Newborn dried blood spot PCR to identify infants with congenital cytomegalovirus-associated sensorineural hearing loss

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Abstract

Objective—To determing the utility of dried blood spot (DBS) PCR in identifying infants with cytomegalovirus infection (CMV)-associated sensorineural hearing loss (SNHL).

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The authors declare no conflicts of interest.

Study design—Newborns at 7 U.S. hospitals from March 2007-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, positive and negative likelihood ratios of DBS PCR for identifying CMV-associated SNHL.

Results—DBS at birth was positive in 11/26 (42%) children with SNHL at 4 years of age and 72/270 (27%) children with normal hearing (p=0.11). 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 4 years of age. The positive and negative likelihood ratios of DBS PCR positivity to detect CMV-associated SNHL at 4 years of age 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 and 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 neither identifies the majority of CMV-infected newborns nor those with CMV-associated SNHL early in life.

Keywords

cytomegalovirus; dried blood spot; hearing loss

Cytomegalovirus (CMV) is an important cause of congenital infection and congenital CMV infection (cCMV) is a significant non-genetic 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 therefore, 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 (DBS) are collected routinely from all infants in the U.S. 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 when compared with saliva rapid culture[19] and therefore would not be a suitable screening method. Although DBS PCR failed to identify the majority of infants with cCMV, the possibility that it would detect infants at increased risk for CMV-associated SNHL remained. The present study is aimed at determining the ability of the DBS PCR assay to identify infants with cCMV at risk for disease and sequelae.

METHODS

Between March 2007 and March 2012, infants born at seven 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 carried out 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, 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 NIH definitions for race and ethnicity.[21] DBS 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/CNS abnormalities (e.g., microcephaly, seizures, focal or generalized neurologic deficits), or chorioretinitis. Clinical decisions about evaluation and antiviral treatment of the CMV-infected infants were made by the physicians at each study site and were not part of the CHIMES study. Seventeen infants were treated with antiviral therapy, with nine having SNHL at birth and none having 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 4 years of age.

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. Also, 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 Congenital CMV 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 per 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 audiological evaluation between 3 and 8 weeks of age, the study children were monitored for hearing loss using age and developmentally appropriate audiological testing protocols every 6 months during the first 4 years of life.[7]

Statistical Analyses

All statistical analyses were performed using SAS software, version 9.3 (SAS Institute). Two-tailed Fisher's 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 4 years of age by the Wilcoxon Rank-Sum test. Sensitivity, specificity, and predictive values for the DBS positivity were calculated using standard methods for proportions and exact 95% confidence limits. Likelihood ratios (LRs) were calculated to summarize the diagnostic accuracy of the DBS PCR. Positive LR was sensitivity/(1-specificity) and the negative LR was (1-sensitivity)/specificity. Confidence intervals (CIs) for LRs were determined using the method described by Simel et al.[23]

RESULTS

During the study period 100,332 infants were enrolled and screened for cCMV. Screening DBS samples were available from 313/391 (80.1%) infants with confirmed cCMV, and these infants constituted this study population (Figure 1; available at www.jpeds.com). Reasons for unavailability of DBS specimens included: the collection of DBS for the routine metabolic screening before study consent was obtained (72); insufficient blood for an additional study filter card (2); loss of DBS sample (3); and refusal by mother (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 half were female (48.6%, CI 42.9%–54.3%) and of black race (47.0%, CI 41.3%–52.7%). The racial make-up of the remaining population was 23.0% (CI 18.5%–28.1%) non-Hispanic white, 25.6% (CI 20.8%–30.8%) Hispanic white, 3.2% (CI 1.5%–5.8%) multi-racial and 1.2% (CI 0.35%–3.2%) Asian. Most infants (93.0%, CI 89.6%–95.5%) were from the well-baby nurseries and had public or no insurance (80.8%, CI 76.0%–85.0%). The mean age of DBS sample collection was 2.29 ± 2.19 days of life.

Among the 313 infected infants, 90 DBS samples (28.8%, CI 23.8%–34.1%) were positive for CMV. DBS PCR was positive in 9/28 (32.1%) symptomatic infants compared with 81/285 (25.9%) of infants with asymptomatic cCMV at birth (p=0.7, Figure 1). To determine whether DBS PCR has a role in identifying infants with CMV-associated SNHL at birth, the results of DBS PCR were compared between infants with hearing loss at birth and those with normal hearing (Table). DBS was positive in 12/26 (46%) infants with SNHL at birth compared with 78/287 (27%) children with normal hearing at birth (p=0.07). Among the 285 infants with asymptomatic cCMV, DBS PCR was positive in 6/15 (40%) children with SNHL at birth compared with 75/270 (28.0%, p=0.38) infants with normal hearing. For infants with symptomatic cCMV (n=28), DBS PCR was positive in 6/11 (55%) infants with hearing loss at birth, compared with 3/17 (18%) infants with normal hearing (p=0.10). The sensitivity and specificity of PCR of newborn DBS for detecting infants with SNHL at birth were 46.2% (95% CI, 26.6%–66.6%) and 72.8% (95% CI, 67.3%–77.9%), respectively (Table). The positive likelihood ratio (LR) for DBS positivity was 1.7 (95% CI, 1.1–2.7) and

