The Brief Case: Nystagmus in a 3-Month-Old Leading to a Diagnosis of Congenital Cytomegalovirus Infection

Priyanka Uprety,a Erin H. Graf

aDepartment of Laboratory Medicine, Rutgers Robert Wood Johnson Medical School, New Brunswick, New Jersey, USA
bDepartment of Laboratory Medicine and Pathology, Mayo Clinic Arizona, Phoenix, Arizona, USA

KEYWORDS congenital infections, cytomegalovirus, pediatric infectious disease

CASE

A previously healthy 3-month-old female presented to her pediatrician with nystagmus. The infant was born via spontaneous vaginal delivery to a gravida 1 para 0 mother at 41 weeks of gestation. The pregnancy was uncomplicated until 22 weeks of gestation, when the mother developed hypertension, which continued through the duration of her pregnancy but did not require medical management. The mother also noted an upper respiratory tract infection during her first trimester. Her 20-week fetal anatomy scan was unremarkable, and all standard infectious disease screening was negative. After delivery, the neonate was admitted to the intensive care unit due to a 1-min Apgar score of 2 and potential meconium aspiration. Following 6 h of monitoring, she was allowed to return to the postpartum unit and was discharged at day 2 of life. Prior to discharge, she had a normal hearing screen and received her hepatitis B vaccination. Her newborn screening, in the state of Pennsylvania, USA, was reported as normal.

The nystagmus was first noted by the mother at around 8 weeks of life but was not brought to medical attention until the baby was 3 months old. The family was referred by their pediatrician to an ophthalmologist who diagnosed the infant with congenital nystagmus and recommended further consultation with a neurologist, as well as magnetic resonance imaging (MRI) of her brain. An MRI showed diffuse white matter signal abnormality throughout her brain, which was worse on the right side (Fig. 1, right image), and extensive cerebral hemisphere polymicrogyria (Fig. 1, left image) with cysts along the bilateral anterior temporal horns, as well as bilateral optic nerve atrophy. These findings were reported to be consistent with cystic leukoencephalopathy or congenital infection. The family was referred for specialized follow up with leukodystrophy and infectious disease clinics.

Upon arrival at the infectious disease clinic, a physical exam revealed a well-appearing infant with normal head circumference and no evidence of chorioretinitis or hearing loss. In addition to the nystagmus, she was also noted to have hypotonia throughout her body, particularly on her left side. Serum antibody testing of the infant was ordered for common congenital infections, including rubella virus (IgM and IgG), Toxoplasma gondii (IgM and IgG), herpes simplex virus (HSV) (HSV-1/2 IgM, HSV-1 IgG, and HSV-2 IgG), and cytomegalovirus (CMV) (IgM and IgG). The family was also referred to a genetic counselor to discuss options for inherited disorder testing. In the interim, all infectious disease serologies from the child came back negative except for a positive CMV IgG, which could represent transfer of maternal antibodies versus in utero or a postpartum CMV infection. A CMV whole-blood viral load was then ordered on the infant, which came back as 21,127 international units (IU) per ml (reference range: not detected). At this point, given the possibility that CMV was acquired postnatally, it was not clear whether CMV infection or an inherited genetic disorder was the cause of her...
To pursue the diagnosis of congenital CMV further and avoid unnecessary genomic testing, the infant’s newborn screening dried blood spot (DBS) card was requested to be sent from the Pennsylvania public health laboratory to the Centers for Disease Control and Prevention for research-based CMV molecular testing. The blood spot tested positive for CMV, confirming the diagnosis of congenital CMV. Given this diagnosis, further genetic testing was not pursued.

Treatment with valganciclovir was discussed but was ultimately found to be unnecessary given the length of time that had passed since birth and potential toxicities. The infant was scheduled to have hearing screens every 3 months for the first 3 years of life, an annual eye exam to evaluate for chorioretinitis, and extensive physical and occupational therapy. At her most recent follow-up, at 31 months old, she had global developmental delays, including gross motor and language deficits. However, she had significantly improved nystagmus and normal hearing and vision screens.

