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Educational Case

Educational Case: Infections during pregnancy: Congenital cytomegalovirus infection

Jennifer A. Wintringham, BS, Richard M. Conran, MD, PhD, JD *

Eastern Virginia Medical School, Norfolk, VA, 23507, USA

The following fictional case is intended as a learning tool within the Pathology Competencies for Medical Education (PCME), a set of national standards for teaching pathology. These are divided into three basic competencies: Disease Mechanisms and Processes, Organ System Pathology, and Diagnostic Medicine and Therapeutic Pathology. For additional information, and a full list of learning objectives for all three competencies, see https://www.journals.elsevier.com/academic-pathology/news/pathology-competencies-for-medical-education-pcme.[1]

Keywords: Pathology competencies, Organ system pathology, Female reproductive tract, Disorders of pregnancy, Infections during pregnancy, Hematogenous infection, Ascending infection, Congenital cytomegalovirus infection

Primary objective

Objective FDP1.4: Infections during pregnancy. Discuss the ascending and hematogenous infections occurring during pregnancy in terms of etiology, pathogenesis, morphology, methods of diagnosis, prognosis, and treatment.

Competency 2. Organ system pathology; Topic: Female reproductive-disorders of pregnancy (FDP); Learning goal 1: Disorders of pregnancy.

Patient presentation

A 29-year-old G3P2 woman with a past medical history of asthma presents to the emergency department at 34 weeks gestation by dates with active contractions and leakage of vaginal fluid. The patient is admitted for examination and fetal monitoring which confirms active premature labor. She receives intramuscular betamethasone and intrapartum ampicillin for unknown group B *Streptococcus* status. Her obstetric history is remarkable for mild fatigue and sore throat during the second trimester of pregnancy which resolved spontaneously. Prenatal care was sporadic due to financial instability.

Diagnostic findings, Part 1

A small for gestational age male infant with generalized edema (fetal hydrops) and an abnormally thickened placenta are delivered vaginally. The skin is notable for generalized yellowish discoloration and petechiae. There is scleral icterus. Palpation of the abdomen reveals hepatosplenomegaly. Microcephaly is also seen. The infant's vital signs and measurements are listed in Table 1.[2,3]

Questions/discussion points, Part 1

Based on the clinical presentation, What disorders are included in the differential diagnosis of an infant with petechiae, jaundice, hepatosplenomegaly, and microcephaly?

The differential diagnosis of an infant with these findings includes congenital infections (cytomegalovirus, rubella, toxoplasmosis, syphilis, Zika, parvovirus B19, herpes simplex virus, hepatitis B, HIV), noninfectious hemolytic diseases related to Rh or ABO incompatibility, immune thrombocytopenia, neonatal sepsis, and rare metabolic disorders (galactosemia, organic acidemia, inherited leukodystrophies).[4] Occasionally, multiple infections may coexist in the patient thus a thorough workup to refine the differential diagnosis is essential.

Based on the clinical presentation, what additional diagnostic tests or imaging should be ordered in the infant and mother?

Further evaluation of a neonate presenting with this differential diagnosis should begin with maternal infectious serologies for common congenital infections (e.g., cytomegalovirus, syphilis, toxoplasmosis, parvovirus B19, rubella, human immunodeficiency virus (HIV), hepatitis viruses). Positive maternal serologies should prompt further investigation in the infant with either viral cultures, PCR testing, or

* Corresponding author. Eastern Virginia Medical School, Pathology & Anatomy, 700 West Olney Road, Norfolk, VA, 23507, USA.
E-mail address: conranrm@evms.edu (R.M. Conran).

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Table 1
Infant vital signs and measurements.[2,3]

Exam fnding	Patient	Reference value 50th percentile	Reference value 10th to 90th percentile	Growth chart percentile
Weight (kg)	1.75	2.2	1.8–2.6	< 10th
Length (cm)	42	45	41.5–48	< 15th
Head circumference (cm)	28.5	31	29–33	< 10th
Temperature (°C)	38.5	36.5–37.5		
Heart rate (beats per min)	174	123–164		
Respiratory rate (breaths per min)	35	34–57		
Arterial blood pressure (mm Hg)	65/40	60–90/20–60		

Measurement reference values (weight, length, head circumference) are for a live born male infant at 34 weeks gestation.

serologies, as indicated for specific pathogens. Additional workup should include, a complete blood count (CBC) and peripheral blood smear, comprehensive metabolic panel (CMP), fundoscopic exam, ABO compatibility, audiologic screening, and neuroimaging.