the negative LR was 0.74 (95% CI, 0.5–1.1). The positive predictive value of the DBS PCR for detecting SNHL at birth was 13.3% (95% CI 7.1%–22.1%) and the negative predictive value was 93.7% (95% CI 89.7%–96.5%).

DBS at birth was positive in 11/26 (42%) children with SNHL at 4 years of age compared with 72/270 (27%) children with normal hearing (p=0.11, Table). Among children with asymptomatic infection (n=277), DBS obtained at birth was positive for CMV in 9/20 (45%) children who developed SNHL by 4 years of age compared with 70/257 (27%) with normal hearing (p=0.12). Among those with symptomatic infection, DBS PCR was positive for CMV in 2/6 (33.3%) of children with SNHL at 4 years of age compared with 2/13 (15.4%) of those with normal hearing (p=0.56). The sensitivity (42.3%, 95% CI 23.4%–63.1%) and specificity (73.3%, 95% CI 67.6%–78.5%) remain low for DBS PCR in identifying children with SNHL at 4 years of age (Table). The positive and negative likelihood ratios (LR) of DBS PCR positivity to detect CMV-associated SNHL at 4 years of age were 1.59 (95% CI, 0.97–2.6) and 0.79 (95% CI, 0.6–1.1), respectively. The positive and negative predictive values of the DBS PCR for detecting SNHL at 4 years of age were 13.3% (95% CI 6.8%–22.5%) and 93.0% (95% CI 88.7%–96.0%), respectively.

We also examined whether the viral load in DBS is predictive of symptomatic infection and CMV-associated SNHL. There was no difference in median DBS CMV viral load between infants with symptomatic infection (3.0×10^3 IU/ml, range 3.3×10^2–6.5×10^3) and those without clinical findings at birth (2.3×10^3 IU/ml, range 1.7×10^2–1.9×10^5; p=1.00, Figure 2). DBS viral load at birth did not differ between those children with SNHL at birth (3.1×10^3 IU/ml, range 8.3×10^2–1.9×10^5) and those with normal hearing (2.3×10^3 IU/ml, range 1.7×10^2–6.9×10^4, p=0.51) Similarly, the median DBS viral load was not different between the group of children who developed SNHL by 4 years of age (2.0×10^3 IU/ml, range 8.3×10^2–7.1×10^4) and those with normal hearing (2.5×10^3 IU/ml, range 1.7×10^2–6.9×10^4, p=0.44, Figure 2).

DISCUSSION

Our findings demonstrate that PCR testing of newborn DBS for CMV DNA has low sensitivity and specificity for identifying infants with CMV-associated hearing loss at birth as well as at 4 years of age. We have previously reported that our PCR method to detect CMV in DBS specimens has low sensitivity (~32%) for identifying infants with cCMV[19]. Although cCMV is an important non-genetic cause of SNHL in children, most infected infants are not recognized at birth. The identification of predictors of adverse outcome in cCMV are urgently needed, especially for infants with asymptomatic infection at increased risk for SNHL. The findings of the current study together with our previous study demonstrate that DBS PCR is not useful in identifying most congenitally infected infants nor does DBS PCR identify the majority of infants at risk for CMV-associated SNHL. Among infants with asymptomatic cCMV, DBS PCR failed to identify over half of the children who developed SNHL. Furthermore, a positive likelihood ratio close to 1 for both SNHL at birth and at 4 years indicates that a positive DBS result at birth has poor diagnostic accuracy for detecting hearing loss in cCMV. Negative LRs for DBS positivity at birth are not sufficiently

small enough to rule out the development of hearing loss in infants with a negative DBS at birth.

