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SUPPLEMENT ARTICLE

Congenital Cytomegalovirus Infection

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Congenital cytomegalovirus (cCMV) infection is a leading cause of hearing loss and neurological disabilities in children, with the disease burden and disabilities due to cCMV greater than many other well recognized childhood conditions. A minority of infants with cCMV will have symptoms at birth. Infants with symptomatic cCMV are at higher risk for sequelae than those born without symptoms. The majority of infants with cCMV are asymptomatic at birth, but 10%–15% will develop hearing loss. Although clinical symptoms can help predict which infants will have sensorineural hearing loss, among asymptomatic cCMV there are currently no predictors of adverse outcome. The identification of a biomarker to identify those at highest risk of sequelae is highly desirable to target interventions to those who could potentially benefit. Because there is increasing rationale for establishing both targeted and universal screening programs for cCMV in the United States and worldwide, this is an urgent priority.

Keywords. congenital infection; cytomegalovirus; hearing loss.

Cytomegalovirus (CMV) is the most common congenital viral infection in the United States. The disease burden due to CMV is tremendous, because disabilities due to congenital CMV (cCMV) infection are estimated to be more common than many other well recognized conditions such as Downs Syndrome, spina bifida, or fetal alcohol syndrome [1]. Congenital CMV-related sequelae affect over 5000 children annually, and costs are greater than $1 billion annually in direct medical care in the United States [2].

Congenital cytomegalovirus is a leading cause of sensorineural hearing loss (SNHL) and neurodevelopmental delay in children [3, 4]. Most congenitally infected infants (~90%) do not show any obvious clinical abnormalities at birth, and thus they are asymptomatic. Infants with symptomatic infection are those who have clinical findings at birth suggestive of congenital infection [5]. There are important prognostic implications associated with this categorization, because infants with symptomatic infection are more likely to have neurodevelopmental sequelae and SNHL.

EPIDEMIOLOGY

Cytomegalovirus is a leading cause of congenital infections worldwide, occurring in 0.2%–6.1% of live births [6, 7]. It is also the most common congenital viral infection in the United States, affecting an estimated 20 000 to 30 000 infants annually [8]. In the United States, Canada, Australia, and Western Europe, cCMV occurs in approximately 5–7 per 1000 live births [7, 9, 10]. In South America, Africa, and Asia, the rates of cCMV tend to be higher, at approximately 10–20 per 1000 live births [11–13]. Within the United States, there are racial and ethnic differences in the prevalence of cCMV, with black infants having the highest prevalence at 9.5 per 1000 live births [14].

SYMPTOMATIC CONGENITAL CYTOMEGALOVIRUS INFECTION

Approximately 10% of infants with cCMV will have clinical abnormalities at birth and thus are classified as symptomatic [5]. There is no standard definition for symptomatic cCMV infection, because studies will include different clinical manifestations. In general, infants with symptomatic cCMV infection can have a broad range of disease manifestations. Severely symptomatic infection can manifest as thrombocytopenia, petechiae, hepatomegaly, splenomegaly, intrauterine growth restriction, and hepatitis. Central nervous system (CNS) involvement of cCMV manifests as microcephaly, neuroimaging abnormalities (ventriculomegaly, intracerebral calcifications, periventricular echogenicity), and seizures [5, 15]. Ophthalmologic examination may reveal chorioretinitis or optic atrophy [16]. Mortality due to cCMV is generally low, with approximately 4% of infants dying as a result of cCMV [17].

Laboratory findings in infants with cCMV infection will mirror the organ systems involved. As such, more than 50% of children with symptomatic infection may have conjugated hyperbilirubinemia, hepatic transaminases elevation, or thrombocytopenia [15]. Bilirubin levels and transaminases will peak within the first 2 weeks of life, but they can continue to remain elevated for several weeks. Thrombocytopenia tends to reach its nadir by the second week of life, and it will completely normalize within 3–5 weeks of life [5].