Diagnostic findings, Part 2

A comprehensive maternal and neonatal workup is ordered. The mother's serum CMV IgG and anti-HAV IgG antibodies are positive. The CMV IgM test is negative. All other maternal serologies are negative including hepatitis B, hepatitis C, parvovirus B19, HIV, syphilis, toxoplasmosis, and herpes simplex virus. The infant's urine CMV DNA PCR was elevated at 1.6×10^5 copies/mL. Both the maternal and infant blood types are B+. Bilateral chorioretinitis is observed on ophthalmological examination. There is moderate hearing impairment on brainstem evoked audiometry testing. Results of the infant's CBC and CMP are listed in Table 2.[5]

Table 2
Laboratory test results.

Laboratory test	Patient	Reference interval (male neonate 0–14 days)
Complete blood count (CBC)		
RBC ($x10^6$/μL)	4.4	4.1–5.55
Hemoglobin (g/dL)	13.5	13.0–19.1
Hematocrit (%)	40.5	39.8–53.6
MCV (fL)	94	91.3–103.1
RDW (%)	16	14.8–17.0
Platelets ($x10^3$/μL)	99	218–419
WBC ($x10^3$/μL)	8.5	8.0–15.4
Neutrophils ($x10^3$/μL)	4.0	1.6–6.06
Lymphocytes ($x10^3$/μL)	4.4	2.07–7.53
Monocytes (%)	0.6	0.52–1.77
Eosinophils ($x10^3$/μL)	0.4	0.12–0.66
Basophils ($x10^3$/μL)	0.07	0.02–0.11
Comprehensive metabolic panel (CMP)		
Glucose, serum (mg/dL)	72	40–99
BUN (mg/dL)	10	7–20
Creatinine, serum (mg/dL)	0.6	0.3–1.0
Sodium, serum (mg/dL)	137	130–140
Potassium, serum (mEq/L)	4.1	3.5–6.0
Chloride, serum (mmol/L)	100	95–108
Carbon dioxide, total (mEq/L)	21	20–30
Calcium, serum (mg/dL)	8.8	8.5–10.6
Protein, total, serum (g/dL)	4.9	4.1–6.3
Albumin, serum (mg/dL)	3.0	2.8–4.4
Globulin, total (g/dL)	1.9	1.3–1.9
Bilirubin, total (mg/dL)	8.8	≤6.0
Alkaline phosphatase, serum (U/L)	260	83–248
AST (SGOT) (U/L)	66	8–60
ALT (SGPT) (U/L)	64	7–55

CBC and CMP results. Reference values given in this table are adapted from commonly accepted reference ranges and extrapolated from Mayo Clinic Laboratories.[5] Patient-specific values may differ depending on age, sex, clinical condition, and the laboratory methodology used to perform the test.

Questions/discussion points, Part 2

What is the interpretation of the lab results and physical exam findings?

Maternal serologies are suggestive of congenital CMV infection, which was confirmed with the infant's significantly elevated urine CMV DNA PCR test. The mother's positive CMV-IgG and negative IgM are suggestive of previous infection with CMV, although the definitive timeline of infection cannot be elucidated. Thrombocytopenia and elevated liver enzymes are consistent with petechiae and hepatosplenomegaly on physical examination. The anemia, blood type compatibility, jaundice, and generalized edema suggest a non-immune etiology for the fetal hydrops. There are non-specific findings, for example, hearing loss and chorioretinitis observed on physical examination suggestive of congenital infections including toxoplasmosis, rubella, CMV, and syphilis.

What imaging modalities can be used to visualize the structural neurologic abnormalities associated with congenital infections? What abnormalities are associated with congenital CMV infection?

Cranial ultrasonography (US), computed topography imaging (CT), and magnetic resonance imaging (MRI) have all been used for diagnosis and characterization of neurological abnormalities in congenital infections, each with associated advantages and disadvantages. Cranial ultrasonography is an attractive option as it is readily available, poses low risk to the neonate, and can be performed at the bedside if the patient is critically ill. CT is highly sensitive for depiction of intracranial calcifications but exposes the child to ionizing radiation.[6] MRI provides excellent resolution of structural abnormalities without the use of ionizing radiation but may require sedation or anesthesia for optimal results. Common neuroimaging abnormalities in congenital CMV infection include periventricular calcifications, ventriculomegaly, neuronal migrational abnormalities, cerebellar and cerebral volume loss, microencephaly, and white matter disease.[6] Among these, intracranial calcifications are the most common imaging finding, and are present in 70% of infants with congenital CMV infection.[7] Imaging findings are most predictive of adverse neurodevelopmental outcomes when performed within the first month of life.