In an attempt to identify a marker for hearing outcome, studies have examined the relationship between blood viral load and SNHL in cCMV and reported conflicting findings.[24–27]. Most of these studies have performed PCR of whole blood to assess viral load, which may be different from DBS viral load. In a retrospective study, Walter et al examined the association between DBS viral load and hearing loss in 39 children with known cCMV. DBS viral load was higher among children with hearing loss (2.69 logs) than those with normal hearing (1.64 logs).[27] In contrast, the findings of our large prospective study that included 313 children with cCMV show that median viral load was not different between children with SNHL and those with normal hearing. Of note, in the Walter et al study, DBS PCR was negative in 11/39 infants with cCMV and 5 of the infants with negative DBS developed SNHL.[27] These data, along with the current findings, indicate that neither DBS positivity nor viral load levels are useful prognostic markers of hearing outcome in cCMV.

A limitation of this study is that DBS specimens were not available from all infants who tested positive for CMV on newborn screening. However, specimens from most (313/391, 80%) infants with confirmed cCMV including those with asymptomatic infection could be tested. In addition, there were no differences in the frequency of symptomatic infection and hearing loss in children with unavailable DBS compared with the study population. Therefore, unavailability of DBS from approximately 20% of infants is unlikely to have influenced the findings of the study. An additional limitation is that the study children were only monitored for hearing function and therefore, the association between DBS PCR assay and other long-term outcomes such as cognitive and motor deficits could not be determined. However, the frequency of cognitive and motor deficits among children with cCMV is significantly lower than hearing impairment, particularly in children with asymptomatic infection, which will require screening and follow-up of much larger number of children.

Another potential limitation is the sensitivity of our DBS PCR assay to detect cCMV, which is less than 40%.[19] The findings of our study show that the sensitivity of DBS PCR in identifying infants with CMV-associated SNHL at birth as well as at 4 years of age remain low (42%). It is possible that the sensitivity of the DBS PCR assay could be improved in the future by utilizing different DNA extraction protocols and by further optimizing the PCR assay. Such an improved DBS PCR method may have a better predictive value for identifying children at increased risk for CMV-associated SNHL. However, even if different extraction protocols or other assay enhancements improve DBS PCR detection in the laboratory, it is essential that these protocols to be evaluated in large screening cohorts of newborns to confirm the increased sensitivity.

In summary, the findings of our study showed the lack of utility of testing of newborn DBS specimens for the identification of infants with CMV-associated SNHL. These findings together with those from our previous report demonstrate that DBS PCR neither identifies the majority of CMV-infected newborns nor those with CMV-associated SNHL early in life.

Acknowledgments

Appendix

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Abbreviations

cCMV	congenital cytomegalovirus infection
SNHL	sensorineural hearing loss
DBS	dried blood spot