**DISCUSSION**

Human cytomegalovirus (CMV) is a betaherpesvirus with a high seroprevalence across the globe. It is estimated that roughly 60% of individuals in the United States have been infected, with significantly higher seropositivity among those above 60 years of age (1). CMV is transmitted by direct contact with infected body fluids, sexual contact, organ transplantation, and blood product transfusion. CMV can also be transplacentally transmitted from a pregnant mother to the unborn fetus (congenital CMV [cCMV]), as well as transmitted in the intrapartum period via contact with the mother’s genital secretions during delivery and in the postnatal period through infant ingestion of CMV-infected breast milk. CMV is the most common congenital viral infection, with a global incidence of 1 infection in every 200 live births, or 40,000 congenital infections per year in the United States (1). Once infected, the virus establishes latency, which persists for the life of the host, and reactivations occur frequently. Importantly, infection does not provide immunity against all types of CMV. Transplacental transmission rates are highest during primary infection of the mother, during which roughly a third of mothers will pass the virus to their fetus (2). Reactivation or reinfection can also lead to transplacental transmission at much lower rates (2).

CMV causes a wide spectrum of disease, which is most severe in the developing fetus and immunocompromised host. The majority of fetal infections are asymptomatic, with only 10% leading to symptoms in utero and/or at birth (3). The clinical manifestations of cCMV disease include hepatosplenomegaly, petechiae and purpura of the skin, and jaundice at birth. More than half of infants infected transplacentally will have neurologic involvement, including microcephaly, seizures, abnormal neurologic examination findings, feeding difficulties, and developmental delay. Sensorineural hearing loss is also present in more than two-thirds of symptomatic newborns. Importantly, up
to 15% of neonates who pass their hearing screens go on to develop hearing loss over the first years of life (2, 3). Similarly, ocular abnormalities, including chorioretinitis, retinal scarring, optic atrophy, and vision loss, are present in roughly a quarter of cases with symptomatic cCMV and can be progressive over the first years of life. Ocular findings in otherwise asymptomatic cases of cCMV, like the case presented here, are quite rare (3). As with the patient presented in this case, frequent vision and hearing tests are recommended for at least the first 3 years (2).

Despite being the most common cause of nonhereditary hearing loss in children in the United States, screening for CMV seropositivity is only currently performed for individuals undergoing transplant, along with their donors, and is not part of routine prenatal diagnostic testing. As a result, most pregnant women do not know their CMV status. Prenatal testing may be considered when abnormalities are observed in the developing fetus. Ultrasound findings can include neurologic manifestations, including intracranial calcifications, ventricular dilation, migrational abnormalities, and atrophy, as well as organomegaly, fetal hydrops, and/or restricted fetal growth. Unfortunately, most pregnant women only receive a 20-week anatomy scan, and cCMV manifestations may not appear until later in pregnancy, as in the case presented here.

If suspected based on ultrasound abnormalities, prenatal diagnosis of cCMV can be established if there is maternal interest and comfort with potential procedural risks (Table 1). One of the least invasive approaches, enzyme immunoassay (EIA) testing of the mother’s serum for IgG and IgM antibodies to CMV, is generally neither sensitive nor specific for cCMV diagnosis. This is due to the fact that CMV-specific IgM antibodies peak at 1 to 3 months postinfection, can persist for several months, or can appear during reactivation (3). Thus, a negative result does not exclude recent infection, and a positive result does not necessarily mean infection occurred postconception. Furthermore, there is well-known cross-reactivity of the IgM EIA with other herpesviruses (2, 3). Similar to the issues with IgM, CMV-specific IgG antibodies rise around the same time as IgM antibodies and persist for life, complicating interpretation of either a positive or negative IgG EIA in the context of ultrasound abnormalities. For ideal serologic diagnosis, acute and convalescent-phase sera would be run simultaneously via CMV IgG EIA to detect a 4-fold or greater rise in titer. This is generally not practical, as many women are asymptomatic during the acute phase and thus their sera are not collected until fetal abnormalities are detected later in the course of CMV infection. Furthermore, titers are not provided by most currently used EIAs. Quantitative CMV viral load testing of the mother’s whole blood, plasma, or serum, performed via PCR, also lacks sensitivity and specificity for cCMV diagnosis. This is due to the fact that viral loads during acute infection are typically very low, often below the limit of detection of PCR assays (4), and reactivation leading to low-level viremia is common in all individuals.