Diagnostic findings, Part 3

A pale, 550 g (411 g-mean weight at 34 weeks gestation, (331–491 g; 10th to 90th percentile)) placenta measuring $15 \times 15 \times 3$ cm is sent to the pathology laboratory.[8] The edematous 3-vessel umbilical cord is 60 cm in length. Sharp demarcation of the cotyledons is obscured on examination of the maternal surface. Placental membranes are thin and translucent.

Questions/discussion points, Part 3

Describe the histological findings associated with the placenta

Figs. 1–4 are sections of the submitted placenta sent for pathological examination. The chorionic villi have decreased vascularity, are large and edematous with increased stromal fibrosis compared to normal term villi that contain numerous capillaries and the presence of vasculosyncytial membranes (Fig. 1). Many of the villi show numerous

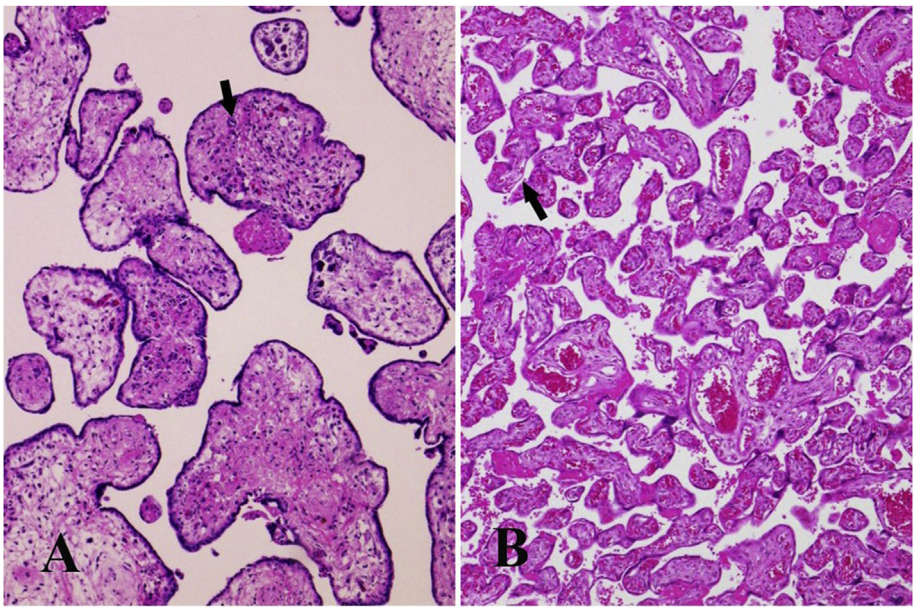

Fig. 1. Placenta. A. Several enlarged villi show an increase in cellularity (villitis) and decreased vascularity. A CMV inclusion is seen (arrow). (H&E, 100× magnification) B. Normal small term villi with prominent capillaries and vasculosyncytial membranes (arrow) are shown for comparison. (H&E, 100× magnification).

large intranuclear inclusions with or without cytoplasmic basophilic inclusions, necrosis and a lymphoplasmacytic infiltrate (villitis) (Figs. 2 and 3). The placental membranes composed of the chorion and amnion are normal compared to the acute chorioamnionitis seen in ascending bacterial infections (Fig. 4). Transplacental (hematogenous) infections, such as cytomegalovirus, generally have unremarkable placental membranes, Depending on the interval between infection and delivery, CMV inclusions may not be as visible as seen in Fig. 2. Immunohistochemistry can be performed to document the CMV organism when not readily apparent. in tissue sections (Fig. 5). PCR for cytomegalovirus on formalin fixed paraffin embedded tissue is also reliable. Three features characteristic of CMV placental infections are prominent villous fibrosis, lymphoplasmacytic villitis and the diagnostic intranuclear inclusions.[8]

What is the underlying pathogenesis of transplacental (hematogenous) infections during pregnancy?

During pregnancy, the maternal immune system is altered to decrease the maternal inflammatory response and allow for fetal antigen tolerance. However, this dampening can also increase maternal and fetal vulnerability to many infectious diseases.[9]

Fetal and perinatal infections are most often acquired through one of two routes—transcervically (ascending) or transplacentally (hematologic).