In a recent report, the International Congenital Cytomegalovirus Recommendations Group outlined

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categories of cCMV disease in an effort to standardize definitions for recommendations regarding antiviral treatment [18]. Symptomatic infection was categorized as either (1) moderately to severely symptomatic cCMV disease defined as multiple manifestations attributable to cCMV and/or CNS involvement or (2) mildly symptomatic cCMV defined as 1 or 2 isolated manifestations of cCMV infection that are mild and transient, such as petechiae or mild hepatomegaly [18]. Infants who manifest none of the above symptoms but have isolated SNHL in the newborn period are considered asymptomatic [18].

COGNITIVE SEQUELAE AND SENSORINEURAL HEARING LOSS

Approximately 40% to 60% of infants with symptomatic cCMV will have permanent sequelae due to disease, most commonly SNHL, followed by cognitive impairment, chorioretinitis, and cerebral palsy [3, 5, 15, 19]. Dreher et al [15] found that 38% of the children with symptomatic cCMV had an intelligence quotient (IQ) <70. The study also found that 23% of infants with cCMV had motor deficits and approximately 19% had seizures [15]. Another study found that significantly more children with CNS involvement had IQ <70 compared with those without CNS involvement [20]. These findings mirror the results of other studies that report microcephaly, chorioretinitis, and neuroimaging abnormalities in the newborn period are associated with poor cognitive outcomes [3, 21, 22].

Although approximately 90% of children with cCMV will be asymptomatic at birth, 10%–15% will develop sequelae, with neurodevelopmental sequelae uncommon in asymptomatic infection. Lopez et al [23] systemically evaluated children with asymptomatic cCMV and measured their cognitive outcomes and academic performance through 18 years of age. This study reported that asymptomatic children who had normal hearing before age 2 had no difference in their IQ, vocabulary, or academic achievement scores through childhood compared with normal controls [23]. In contrast, among asymptomatic children who developed SNHL by age 2, intelligence and receptive vocabulary scores were lower compared with normal controls [23].

Hearing loss is the most common sequela in both symptomatic and asymptomatic cCMV (Table 1). Among children with symptomatic cCMV, 30%–40% will have SNHL in the newborn period or in the first years of life. Delayed-onset hearing loss is common as 18%–27% of symptomatic infants develop hearing loss after birth. Progression of hearing loss over time occurs in 20%–54% of symptomatic infants, and 43% of SNHL is bilateral and severe/profound [19, 24].

Asymptomatic infants with cCMV are also at increased risk for hearing loss, particularly delayed-onset and progressive SNHL (Table 1). Approximately 10% of asymptomatic infants have SNHL at birth or develop hearing loss during the first several years of life, with over half having involvement of only 1 ear. Delayed-onset loss in asymptomatic children occurs later than in symptomatic cCMV, with 38% children developing SNHL at a median age of 44 months. Fluctuation of SNHL is also a common feature in asymptomatic cCMV with approximately half of asymptomatic children having improvement in their hearing threshold [19, 24]. Delayed onset and progression of hearing loss in both asymptomatic and symptomatic children with cCMV necessitates long-term follow-up for prompt diagnosis and management to improve outcomes.

NEWBORN CYTOMEGALOVIRUS SCREENING

As noted, the majority of infants with cCMV are asymptomatic at birth, thus they will not be identified as congenitally infected in the newborn period. Because approximately half of hearing loss in asymptomatic children is delayed [19], screening for cCMV in the newborn period will assure timely diagnosis and intervention to improve outcomes for children with SNHL. To diagnose cCMV, testing must be performed within the first 3 weeks of life, because testing past this period does not differentiate intrauterine from perinatal acquisition of CMV infection. The application of polymerase chain reaction (PCR) evaluation of dried blood spots to screen newborns for cCMV has demonstrated center to center variability in sensitivity [9, 25–29]. Because

Table 1. Hearing Outcomes in Congenital CMV[a]

Hearing Loss Characteristics	Asymptomatic	Symptomatic
Sensorineural hearing loss	7%–10%	32%–41%
Characteristics of Loss		
Unilateral	52%–57%	29%–33%
Bilateral	43%–48%	67%–71%
Progressive loss	20%–54%	18%–54%
Fluctuating loss	24%–54%	22%–29%
High-frequency loss only	38%	27%
Bilateral severe to profound loss	43%	75%
Delayed-onset loss	9%–38%	18%–27%
Median age (range) of delayed-onset loss	44 months (24–182)	33 months (6–197)

