Performance of Dried Blood Spots for the Diagnosis of Congenital Cytomegalovirus in a Single Center: A Real-World Experience

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INTRODUCTION

- Congenital Cytomegalovirus (cCMV) is the most common congenital viral infection worldwide.
- cCMV can be difficult to diagnose given its lack of, or subtle symptoms at birth, as well as its strict diagnostic timing of the first 21 days of life.
- The newborn Dried Blood Spot (DBS) has been used for the retrospective diagnosis as well as a screening method for cCMV.
- We aimed to assess the sensitivity and specificity of this test compared to traditional methods, for the diagnosis of children suspected to have cCMV in the first 21 DOL.

METHODS

- In this single-center retrospective cohort study, all patients (N= 304) with a request for DBS testing between 2013 and 2021 were reviewed.
- Those with a DBS available for testing, a medical record at our institution, and with at least one diagnostic test for cCMV obtained within the first 21 days of life (DOL) available for review were included in the study (N=86).
- DBS were requested from the Texas State Health Department and sent to the Center for Disease Control for testing by DNA CMV PCR.
- DBS results were compared to patients whose diagnoses were made via traditional testing with at least one accepted method (urine culture, urine PCR, blood PCR and/or saliva culture) obtained within the first 21 DOL. Sensitivity, specificity, positive predictive value, and negative predictive value were calculated.
- Due to non normal distribution, viremia levels were compared with Wilcoxon Rank Sum test.
- Definitions, as published by Kimberlin and colleagues with minor emendations from discussions of the International cCMV Recommendations Group (*Lancet Infect Dis*. 2017; 17:e177-88):
  - Asymptomatic cCMV: no apparent abnormalities to suggest cCMV and normal hearing.
  - Asymptomatic cCMV with isolated SNHL: no apparent abnormalities to suggest cCMV, but SNHL present.
  - Mild symptomatic cCMV: one or two isolated manifestations of cCMV infection that are mild or transient (eg, mild hepatomegaly or a single measurement of low platelet count).
  - Moderate to severe symptomatic cCMV: multiple manifestations attributable to cCMV or CNS involvement such as microcephaly, radiographic abnormalities consistent with CMV CNS disease (ventriculomegaly, intracerebral calcifications, periventricular echogenicity, cortical or cerebellar manifestations), abnormal cerebrospinal fluid (CSF) indices for age, chorioretinitis, SNHL or the detection of CMV DNA in CSF.

Fig 1. Inclusion and exclusion criteria of study population

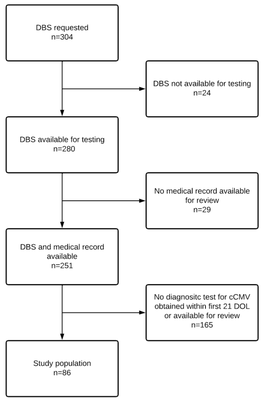


Table 1. Patient demographics

Characteristic	All patients, n=86	
Gender		
Male	41	49%
Female	45	41%
Ethnicity		
Hispanic	22	26%
Non-Hispanic	63	73%
Unknown	1	1%
Race		
White	60	70%
Asian	5	6%
Black/AA	19	22%
Other	2	2%
Gestational age (weeks)		
> 40	1	1%
37-40	66	77%
34-36	11	13%
<34	8	9%
Birth weight (gm)		
≥2500	49	57%
1500-2500	28	33%
<1500	6	7%
Unknown	3	3%
Maternal age		
≤20	16	19%
21-30	47	55%
31-40	22	25%
Unknown	1	1%
Reason for screening		
History of maternal infection	28	32%
Failed hearing screen	32	37%
History of IUGR	28	32%
Signs and symptoms suggestive of cCMV	55	64%
cCMV classification	n=59	
Asymptomatic at birth	9	15%
Asymptomatic with isolated hearing loss	4	7%
Mild disease	11	19%
Moderate/severe disease	35	59%

Fig 2. Sensitivity and Specificity of DBS when compared to patients with a diagnosis of cCMV by conventional testing