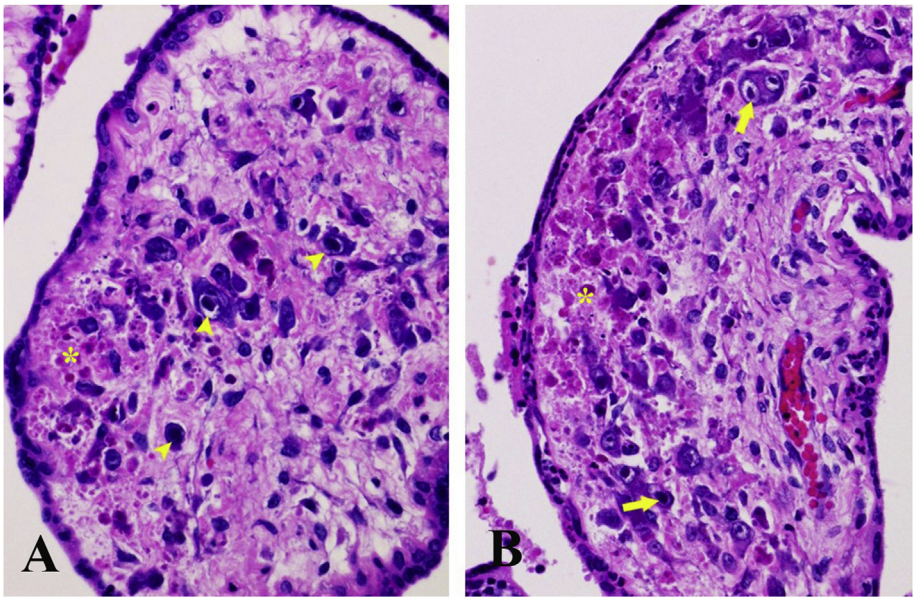

Fig. 2. A, B. The enlarged villi show necrosis (*), stromal fibrosis, increased cellularity and numerous CMV inclusions with prominent basophilic cytoplasm (arrowheads). (H&E, high magnification).

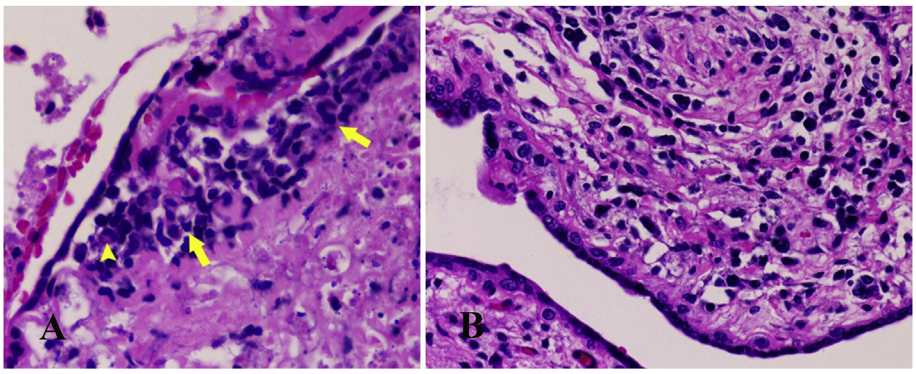

Fig. 3. A prominent lymphocytic (arrows) and plasmacytic (arrowhead) infiltrate is present at the periphery of the villus in Fig. 3A and diffuse throughout the villus in Fig. 3B. (H&E, intermediate magnification).

Most parasitic and viral infections, and some bacterial infections (*Listeria monocytogenes*, *Treponema pallidum*), gain access to the fetal bloodstream transplacentally through the chorionic villi.[10] Hematogenous transmission may occur at any time during gestation or rarely at the time of delivery via maternal-to-fetal transfusion.

What is the underlying pathogenesis of ascending infections during pregnancy? How does this differ from transplacental infections?

Most bacterial (e.g., group B *Streptococcus*, *Escherichia coli*) and a few viral (e.g., *Herpes simplex II*) infections are acquired by the cervicovaginal route.[10] Herpes simplex virus is most often acquired as an ascending infection through direct intrapartum exposure, although transplacental infections rarely occur.

Commonly, ascending infections lead to acute chorioamnionitis, which may lead to premature rupture of the membranes and preterm delivery. Histologically, inflammation of the placental membranes (acute chorioamnionitis) and cord (funisitis) are usually seen, but the severity of chorioamnionitis does not necessarily parallel the severity of the fetal infection (Fig. 4B).[10] Congenital pneumonia can be acquired through inhaling infected amniotic fluid into the fetal lungs or through direct contact with an infected birth canal during delivery.

What are the key features among the transplacental (TORCH) infections?