[a]Data adapted from references [19, 21, 24].

high viral loads are shed in both urine and saliva in infants with cCMV, both sites have been shown to be reliable for diagnosis of cCMV in newborns [30–32]. Because saliva specimens are readily obtained, saliva CMV PCR is the preferred diagnostic test for newborn cCMV screening [18]. Although CMV present in breast milk could lead to false-positive results when using saliva PCR for newborn CMV screening, one large study demonstrated an acceptably low false-positive rate [33]. Obtaining a saliva sample at least 1 hour after breastfeeding could avoid potential contamination with CMV from breastmilk.

With the availability of a reliable screening test and recognition of the importance of identifying infants at risk for SNHL early, more than 100 hospitals in 18 states are performing targeted CMV screening of newborns that refer on newborn hearing screening [34, 35]. This approach identifies infants with asymptomatic cCMV and cCMV-related SNHL that would have otherwise not been detected. A study from Utah showed the added benefit of improving the 3-month diagnostic hearing evaluation rate for the newborn hearing screening program [34]. However, a recent large multicenter study demonstrated that more than 40% of infants with CMV-related SNHL would be missed by the targeted CMV screening approach [36]. Universal newborn screening for CMV would identify all infants at risk for hearing loss, with a recent study showing the cost-effectiveness of such approach [37].

PREDICTORS OF HEARING LOSS

Both universal and targeted newborn CMV screening approaches will lead to identification of many more infants with cCMV infection than are currently identified because of clinical signs [19, 38]. Among infants with symptomatic cCMV, CNS involvement as evidenced by clinical findings (microcephaly, seizures, abnormal tone) or neuroimaging findings (intracerebral calcifications, ventriculomegaly, and white matter changes) has been shown to be a strong predictor of adverse neurodevelopmental outcome including SNHL [15, 20, 39–41]. A few small studies including primarily symptomatic infants have used a neuroimaging scoring system to better define the severity of neuroimaging abnormalities and suggested that such a scoring system leads to improved prediction of neurodevelopment outcome and SNHL [22, 42]. Likewise, a limited number of studies that mostly included selected infants with symptomatic infection have examined the role of cerebral ultrasound in identifying CNS involvement [40, 43]. A recent Italian study that included 56 infants with asymptomatic cCMV who underwent cranial ultrasound [43] identified 18 infants who had abnormal neuroimaging. Lenticulostriate vasculopathy was the most common abnormal finding detected in 13 of 56 (23%) of the asymptomatic infants. Calcifications, pseudocysts, and ventriculomegaly were also observed in an additional 5 infants. Among the entire study population, the presence of lenticulostriate vasculopathy with or without other findings was not associated with SNHL. However, lenticulostriate vasculopathy is not specific to cCMV, and it has been associated with other perinatal and neonatal abnormalities. A study of a large group of symptomatic infants with long-term hearing follow-up demonstrated that the risk of SNHL and adverse cognitive outcome varied depending on the clinical presentation at birth. Infants with evidence of CNS involvement at birth had a significantly higher risk for SNHL and cognitive deficits than those with transient symptoms such as hepatomegaly or splenomegaly or those with an isolated petechial rash, suggesting a direct relationship between the severity of disease at birth and the risk for adverse outcomes [20].

The prognostic value of viral load in neonatal samples for identifying infants at risk for SNHL, especially those with asymptomatic infection, is unclear. Studies with primarily symptomatic children demonstrated that higher viral load during infancy was associated with an increased risk of SNHL [44–46]. A subsequent study with a larger group of children with cCMV, including those with asymptomatic infection (n = 135), found a large overlap in blood viral load between children with SNHL and those with normal hearing [47]. In a recent antiviral trial in infants with symptomatic cCMV, area under the curve (AUC) analysis suggested that lower viral loads at baseline correlated with better hearing outcomes [48]. Thus, the role of virus burden in the peripheral blood, urine, and saliva in disease and outcome needs further study.

