Newborn Dried Blood Spot Polymerase Chain Reaction to Identify Infants with Congenital Cytomegalovirus-Associated Sensorineural Hearing Loss

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Objective To determine the utility of dried blood spot (DBS) polymerase chain reaction (PCR) in identifying infants with cytomegalovirus (CMV) infection–associated sensorineural hearing loss (SNHL).

Study design Newborns at 7 US hospitals between March 2007 and March 2012 were screened for CMV by saliva rapid culture and/or PCR. Infected infants were monitored for SNHL during the first 4 years of life to determine sensitivity, specificity, and positive and negative likelihood ratios of DBS PCR for identifying CMV-associated SNHL.

Results DBS at birth was positive in 11 of 26 children (42%) with SNHL at age 4 years and in 72 of 270 children (27%) with normal hearing ($P = .11$). The sensitivity (42.3%; 95% CI, 23.4%-63.1%) and specificity (73.3%; 95% CI, 67.6%-78.5%) was low for DBS PCR in identifying children with SNHL at age 4 years. The positive and negative likelihood ratios of DBS PCR positivity to detect CMV-associated SNHL at age 4 years were 1.6 (95% CI, 0.97-2.6) and 0.8 (95% CI, 0.6-1.1), respectively. There was no difference in DBS viral loads between children with SNHL and those without SNHL.

Conclusions DBS PCR for CMV has low sensitivity and specificity for identifying infants with CMV-associated hearing loss. These findings, together with previous reports, demonstrate that DBS PCR does not identify either the majority of CMV-infected newborns or those with CMV-associated SNHL early in life. *(J Pediatr 2017;184:57-61).*

Cytomegalovirus (CMV) is an important cause of congenital infection, and congenital CMV infection (cCMV) is a significant nongenetic cause of sensorineural hearing loss (SNHL) in children.[1-4] Most congenitally infected infants (~90%) do not have obvious clinical abnormalities at birth (asymptomatic cCMV), and thus are not identified in the newborn nursery[5,6]; however, approximately 15% of children with asymptomatic cCMV develop SNHL.[1,7,8] Although CMV-associated SNHL may be present at birth, a substantial proportion of children with cCMV develop late-onset and/or progressive SNHL.[1,9] Therefore, most children with cCMV and a significant number of those with CMV-associated SNHL are not identified on routine physical examination or hearing screening in the newborn nursery.

The need to develop rapid and reliable methods to screen newborns for CMV so that infants at increased risk for hearing loss can be identified for targeted monitoring and early intervention has been recognized.[10-12] Because dried blood spots (DBSs) are collected routinely from all infants in the US for newborn metabolic screening, and several initial studies have shown promise, the hope was that DBS polymerase chain reaction (PCR) would facilitate the development of effective strategies to screen all newborns for CMV.[13-18] However, in a CMV screening study of 20 446 newborns, we demonstrated that the sensitivity of DBS PCR in identifying infants with cCMV was unacceptably low compared with saliva rapid culture,[19] and thus would not be a suitable screening method. Although DBS PCR failed to identify the majority of infants with cCMV, the possibility that it could detect infants at increased risk for CMV-associated SNHL remained. The present study aimed to determine the ability of the DBS PCR assay to identify infants with cCMV at risk for disease and sequelae.

CHIMES	Congenital Cytomegalovirus and Hearing Multicenter Screening
CMV	Cytomegalovirus
cCMV	Congenital cytomegalovirus
DBS	Dried blood spot
LR	Likelihood ratio
PCR	Polymerase chain reaction
SNHL	Sensorineural hearing loss

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Methods

Between March 2007 and March 2012, infants born at 7 US hospitals were enrolled prospectively in the CMV and Hearing Multicenter Screening (CHIMES) study supported by the National Institutes on Deafness and Other Communication Disorders.[19,20] Newborn CMV screening was performed by testing saliva specimens (rapid culture or PCR) and DBS PCR as described previously.[19,20] Between March 2007 and May 2008, all DBS specimens collected from screened infants were tested by PCR.[19] After May 2008, only DBS specimens from infants who were positive for CMV by rapid culture or PCR of saliva were tested. Demographic information, newborn hearing screening results, and saliva and DBS specimens were collected from the screening cohort. Race and ethnicity were self-reported by the parents of the infants and categorized by the National Institutes of Health's definitions for race and ethnicity.[21] DBSs for this study were collected on a separate card after obtaining the sample for routine newborn metabolic screening without an additional heel stick. The parents /guardians of the screened infants were provided the CMV screening results.