More recently developed CMV IgG avidity EIAs offer improved specificity over conventional EIAs for the diagnosis of primary CMV infection during pregnancy. In the early postinfection period, roughly the first 2 to 3 months after exposure to CMV, virus-specific IgG antibodies bind to their antigen epitope with low strength. As B cells mature in the postinfection period, greater than 3 months after virus exposure, they produce antibodies with high avidity. An avidity index is determined by comparing wells of an EIA with a denaturing agent that disrupts CMV antigen-antibody binding of low affinity to wells with a regular buffer (3). A low index suggests recent infection, while a high index suggests remote infection. There are currently no FDA-cleared CMV IgG avidity assays available, and testing is limited to reference laboratories. Low-avidity results should be interpreted with caution, as low-avidity CMV IgG may persist beyond the early postinfection period. High avidity results can be helpful in ruling out cCMV if testing is performed early in pregnancy (3). These results should not be used alone but may help guide subsequent confirmatory testing.

Although it poses risks to the fetus, amniocentesis is generally recommended if a specific diagnosis is desired by the mother and is important for treatment and/or appropriate monitoring. As CMV DNA accumulates in amniotic fluid after excretion from the fetal kidneys, detection via qualitative PCR (PCR) is highly specific for cCMV.
There can be a lag between maternal infection, vertical transmission, and viral shedding from the fetus, and thus the timing of amniocentesis relative to maternal infection is an important consideration to avoid false-negative results. As with the avidity assays, there are no FDA-cleared assays to detect CMV DNA in amniotic fluid, and testing is generally limited to reference laboratories. Viral culture of amniotic fluid, or any other source, for CMV is no longer recommended as the primary method for laboratory diagnosis due to lengthy turnaround times and inferior sensitivity compared to molecular methods. Culture can be considered if demonstration of active virus replication is of clinical utility (3).

After delivery, testing for cCMV is often only performed based on observed symptoms. Since hearing loss is the most common sequela, infants who fail hearing screens are often worked up for cCMV. Many states now recommend qualitative PCR testing for neonates who fail their newborn hearing screen administered in the hospital, and a few states have enacted laws to require such testing. Collection of urine is the preferred method, but this requires temporary catheterization. Saliva, collected via swab, is a less Table 1

<table>
<thead>
<tr>
<th>Method</th>
<th>Method detail</th>
<th>Specimen type(s)</th>
<th>Collected from mother</th>
<th>Collected from neonate/infant</th>
<th>Additional limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>CMV-specific enzyme immunoassay</td>
<td>IgM</td>
<td>Serum</td>
<td>Low sensitivity/specificity; could represent acute, recent or remote infection or reactivation; cross-reactivity with other herpesviruses</td>
<td>Low sensitivity/specificity; cross-reactivity with other herpesviruses</td>
<td></td>
</tr>
<tr>
<td></td>
<td>IgG</td>
<td>Serum</td>
<td>Low sensitivity/specificity; could represent recent or past infection</td>
<td>Poor specificity; could represent maternal IgG or intra- or postpartum acquisition</td>
<td></td>
</tr>
<tr>
<td></td>
<td>IgG avidity</td>
<td>Serum</td>
<td>Can discriminate positive IgG as recent or past</td>
<td>NA</td>
<td>Low-avidity antibodies can persist beyond 3 weeks</td>
</tr>
<tr>
<td>CMV DNA detection</td>
<td>Qualitative</td>
<td>Amniotic fluid</td>
<td>Preferred method for specific fetal diagnosis of cCMV; highly sensitive and specific; may be falsely negative if collected too early</td>
<td>NA</td>
<td>Requires invasive procedure that poses risks to the fetus; could consider IgG avidity testing first to rule out recent infection</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Urine, saliva</td>
<td>Unclear significance</td>
<td>Preferred method for confirmatory diagnosis for neonates aged &lt;3 weeks if fetal diagnosis was not established</td>
<td>Potential false positives in neonate/infant saliva due to maternal shedding via breast milk</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dried blood spot (newborn screening card)</td>
<td>NA</td>
<td>Can be considered for confirmatory diagnosis for infants &gt;3 weeks due to potential for intrapartum/postpartum infection</td>
<td>Cards are discarded at variable intervals by state, as early as 1 month after storage</td>
</tr>
<tr>
<td></td>
<td>Quantitative</td>
<td>Whole blood, plasma, serum</td>
<td>Limited sensitivity and specificity</td>
<td>NA (urine preferred)</td>
<td></td>
</tr>
<tr>
<td>CMV culture</td>
<td></td>
<td>Any</td>
<td>No longer recommended as primary means for laboratory diagnosis due to inferior sensitivity and turnaround time compared to those of molecular tests</td>
<td>NA</td>
<td></td>
</tr>
</tbody>
</table>