The timing of fetal infection and risk of long-term sequelae has been assessed in multiple studies in women who acquire primary CMV infection during pregnancy [49–52]. Sensorineural hearing loss has been documented as a result of primary maternal infection in all trimesters of pregnancy, demonstrating the need for routine hearing follow-up of all infants with cCMV. However, nonprimary maternal infection is estimated to account for the majority of congenital infections worldwide [53], and it can result in CMV-related SNHL in infected infants [54]. Because routine antenatal screening for CMV in pregnancy is not routinely performed [18], the ascertainment of the type of maternal infection and timing during gestation is often challenging in clinical practice.

Cytomegalovirus is highly genetically diverse, and many studies have explored whether variants of individual CMV glycoproteins and other loci can serve as prognostic markers for CMV-associated SNHL. These have produced conflicting results with no clear relationship found between particular CMV genotypes and disease [55–58]. Mixed infection with multiple CMV genotypes and compartmentalization of genotypes has also been reported in cCMV infection [59], but it is unclear whether infection with more than 1 CMV strain is related to hearing outcome. Studies utilizing new ultra deep sequencing technology have illustrated that the diversity of CMV strains in a single host is far greater than what was demonstrated by traditional genotyping methods [60, 61]. Next-generation sequencing (NGS) of samples from infants with cCMV

infection show intrahost diversity of CMV to be quite large and comparable to that of ribonucleic acid viruses. Most intriguing was the observation that some open reading frames were targets of positive selection [60]. More recent bioinformatic analyses demonstrate that high CMV diversity in some samples is attributed to coinfection with multiple distinct CMV strains [62, 63]. These findings raise important questions about the role of virus diversity in outcome and demonstrates the need for further studies using NGS in a well characterized cohort of children with cCMV in whom outcome data are available.

CONCLUSIONS

Congenital CMV infection is an important cause of hearing loss and neurologic disabilities in children worldwide. Children with both asymptomatic and symptomatic cCMV are at risk of sequelae, with a significant proportion of infants with asymptomatic cCMV developing hearing loss months to years after birth. However, there are currently no predictors of outcome in asymptomatic cCMV. Because there is increased interest towards both targeted and universal newborn screening for cCMV, future studies of infants identified on newborn screening will need to address the identification and management of children with cCMV at risk for adverse outcome.