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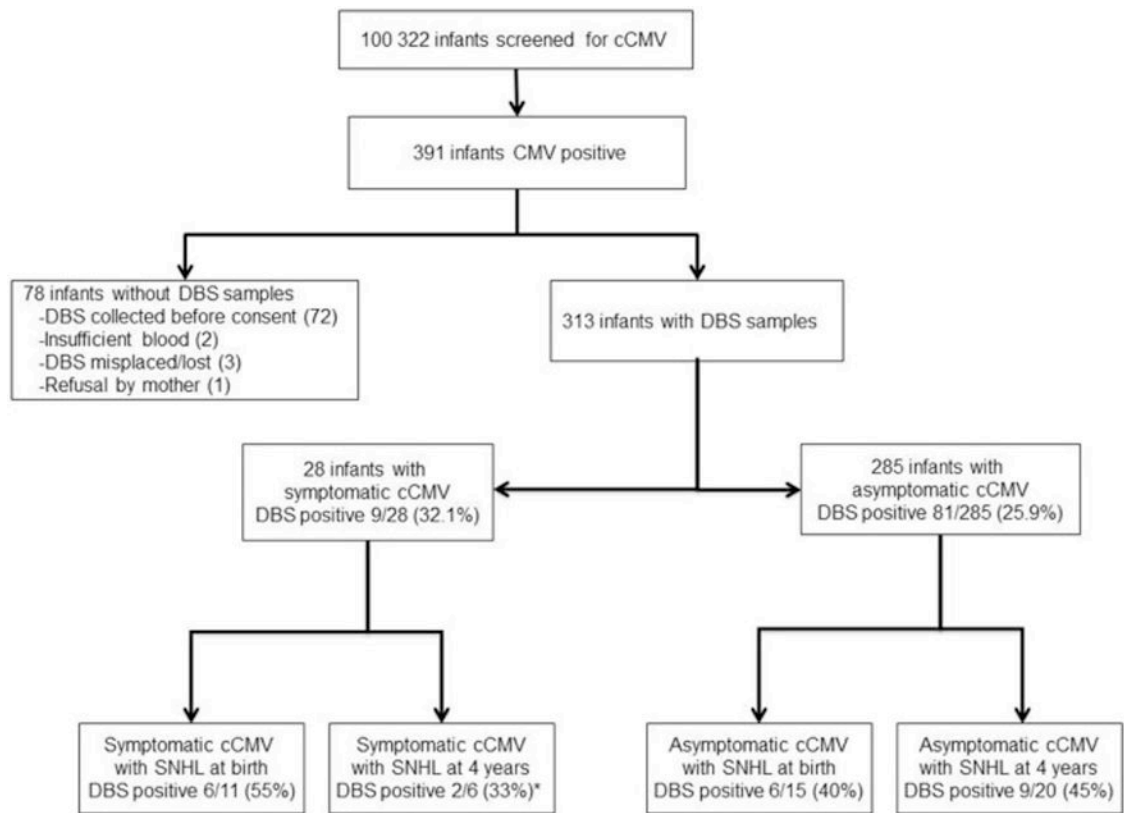
Figure 1.
Dried blood spot (DBS) PCR to identify infants with hearing loss in congenital CMV
infection. *Infants who received antiviral therapy were excluded from the number of infants
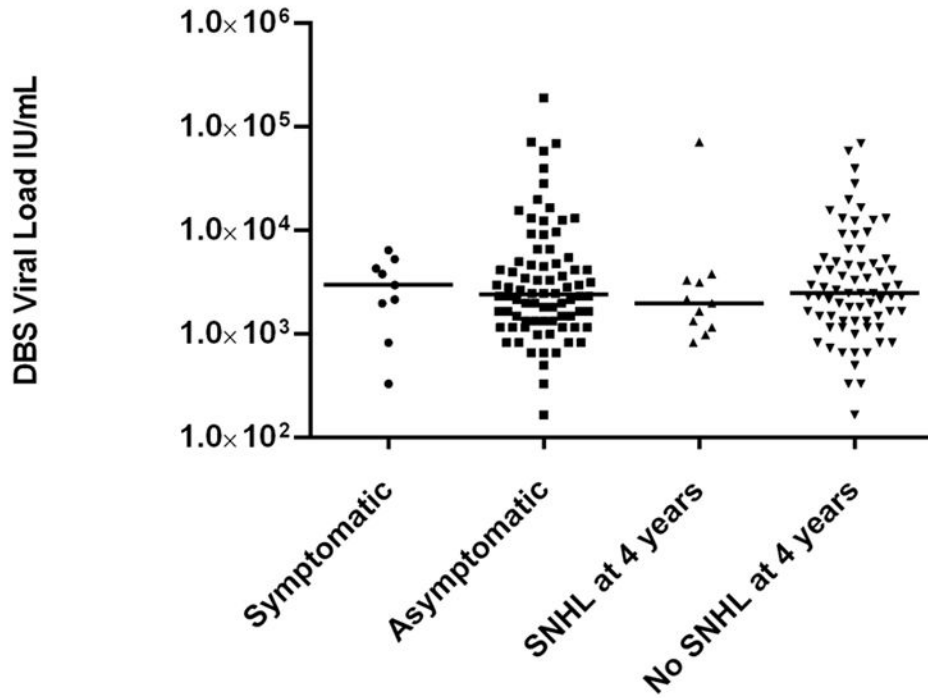with symptomatic cCMV who had hearing loss at 4 years of age.

Figure 2.
Dried blood spot (DBS) viral load levels at birth in infants with congenital CMV infection. Median viral load values do not differ between infants with symptomatic infection and asymptomatic infection, or between infants that develop SNHL and those with normal hearing.

Table 1

DBS PCR at birth to identify sensorineural hearing loss at birth and at 4 years of age in children with congenital CMV infection

	DBS obtained at birth						
SNHL at birth	Positive	Negative	Total	SNHL at 4 years *†	Positive	Negative	Total
Yes	12	14	26	Yes	11	15	26
No	78	209	287	No	72	198	270
Total	90	223	313	Total	83	213	296 *

Other analyses % (95% CI)		
Sensitivity	46.2 (26.6–66.6)	42.3 (23.4–63.1)
Specificity	72.8 (67.3–77.9)	73.3 (67.6–78.5)
Positive likelihood ratio	1.70 (1.08–2.68)	1.59 (1.97–2.59)
Negative likelihood ratio	0.74 (0.51–1.06)	0.79 (0.56–1.1)
Positive predictive value	13.3 (7.1–22.1)	13.3 (6.8–22.5)
Negative predictive value	93.7 (89.7–96.5)	93.0 (88.7–96.0)

*
17 infants treated with antiviral therapy were removed from the analysis

†
All children with SNHL at birth also had SNHL at 4 years of age