*NA, not applicable.
invasive alternative, but this specimen type can produce false-positive results in neonates who have recently ingested breast milk from a mother shedding CMV. In late 2018, the FDA approved one manufacturer’s test system for CMV DNA detection in swab-collected saliva from neonates. In addition to several state public health laboratories, neonatal saliva and urine CMV DNA testing is also available at commercial reference and many hospital laboratories.

Diagnosis can be challenging if symptoms present more than 3 weeks after delivery, like in the case presented above, as CMV antibody and/or DNA detection can reflect intra- or postpartum infection. Detection of CMV DNA in dried blood spots (DBS) from newborn screening cards can be considered for supportive diagnosis in such cases. A meta-analysis on the use of DBS for diagnosis of cCMV showed that the accuracy of the assay was excellent, with a pooled sensitivity of 84.4% and specificity of 99.9% (5). Similar sensitivity and specificity results have been described with the CDC assay used in the case presented here (6). One older study found inferior sensitivity and specificity, although the extraction method used likely contributed to this difference (7). Several public health laboratories and a few commercial reference laboratories offer CMV PCR from dried blood spots. Unfortunately, the period of newborn screening card retention varies by state, with some being as short as 1 month of storage. Given that symptoms may not arise until several months to years after delivery, there is a push to have CMV testing performed on the DBS as part of the newborn screening panel. However, universal screening for CMV would require an improvement in sensitivity beyond what is currently described for DBS CMV PCR (5, 7).

There is utility in both prenatal and postpartum cCMV diagnosis to inform treatment and/or management. Neonates with confirmed cCMV disease may benefit from the use of valganciclovir or ganciclovir when initiated during the first 30 days of life (8). Antiviral treatment of the fetus or of infants diagnosed with cCMV infection after 30 days is generally not recommended due to the lack of data to support any clinical benefit, as well as the potential side effects. Administration of CMV immunoglobulin to pregnant mothers with fetal evidence of cCMV was shown to provide benefit in one study (9), and clinical trials are now underway. cCMV diagnosis also helps focus surveillance activities for hearing and vision issues and helps avoid additional costs related to pursuit of genetic causes for hearing or vision loss once detected.

SELF-ASSESSMENT QUESTIONS

1. Which of the following is the most sensitive and specific laboratory test to diagnose congenital CMV (cCMV) in a pregnant woman who has fetal abnormalities detected during her 20-week ultrasound?
   a. CMV IgG enzyme immunoassay from the mother’s serum
   b. CMV IgM enzyme immunoassay from the mother’s serum
   c. CMV PCR of the mother’s blood
   d. CMV PCR of amniotic fluid

2. Which of the following is the most sensitive and specific laboratory test to diagnose cCMV in a newborn who has failed their first hearing screen at day 1 of life?
   a. CMV PCR of urine
   b. CMV PCR of swab-collected saliva
   c. CMV IgG testing of the neonate’s serum
   d. CMV IgG testing of the mother’s serum

3. Which of the following could be considered to support a diagnosis of cCMV in a 6-month-old infant who has developmental delays and abnormal MRI findings?
   a. CMV PCR of urine
   b. CMV PCR of swab-collected saliva
   c. CMV PCR of the 6-month-old infant’s dried blood spot collected 24 hours after birth
   d. CMV IgG testing of the 6-month-old infant’s serum
REFERENCES