Notes

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References

1. Cannon MJ. Congenital cytomegalovirus (CMV) epidemiology and awareness. J Clin Virol **2009**; 46(Suppl 4):S6–10.
2. Stratton KR, Durch JS, Lawrence RS, eds. Vaccines for the 21st Century: A Tool for Decisionmaking. Washington DC: National Academies Press; **2000**.
3. Dollard SC, Grosse SD, Ross DS. New estimates of the prevalence of neurological and sensory sequelae and mortality associated with congenital cytomegalovirus infection. Rev Med Virol **2007**; 17:355–63.
4. Morton CC, Nance WE. Newborn hearing screening–a silent revolution. N Engl J Med **2006**; 354:2151–64.
5. Boppana SB, Pass RF, Britt WJ, Stagno S, Alford CA. Symptomatic congenital cytomegalovirus infection: neonatal morbidity and mortality. Pediatr Infect Dis J **1992**; 11:93–9.
6. Lanzieri TM, Dollard SC, Bialek SR, Grosse SD. Systematic review of the birth prevalence of congenital cytomegalovirus infection in developing countries. Int J Infect Dis **2014**; 22:44–8.
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. Britt W. Cytomegalovirus. In: Remington JS, Klein JO, Wilson CB, Baker CJ, eds. Infectious Diseases of the Fetus and Newborn Infant. 7th ed. Philadelphia: W.B. Saunders Company; **2011**: pp 704–53.
9. Kharrazi M, Hyde T, Young S, Amin MM, Cannon MJ, Dollard SC. Use of screening dried blood spots for estimation of prevalence, risk factors, and birth outcomes of congenital cytomegalovirus infection. J Pediatr **2010**; 157:191–7.
10. Puhakka L, Lappalainen M, Lonnqvist T, et al. The burden of congenital cytomegalovirus infection: a prospective cohort study of 20 000 infants in Finland. J Pediatric Infect Dis Soc **2018**; 8:205–212.
11. Dar L, Pati SK, Patro AR, et al. Congenital cytomegalovirus infection in a highly seropositive semi-urban population in India. Pediatr Infect Dis J **2008**; 27:841–3.
12. Mussi-Pinhata MM, Yamamoto AY, Moura Brito RM, et al. Birth prevalence and natural history of congenital cytomegalovirus infection in a highly seroimmune population. Clin Infect Dis **2009**; 49:522–8.
13. Pathirana J, Groome M, Dorfman J, et al. Prevalence of congenital cytomegalovirus infection and associated risk of in-utero HIV acquisition in a high HIV prevalence setting, South Africa. Clin Infect Dis **2019**.
14. Fowler KB, Ross SA, Shimamura M, et al. Racial and ethnic differences in the prevalence of congenital cytomegalovirus infection. J Pediatr **2018**; 200:196–201.e1.
15. Dreher AM, Arora N, Fowler KB, et al. Spectrum of disease and outcome in children with symptomatic congenital cytomegalovirus infection. J Pediatr **2014**; 164:855–9.
16. Capretti MG, Marsico C, Guidelli Guidi S, et al. Neonatal and long-term ophthalmological findings in infants with symptomatic and asymptomatic congenital cytomegalovirus infection. J Clin Virol **2017**; 97:59–63.
17. Fowler KB, Dahle AJ, Boppana SB, Pass RF. Newborn hearing screening: will children with hearing loss caused by congenital cytomegalovirus infection be missed? J Pediatr **1999**; 135:60–4.
18. Rawlinson WD, Boppana SB, Fowler KB, et al. Congenital cytomegalovirus infection in pregnancy and the neonate: consensus recommendations for prevention, diagnosis, and therapy. Lancet Infect Dis **2017**; 17:e177–88.
19. Dahle AJ, Fowler KB, Wright JD, Boppana SB, Britt WJ, Pass RF. Longitudinal investigation of hearing disorders in children with congenital cytomegalovirus. J Am Acad Audiol **2000**; 11:283–90.