TORCH infections are a large group of hematogenously transmitted infections that can affect the placenta and fetus. They include toxoplasmosis, syphilis, varicella, mumps, parvovirus, HIV, tuberculosis, listeriosis, rubella, cytomegalovirus, and herpes simplex virus (HSV). TORCH infections are grouped together because they result in similar clinical and pathologic manifestations such as fever, hepatosplenomegaly, encephalitis, chorioretinitis, jaundice, pneumonitis, neurological dysfunction, myocarditis, hemolytic anemia, and skin lesions.[10] However, there are some notable unique findings amongst the TORCH infections that are outlined in Table 3.[11–13]

Diagnostic findings, Part 4

The infant dies soon after delivery. An autopsy is performed. In addition to the anasarca, there is significant ascites, pericardial effusion and pleural effusions. The gross brain section shows periventricular necrosis. An x-ray of the brain section shows prominent calcification (Fig. 6). The liver is markedly enlarged (Fig. 7). Cytomegalovirus inclusions and cytomegaly are present in the bile duct epithelium (Fig. 8) and kidney tubular epithelium (Fig. 9).

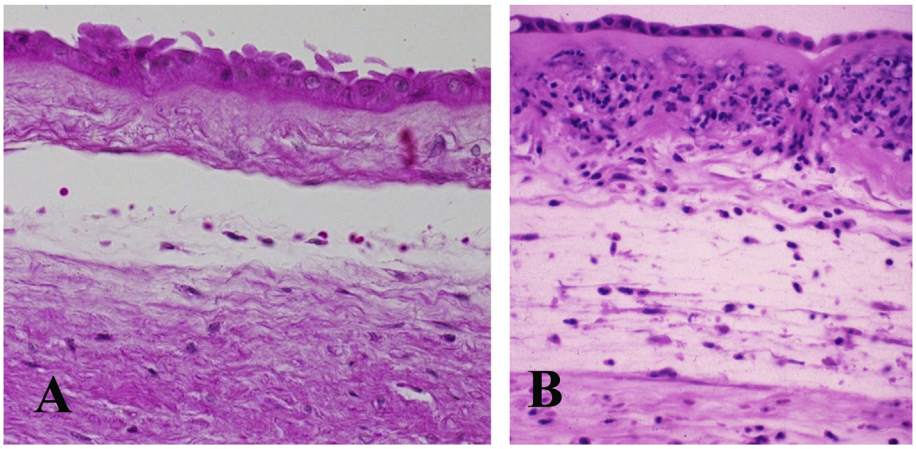

Fig. 4. A. The placental membranes are unremarkable in this patient with congenital CMV infection. (H&E, high magnification). B. The fetal membranes show a prominent neutrophilic infiltrate involving the amnion and chorion (acute chorioamnionitis) in a patient that was positive for colonization of the cervical canal with group B *Streptococcus*. (H&E, high magnification).

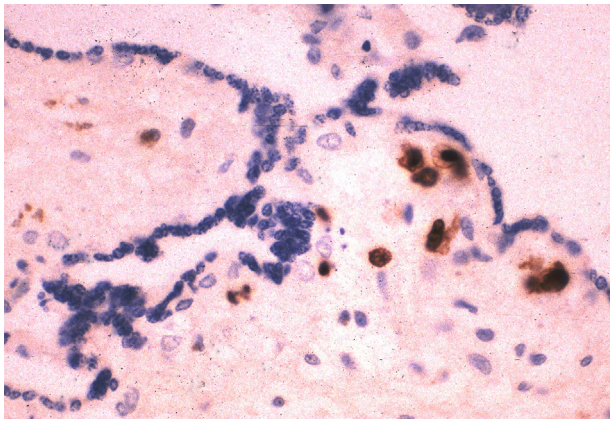

Fig. 5. Immunohistochemistry for CMV. Within the chorionic villi are several CMV organisms that are immunoreactive for antibody to CMV. (CMV, high magnification).

Questions/discussion points Part 4

What is the most likely diagnosis based on the pathological and laboratory findings?

The diagnosis is congenital CMV infection, also referred to as cytomegalic inclusion disease. The pathogenesis of this infant's fetal hydrops is most likely a result of altered erythropoiesis as a consequence of dissemination of CMV to the fetal liver and spleen, the predominant hematopoietic organs in utero.

What is fetal hydrops?

The accumulation of edema fluid within the fetus during the gestational period is referred to as fetal hydrops. It is classified as immune and non-immune. Immune hydrops which accounts for 5% of fetal hydrops cases is due to Rh or ABO incompatibility. Non-immune hydrops is multifactorial. Recognized causes include chromosomal disorders, congenital infections (TORCH agents), cardiovascular malformations, fetal anemia, metabolic disorders, twin pregnancies, and congenital neoplasms. The amount of edema fluid observed is based on the etiology and severity of the underlying disorder.[10]

Congenital CMV infection is associated with fetal hydrops, however, intrauterine growth retardation (IUGR) is a more common presentation in symptomatic patients. If the fluid observed at autopsy is subtracted from the infant's birth weight, the degree of IUGR is more severe.