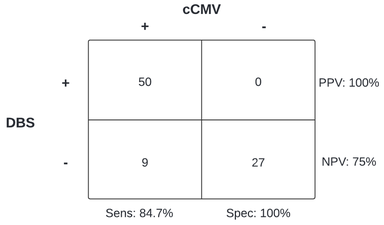


Fig 3. Patients with confirmed cCMV and SNHL

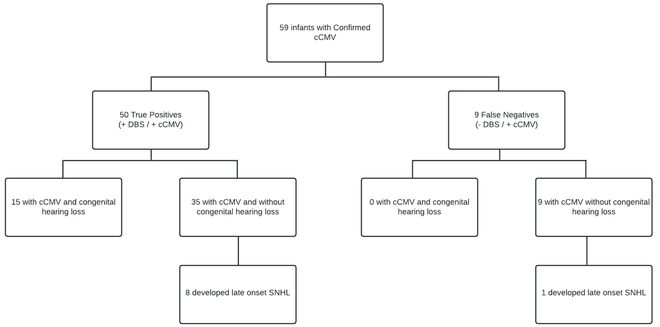
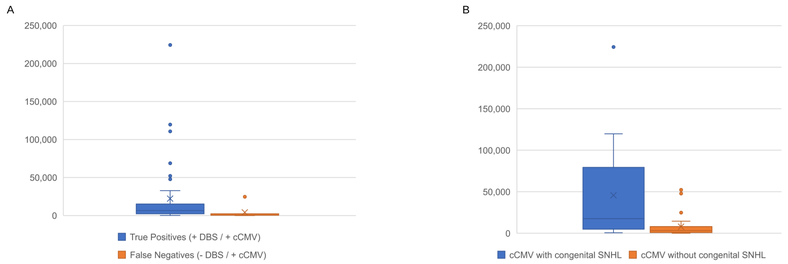


Table 2. Clinical characteristics of infants with a false negative result on DBS

Subject	DBS results	Congenital hearing loss?	Symptoms at birth?	Long term sequelae?	Confirmatory testing for cCMV	Blood PCR value (IU/ml)
38	N	No	M	No	UC, BP	<472
63	D (N/E)	No	M	No	UC, BP	<472
131	N	No	M/S	Learning disabilities, growth delays	UP, BP	2,400
142	D (E/N)	No	M/S	SNHL	UC, BP	24,717
162	N	No	A	No	UP	Neg
189	N	No	A	No	UP, BP	598
213	D (E/N)	No	M	No	UP	ND
221	D (E/N)	No	M	No	UP	ND
254	D (E/N)	No	M/S	Hypertonia and hyperreflexia	UP, BP	<472

E = equivocal, - = negative, + = positive, ND= not done, D=discordant
A= asymptomatic w/o hearing loss M= Mild disease, M/S= moderate to severe disease
UC= urine culture, UP= urine PCR, BP=blood PCR

Fig 4. Comparison of viremia levels (IU/ml) in patients with blood CMV qPCR obtained within first 21 DOL



(A) True positives (N=38) had a median viremia level of 6,487 IU/ml (range: 199-244,293 IU/ml) that was significantly higher when compared to those patients with cCMV and a false negative result (N=7) who had a median viremia level of 471 IU/ml (range: 0-24,717 IU/ml), p=0.012.
(B) Infants with cCMV with congenital SNHL (N=13) had a median viremia level of 17,547 IU/ml (range: 497-224,293 IU/ml) that was significantly higher when compared to those patients with cCMV without congenital SNHL (N=32) who had a median viremia level of 3,175 IU/ml (range: 0-52,220 IU/ml), p= 0.012.

RESULTS

- Of the 86 patients included, 59 had a diagnosis of cCMV established by conventional testing, with 50 of them having a positive DBS and 9 a negative result.
- DBS had a sensitivity of 84.7%, specificity of 100 %, positive predictive value of 100 % and a negative predictive value of 75%.
- Those patients with a true positive DBS had a higher median level of viremia by CMV blood PCR (6487 IU/ml vs 471 IU/ml, p= 0.012) when compared to those with a false negative result.
- All patients with cCMV and congenital hearing loss had a positive DBS (N=15).
- A significant difference was noted in the median viremia levels of those patients with cCMV with congenital hearing loss vs those with cCMV without congenital hearing loss (17,547 IU/ml vs 3,175 IU/ml, p=0.012).
- Of those cCMV patients that developed late onset hearing loss (N=9), 8 had a true positive DBS and 1 had a false negative result.

CONCLUSIONS

- DBS could be useful in the diagnosis of cCMV, and its performance may be related to the level of CMV viremia at birth.
- DBS appears to be able to accurately identify those patients with congenital hearing loss and those who will develop late onset hearing loss. Future studies are needed to determine the best population that can be identified by this test.