20. Pinninti SG, Rodgers MD, Novak Z, et al. Clinical predictors of sensorineural hearing loss and cognitive outcome in infants with symptomatic congenital cytomegalovirus infection. Pediatr Infect Dis J **2016**; 35:924–6.
21. Bartlett AW, McMullan B, Rawlinson WD, Palasanthiran P. Hearing and neurodevelopmental outcomes for children with asymptomatic congenital cytomegalovirus infection: a systematic review. Rev Med Virol **2017**; 27:e1938.
22. Noyola DE, Demmler GJ, Nelson CT, et al. Early predictors of neurodevelopmental outcome in symptomatic congenital cytomegalovirus infection. J Pediatr **2001**; 138:325–31.
23. Lopez AS, Lanzieri TM, Claussen AH, et al. Intelligence and academic achievement with asymptomatic congenital cytomegalovirus infection. Pediatrics **2017**; 140:e20171517.
24. Goderis J, De Leenheer E, Smets K, Van Hoecke H, Keymeulen A, Dhooge I. Hearing loss and congenital CMV infection: a systematic review. Pediatrics **2014**; 134:972–82.
25. Barbi M, Binda S, Primache V, et al. Cytomegalovirus DNA detection in Guthrie cards: a powerful tool for diagnosing congenital infection. J Clin Virol **2000**; 17:159–65.
26. Boppana SB, Ross SA, Novak Z, et al. Dried blood spot real-time polymerase chain reaction assays to screen newborns for congenital cytomegalovirus infection. JAMA **2010**; 303:1375–82.
27. de Vries JJ, Claas EC, Kroes AC, Vossen AC. Evaluation of DNA extraction methods for dried blood spots in the diagnosis of congenital cytomegalovirus infection. J Clin Virol **2009**; 46(Suppl 4):S37–42.
28. Leruez-Ville M, Vauloup-Fellous C, Couderc S, et al. Prospective identification of congenital cytomegalovirus infection in newborns using real-time polymerase chain reaction assays in dried blood spots. Clin Infect Dis **2011**; 52:575–81.
29. Wang S, Wang T, Zhang W, et al. Cohort study on maternal cytomegalovirus seroprevalence and prevalence and clinical manifestations of congenital infection in China. Medicine (Baltimore) **2017**; 96:e6007.
30. Boppana SB, Ross SA, Shimamura M, et al. Saliva polymerase-chain-reaction assay for cytomegalovirus screening in newborns. N Engl J Med **2011**; 364:2111–8.
31. Ross SA, Ahmed A, Palmer AL, et al. Detection of congenital cytomegalovirus infection by real-time polymerase chain reaction analysis of saliva or urine specimens. J Infect Dis **2014**; 210:1415–8.
32. Yamamoto AY, Mussi-Pinhata MM, Marin LJ, Brito RM, Oliveira PF, Coelho TB. Is saliva as reliable as urine for detection of cytomegalovirus DNA for neonatal screening of congenital CMV infection? J Clin Virol **2006**; 36:228–30.
33. Ross SA, Michaels MG, Ahmed A, et al. Contribution of breastfeeding to false-positive saliva polymerase chain reaction for newborn congenital cytomegalovirus screening. J Infect Dis **2018**; 217:1612–5.
34. Diener ML, Zick CD, McVicar SB, Boettger J, Park AH. Outcomes from a hearing-targeted cytomegalovirus screening program. Pediatrics **2017**; 139:e20160789.
35. National CMV Foundation. Newborn screening. Available at: https://www.nationalcmv.org/overview/newborn-screening. Accessed 26 August 2019.
36. Fowler KB, McCollister FP, Sabo DL, et al. A targeted approach for congenital cytomegalovirus screening within newborn hearing screening. Pediatrics **2017**; 139:e20162128.
37. Gantt S, Dionne F, Kozak FK, et al. Cost-effectiveness of universal and targeted newborn screening for congenital cytomegalovirus infection. JAMA Pediatr **2016**; 170:1173–80.
38. Goderis J, Keymeulen A, Smets K, et al. Hearing in children with congenital cytomegalovirus infection: results of a longitudinal study. J Pediatr **2016**; 172:110–5.e2.
39. Alarcon A, Martinez-Biarge M, Cabanas F, Hernanz A, Quero J, Garcia-Alix A. Clinical, biochemical, and neuroimaging findings predict long-term neurodevelopmental outcome in symptomatic congenital cytomegalovirus infection. J Pediatr **2013**; 163:828–34.e1.
40. Capretti MG, Lanari M, Tani G, et al. Role of cerebral ultrasound and magnetic resonance imaging in newborns with congenital cytomegalovirus infection. Brain Dev **2014**; 36:203–11.
41. de Vries LS, Gunardi H, Barth PG, Bok LA, Verboon-Maciolek MA, Groenendaal F. The spectrum of cranial ultrasound and magnetic resonance imaging abnormalities in congenital cytomegalovirus infection. Neuropediatrics **2004**; 35:113–9.
42. Alarcon A, Martinez-Biarge M, Cabañas F, Quero J, García-Alix A. A prognostic neonatal neuroimaging scale for symptomatic congenital cytomegalovirus infection. Neonatology **2016**; 110:277–85.
43. Giannattasio A, Di Costanzo P, Milite P, et al. Is lenticulostriated vasculopathy an unfavorable prognostic finding in infants with congenital cytomegalovirus infection? J Clin Virol **2017**; 91:31–5.
44. Boppana SB, Fowler KB, Pass RF, et al. Congenital cytomegalovirus infection: association between virus burden in infancy and hearing loss. J Pediatr **2005**; 146:817–23.
45. Lanari M, Lazzarotto T, Venturi V, et al. Neonatal cytomegalovirus blood load and risk of sequelae in symptomatic and asymptomatic congenitally infected newborns. Pediatrics **2006**; 117:e76–83.
46. Walter S, Atkinson C, Sharland M, et al. Congenital cytomegalovirus: association between dried blood spot viral load and hearing loss. Arch Dis Child Fetal Neonatal Ed **2008**; 93:F280–5.
47. Ross SA, Novak Z, Fowler KB, Arora N, Britt WJ, Boppana SB. Cytomegalovirus blood viral load and hearing loss in young children with congenital infection. Pediatr Infect Dis J **2009**; 28:588–92.