What are the routes of transmission of cytomegalovirus?

Cytomegalovirus can be transmitted by multiple routes, depending on the age group that is affected and the host's immune status. Transplacental transmission, as in this case, occurs from a newly acquired or primary infection in a mother who lacks protective antibodies, leading to congenital CMV infection.[14] Transplacental transmission occurs in 30–40% of primary maternal infections. Additional routes of transmission include neonatal through maternal tract shedding or breast milk, saliva in preschool-aged children, genital route in older adolescents and adults, and iatrogenic (i.e., blood transfusion, organ transplant).[4,14]

How is congenital CMV infection diagnosed?

Congenital CMV infection is diagnosed by isolation or molecular detection of CMV in urine (lower false positive rate) or saliva within three weeks of birth. Congenital CMV infection is difficult to definitively diagnose after three weeks as testing cannot distinguish between congenital infection and infection acquired during or after delivery.[15] Serologic screening alone is unreliable in the diagnosis of congenital CMV infection. The presence of CMV-IgG antibody may simply reflect passive maternal antibody transfer. Additionally, the sensitivity and specificity of CMV-IgM assays are poor and may report false negatives in up to 50% of infants.[16] Demonstration of CMV inclusions on placental examination or at autopsy is diagnostic.[8]

What are the possible sequelae of a congenital cytomegalovirus infection?

Congenital cytomegalovirus infection is common and is the leading cause of non-genetic sensorineural hearing loss. CMV can cause other long-term neurodevelopmental disabilities, intellectual disability, vision impairment, and seizures. Symptomatic CMV infections have a poor long-term prognosis. Nearly 90% of infants with symptomatic CMV infections experience at least one long-term sequelae. Sensorineural hearing loss is the most common deficit observed and occurs in 50–58% of symptomatic individuals.[4] Studies have indicated petechiae, intrauterine growth restriction, and increased viral load in blood and urine as independent predictors of the hearing outcome in symptomatic individuals.[17,18] The frequency of other long-term neurologic defects is described in Table 4.[4] When classifying congenital CMV infections, "symptomatic" refers to infants with virologically confirmed infection with at least one symptom at birth, "asymptomatic with failed hearing screen" refers to infants with only isolated hearing loss at birth, and "asymptomatic" refers to infants with no clinically apparent symptoms at birth.

Describe the epidemiology and public health impact of congenital CMV infections

Using the current estimate of congenital CMV infection with an average rate of 1% and birth rate of 4 million per annum, an estimated 40,000 infants are born with congenital CMV infections each year in the United States.[4] Approximately 10% of these neonates are symptomatic at birth; symptomatic presentation carries a significant associated increase in morbidity and mortality.[19] The overall disease burden of congenital

Table 3
Neonatal and maternal manifestations of transplacentally-acquired infections.[11–13]

Infection	Maternal manifestations	Neonatal manifestations
Toxoplasmosis	Asymptomatic, +/− lymphadenopathy	Chorioretinitis, intracranial calcifications, hydrocephalus, abnormal cerebrospinal fluid
Rubella	Rash, polyarthralgia, lymphadenopathy	Hearing impairment, cardiac defects (patent ductus arteriosus, peripheral pulmonic stenosis, cataracts
Cytomegalovirus	Usually asymptomatic, mononucleosis-like illness in ~5%	Sensorineural hearing loss, neurodevelopmental abnormalities, chorioretinitis, periventricular calcifications, "blueberry muffin rash", seizures
HIV	Variable with CD4 count	Recurrent infections, chronic diarrhea
Herpes simplex II	Asymptomatic or herpetic lesions	Meningoencephalitis, vesicular skin lesions
Parvovirus b19	Slapped cheek rash, polyarthritis, laticiform macular rash on trunk	Severe anemia, non-immune hydrops fetalis, fetal demise
Syphilis	Chancre (primary), disseminated rash (secondary)	Facial and skeletal abnormalities (notched teeth, saddle nose, saber shins), hydrops fetalis, cranial nerve VII deafness