Infants were considered to have symptomatic cCMV if they had any of the following findings in the newborn period: generalized petechial rash, purpuric rash, hepatomegaly, splenomegaly, jaundice with direct bilirubin ≥3 mg/dL, unexplained neurologic/central nervous system abnormalities (eg, microcephaly, seizures, focal or generalized neurologic deficits), or chorioretinitis. Clinical decisions about evaluation and antiviral treatment of the infants infected with CMV were made by the physicians at each study site and were not part of the CHIMES study. Seventeen infants were treated with antiviral therapy, including 9 with SNHL at birth and none with late-onset SNHL. The 17 treated infants are included in the cohort for evaluating both DBS positivity and symptomatic status and DBS positivity and SNHL at birth; however, because antiviral therapy has been shown to affect hearing outcome,[22] the 17 treated infants were not included in the comparison of DBS positivity and hearing loss at age 4 years.

Medical records were reviewed for family history of hearing loss or other potential etiologies including congenital malformations that could cause SNHL. In addition, the parents were asked at enrollment about any family history of hearing loss. In addition, any new diagnosis possibly related to SNHL was collected from the parents at the follow-up visits. None of the children with SNHL had syndromes or other malformations associated with either SNHL or a family history of SNHL. Local Institutional Review Board approval was obtained at each site.

Follow-Up of Children with cCMV Infection

Infants with a positive saliva or DBS screening test were enrolled in the follow-up component of the study to confirm cCMV and to monitor hearing function during the first 4 years of life in accordance with study protocol. Infants were considered to have confirmed cCMV if the follow-up urine or saliva sample was positive by rapid culture and/or PCR. After the initial diagnostic audiologic evaluation between age 3 and 8 weeks, the study children were monitored for hearing loss using age and developmentally appropriate audiologic testing protocols every 6 months during the first 4 years of life.[7]

Statistical Analyses

All statistical analyses were performed using SAS version 9.3 (SAS Institute, Cary, North Carolina). The 2-tailed Fisher exact test was used to assess the association between DBS positivity and symptomatic infection and hearing outcomes. Viral load levels were compared between the infants with symptomatic infection and asymptomatic infection, and those with and without hearing loss at age 4 years using the Wilcoxon rank-sum test. Sensitivity, specificity, and predictive values for DBS positivity were calculated using standard methods for proportions and exact 95% CIs. Likelihood ratios (LRs) were calculated to summarize the diagnostic accuracy of the DBS PCR. The positive LR was sensitivity/(1-specificity), and the negative LR was (1-sensitivity)/specificity. The 95% CIs for LRs were determined using the method described by Simel et al.[23]

Results

During the study period., a total of 100 332 infants were enrolled and screened for cCMV. Screening DBS samples were available for 313 of 391 infants (80.1%) with confirmed cCMV, and these infants constituted the study population (**Figure 1**; available at www.jpeds.com). Reasons for unavailability of DBS specimens included collection of DBS for the routine metabolic screening before study consent was obtained (n = 72), insufficient blood for an additional study filter card (n = 2), loss of DBS sample (n = 3), and refusal by mother (n = 1). There was no difference in the proportion of infants with symptomatic cCMV and SNHL between those without a screening DBS sample and those with an available DBS. Among the 313 study children, congenital infection was confirmed by rapid culture of saliva or urine samples in 302 infants and by PCR of saliva or urine in the remaining 11 infants.

Of the 313 study children, approximately one-half were female (48.6%; 95% CI, 42.9%-54.3%) and of black race (47.0%; 95% CI, 41.3%-52.7%). The racial makeup of the remaining population was 23.0% (95% CI, 18.5%-28.1%) non-Hispanic white, 25.6% (95% CI, 20.8%-30.8%) Hispanic white, 3.2% (95% CI, 1.5%-5.8%) multiracial, and 1.2% (95% CI, 0.35%-3.2%) Asian. Most infants (93.0%; 95% CI, 89.6%-95.5%) were from the well-baby nurseries and had public insurance or no insurance (80.8%; 95% CI, 76.0%-85.0%). The mean age at DBS sample collection was 2.29 ± 2.19 days.

Among the 313 infected infants, 90 DBS samples (28.8%; 95% CI, 23.8%-34.1%) were positive for CMV. DBS PCR was positive in 9 of 28 (32.1%) symptomatic infants, compared with 81 of 285 (25.9%) infants with asymptomatic cCMV at birth ($P = .70$) (**Figure 1**). To determine whether DBS PCR has a role in identifying infants with CMV-associated SNHL at birth, we compared the results of DBS PCR between infants with hearing loss at birth and those with normal hearing (**Table I**). DBS was positive in 12 of 26 (46%) infants with SNHL at birth, compared with 78 of 287 (27%) infants with normal hearing at