48. Kimberlin DW, Jester PM, Sánchez PJ, et al. Valganciclovir for symptomatic congenital cytomegalovirus disease. N Engl J Med **2015**; 372:933–43.
49. Faure-Bardon V, Magny JF, Parodi M, et al. Sequelae of congenital cytomegalovirus (cCMV) following maternal primary infection are limited to those acquired in the first trimester of pregnancy. Clin Infect Dis **2018**.
50. Gindes L, Teperberg-Oikawa M, Sherman D, Pardo J, Rahav G. Congenital cytomegalovirus infection following primary maternal infection in the third trimester. BJOG **2008**; 115:830–5.
51. Lipitz S, Yinon Y, Malinger G, et al. Risk of cytomegalovirus-associated sequelae in relation to time of infection and findings on prenatal imaging. Ultrasound Obstet Gynecol **2013**; 41:508–14.
52. Pass RF, Fowler KB, Boppana SB, Britt WJ, Stagno S. Congenital cytomegalovirus infection following first trimester maternal infection: symptoms at birth and outcome. J Clin Virol **2006**; 35:216–20.
53. de Vries JJ, van Zwet EW, Dekker FW, Kroes AC, Verkerk PH, Vossen AC. The apparent paradox of maternal seropositivity as a risk factor for congenital cytomegalovirus infection: a population-based prediction model. Rev Med Virol **2013**; 23:241–9.
54. Ross SA, Fowler KB, Ashrith G, et al. Hearing loss in children with congenital cytomegalovirus infection born to mothers with preexisting immunity. J Pediatr **2006**; 148:332–6.
55. Arav-Boger R. Strain variation and disease severity in congenital cytomegalovirus infection: in search of a viral marker. Infect Dis Clin North Am **2015**; 29:401–14.
56. Pignatelli S, Lazzarotto T, Gatto MR, et al. Cytomegalovirus gN genotypes distribution among congenitally infected newborns and their relationship with symptoms at birth and sequelae. Clin Infect Dis **2010**; 51:33–41.
57. Waters A, Hassan J, De Gascun C, et al. Human cytomegalovirus UL144 is associated with viremia and infant development sequelae in congenital infection. J Clin Microbiol **2010**; 48:3956–62.
58. Pati SK, Pinninti S, Novak Z, et al. Genotypic diversity and mixed infection in newborn disease and hearing loss in congenital cytomegalovirus infection. Pediatr Infect Dis J **2013**; 32:1050–4.
59. Ross SA, Novak Z, Pati S, et al. Mixed infection and strain diversity in congenital cytomegalovirus infection. J Infect Dis **2011**; 204:1003–7.
60. Renzette N, Bhattacharjee B, Jensen JD, Gibson L, Kowalik TF. Extensive genome-wide variability of human cytomegalovirus in congenitally infected infants. PLoS Pathog **2011**; 7:e1001344.
61. Renzette N, Pokalyuk C, Gibson L, et al. Limits and patterns of cytomegalovirus genomic diversity in humans. Proc Natl Acad Sci U S A **2015**; 112:E4120–8.
62. Cudini J, Roy S, Houldcroft CJ, et al. Human cytomegalovirus haplotype reconstruction reveals high diversity due to superinfection and evidence of within-host recombination. Proc Natl Acad Sci U S A **2019**; 116:5693–8.
63. Suárez NM, Wilkie GS, Hage E, et al. Human cytomegalovirus genomes sequenced directly from clinical material: variation, multiple-strain infection, recombination, and gene loss. J Infect Dis **2019**; 220:781–91.