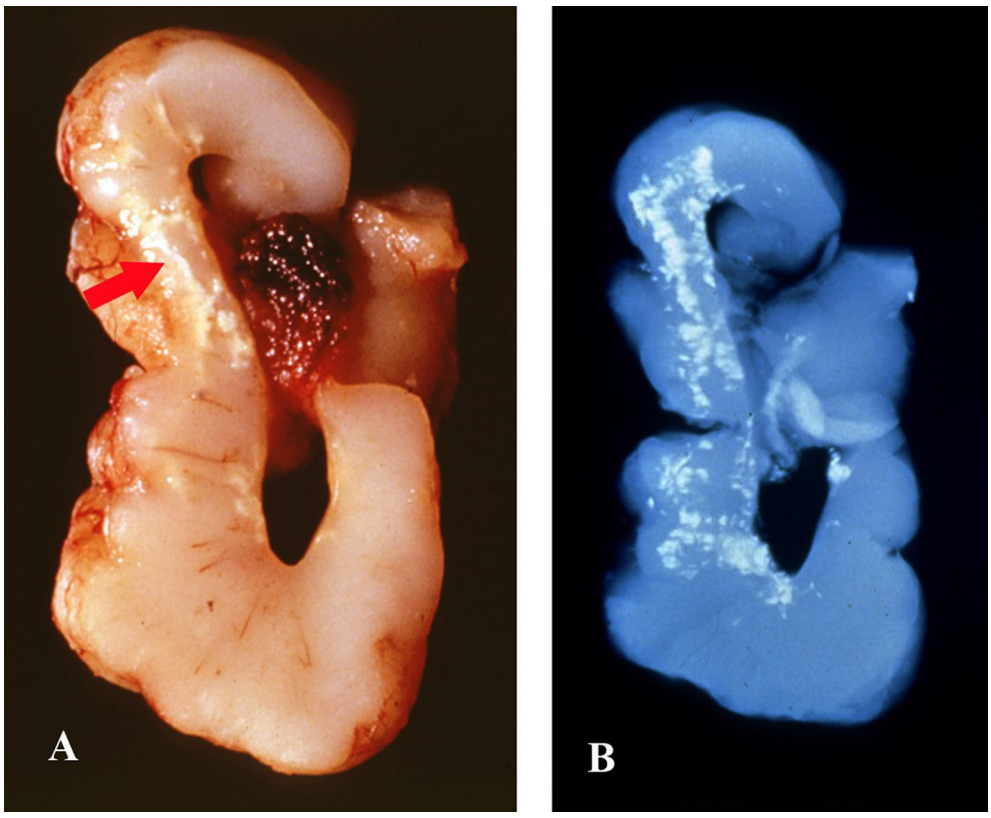

Fig. 6. A. The section of brain demonstrates prominent periventricular necrosis (arrow). B. Prominent calcification is seen in the brain on imaging the brain tissue at autopsy.

CMV infection in the 1990s was estimated to cost the United States $1.86 billion per year with a cost per symptomatic child of $300,000.[20] Several risk factors have been identified and associated with congenital CMV birth prevalence, including infants born to non-Hispanic black and Mexican American women, low socioeconomic status, maternal HIV infection, premature birth, NICU admission, and young maternal age.[21] Therefore, CMV infection prevention strategies need to account for these observed age, race, socioeconomic, and health differences.

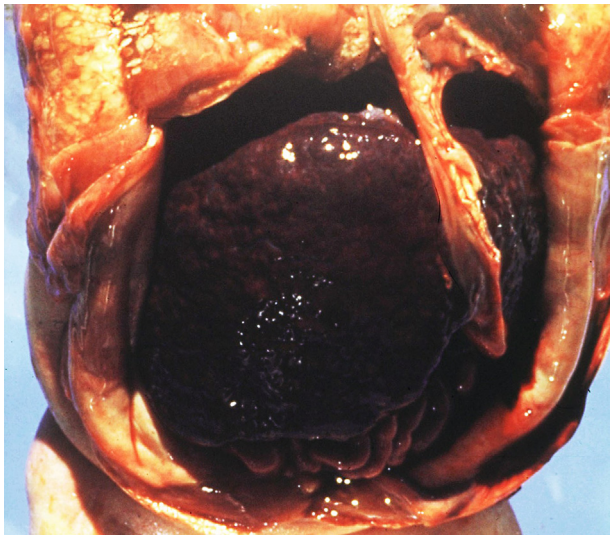

Fig. 7. There is massive enlargement of the liver at autopsy.

What are the current treatment recommendations for an infant with CMV?

In infants with symptomatic congenital CMV infection, treatment with intravenous ganciclovir or valganciclovir is recommended as soon as virologic testing is confirmed. Ganciclovir is preferred for life-threatening illnesses, while valganciclovir can be used for milder disease. Multicenter, randomized control trials show these drugs are most beneficial when given in the first month of life and can improve long-term audiologic and neurodevelopmental outcomes.[22] There is insufficient evidence to support antiviral treatment for infections in utero or asymptomatic individuals.

