testing is performed as a confirmatory test. High IgG avidity is a characteristic of chronic infection (> 4 months old), but low avidity can persist for years and is not diagnostic of recent infection.

b. Invasive Testing

Amniocentesis should be offered to identify Toxoplasma DNA by PCR when the maternal primary infection is diagnosed or when serologic testing cannot negate an acute infection, or in the presence of sonographic features of fetal infection [23]. It is performed after 18 weeks of gestation with confirmed recent maternal infection.

Table 1 summarizes the role of prenatal testing in diagnosis of specific fetal infections (ultrasound and invasive procedures).

Table 2 summarizes ultrasound findings in various other intra-uterine bacterial and fungal infections.

Conclusion

Prenatal diagnosis and assessment of the severity of congenital infection is challenging and varies according to the period of gestation at the time of maternal infection and the underlying infectious pathogen. The foremost and vital step is serological confirmation of underlying maternal infection by testing for pathogen-specific IgG and IgM antibodies. Post-confirmation of maternal infection, the patient may be referred to a fetal medicine expert for further management. Ultrasound surveillance can determine the whole range of wide constellation of subtle to dramatic manifestations but it has limitations in the accurate prediction of postnatal outcome for the baby. The mainstay for prenatal diagnosis of fetal infection is amniocentesis to detect the presence of pathogen-specific RNA/DNA by PCR technique; however, the timing of the test is crucial with reference to the probable point at which transmission occurred.

Keypoints

- Risk of vertical transmission and subsequent fetal abnormalities are dependent on the pathogen and period of gestation.
- Ultrasound can be an important tool to detect various specific and non-specific features of fetal infection.
- Detection of RNA or DNA by PCR can be obtained via amniocentesis and is at the core of the diagnosis of fetal infection.
- PCR has led to an increased accuracy of test results.
- Intrauterine blood transfusion in cases of fetal parvovirus infection and maternal antibiotic therapy in the presence of toxoplasmosis and syphilis infection are the only therapeutic options available in current practice.

Funding None.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Bailão LA, Osborne NG, Rizzi MC, Bonilla-Musoles F, Duarte G, Bailão TC. Ultrasound markers of fetal infection part 1: viral infections. Ultrasound Q. 2005;21(4):295–308.
2. Bailao ND, Rizzi MC, Bonila-Musoles F, et al. Ultrasound markers of fetal infection: an update. Ultrasound Q. 2000;16(4):221–33.
3. Ghidini A, Lynch L. Prenatal diagnosis and significance of fetal infections, in fetal medicine [special issue]. West J Med. 1993;159:366–73.
4. Dontigny L, Arsenault MY, Martel MJ. Rubella in pregnancy. SOGC clinical practice guidelines. J Obstet Gynaecol Can. 2008;30(2):152–8.
5. Best JM. Rubella. Semin Fetal Neonatal Med. 2007;12:182–92.
6. Tang JW, Aarons E, Hesketh LM, Strobel S, Schalasta G, Jauniaux E, Brink NS, Enders G. Prenatal diagnosis of congenital rubella infection in the second trimester of pregnancy. Prenat Diagn. 2003;23(6):509–12.
7. Kenneson A, Cannon MJ. Review and meta-analysis of the epidemiology of congenital cytomegalovirus (CMV) infection. Rev Med Virol. 2007;17:253–76.
8. American College of Obstetricians and Gynecologists. Practice bulletin no. 151: Cytomegalovirus, parvovirus B19, varicella zoster, and toxoplasmosis in pregnancy. Obstet Gynecol. 2015;125(6):1510–25.
9. Guerra B, Simonazzi G, Puccetti C, et al. Ultrasound prediction of symptomatic congenital cytomegalovirus infection. Am J Obstet Gynecol. 2008;198(380):380.e1–7.
10. Khalil A, Heath P, Jones C, Soe A, Ville YG, on behalf of the Royal College of Obstetricians and Gynaecologists. Congenital cytomegalovirus infection: update on treatment. Scientific impact paper no. 56. BJOG. 2018;125:e1–11.
11. Cytomegalovirus in pregnancy. SOGC clinical practice guidelines. No 240, 2018 February. J Obstet Gynaecol Can. 2018;40(2):e134–41.
12. Enders G, Daiminger A, Bäder U, Exler S, Schimpf Y, Enders M. The value of CMV IgG avidity and immunoblot for timing the onset of primary CMV infection in pregnancy. J Clin Virol. 2013;56(2):102–7.
13. Yinon Y, Farine D, Yudin MH. Screening, diagnosis, and management of cytomegalovirus infection in pregnancy. Obstet Gynecol Surv. 2010;65(11):736–43.
14. Harger JH, Ernest JM, Thurnau GR, et al. Risk factors and outcome of varicella-zoster virus pneumonia in pregnant women. J Infect Dis. 2002;185:422–7.
15. Management of varicella infection in pregnancy. SOGC clinical guidelies. No. 274 August 2018. J Obstet Gynaecol Can. 2018;40(8):e652–7.
16. Kimberlin DW. Neonatal herpes simplex infection. Clin Microbiol Rev. 2004;17:1–13.
17. Wald A, Huang M-L, Carrel D, Selke S, Corey L. Polymerase chain reaction for herpes simplex virus DNA on mucosal surfaces: comparison with HSV isolation in cell culture. J Infect Dis. 2003;188:1345–51.
18. de Jong EP, de Haan TR, Kroes AC, et al. Parvovirus B19 infection in pregnancy. J Clin Virol. 2006;36:1.
19. Young NS, Brown KE. Parvovirus B19. N Engl J Med. 2004;350(6):586–97.
20. Yaegashi N. Pathogenesis of nonimmune hydrops fetalis caused by intrauterine B19 infection. Tohoku J Exp Med. 2000;190:65–82.
21. Parvo virus infection in pregnancy. SOGC Clinical guidelines. No. 316 August 2014. J Obstet Gynaecol Can. 2014;36(12):1107–16.
22. Dunn D, Wallon M, Peyron F, Peterson E, Peckhem C, Gilbert R. Mother to child transmission of toxoplasmosis: risk estimates for clinical counselling. Lancet. 1999;353:1–33.
23. Toxoplasmosis in pregnancy: prevention, screening and treatment. SOGC clinical guidelines. No. 285 January 2013. J Obstet Gynaecol Can. 2013;35(1):78–9.