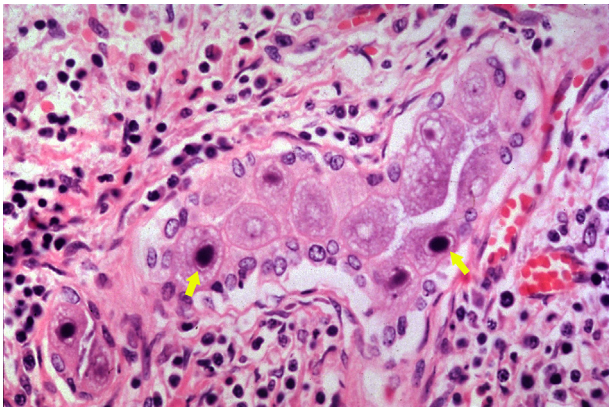

Fig. 8. Liver. CMV inclusions and cytomegaly (arrows) are identified in biliary duct epithelium. (H&E, intermediate magnification).

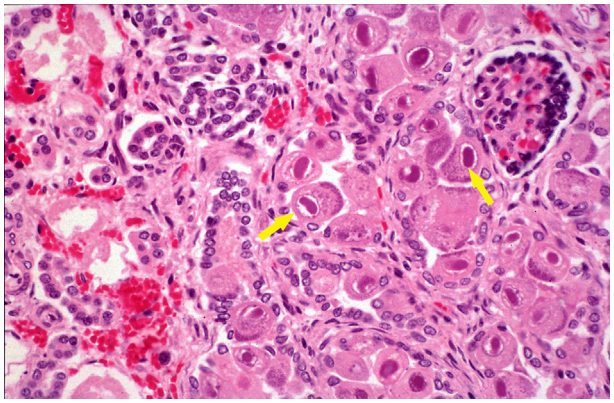

Fig. 9. Kidney. The renal tubular epithelium demonstrates numerous epithelial cells infected with CMV. Note the presence of nuclear and cytoplasmic inclusions (arrows). (H&E, intermediate magnification).

Table 4
Sequelae in children after symptomatic and asymptomatic congenital CMV infection.[4]

Sequelae	% symptomatic (No.)	% Asymptomatic
Sensorineural hearing loss	58 (58/100)	7.4 (22/299)
Chorioretinitis	20.4 (19/93)	2.5 (7/281)
IQ < 70	55 (33/60)	3.7 (6/159)
Microcephaly	37.5 (39/104)	1.8 (6/330)
Seizures	23.1 (24/104)	0.9 (3/330)
Death	5.8 (6/104)	0.3 (1/330)

IQ, Intelligence quotient
Reprinted in part from Britt W. Cytomegalovirus. In: Wilson CB, Nizet V, Maldonado YA, Remington JS, Klein JO. eds. *Remington and Klein's infectious diseases of the fetus and newborn infant*. Table 24–8 Sequelae in Children after Congenital Cytomegalovirus Infection. 8th ed. Elsevier/Saunders; 2016; 724–781 with permission from Elsevier.[4]

Does a vaccine exist to prevent congenital CMV infection?

Despite more than 30 years of research, no vaccine is currently available for the primary prevention of congenital CMV infection. Vaccine progress has been limited as CMV has unique replication, tissue tropism, and pathogenesis in humans that has been difficult to recreate in animal models accurately.[4] Current recommendations from the CDC include educating pregnant women about hygienic practices such as hand washing and decreasing exposure to body fluids from young children.[15] Consistent prenatal care is important for the early detection of congenital infections and abnormalities and may have played a role in the severity and development of this patient's disease.

Teaching points

- Perinatal infections can be acquired by an ascending route (transcervical) or via a hematogenous route (transplacental).
- Most parasitic and viral infections and a few bacterial infections gain access to the fetal bloodstream transplacentally via the chorionic villi. Clinical manifestations of these infections are highly variable depending upon gestational timing and the microorganism involved.
- Most bacterial and a few viral (e.g., *Herpes simplex II*) infections are acquired by the cervicovaginal route. Acute chorioamnionitis and preterm birth are common consequences of infection.
- Transmission of congenital CMV is transplacental and occurs in 30–40% of primary maternal infections. The large majority (80–90%) of infants with congenital CMV infection are asymptomatic at birth. For those who are symptomatic at birth, there is a significant increase in morbidity and mortality.
- Congenital CMV infection is best diagnosed by viral isolation or molecular detection of CMV in saliva or urine within three weeks of birth. Serologies alone are insufficient for diagnosis.
- Congenital cytomegalovirus infection is the leading cause of non-genetic sensorineural hearing loss. CMV can cause other long-term neurodevelopmental disabilities, intellectual disability, vision impairment, and seizures.
- Given the limited success of vaccine development, current prevention efforts involve patient education on careful hygiene practices to prevent the acquisition of infection.

Declaration of conflicting interests

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