Dried Blood Spots and Universal Newborn Screening for Congenital Cytomegalovirus Infection

Robert F. Pass
Departments of Pediatrics and Microbiology, University of Alabama at Birmingham School of Medicine, Birmingham, Alabama

(See the article by Leruez-Ville et al, on pages 575–581.)

Newborns in the United States and in many other developed countries are routinely tested for inherited metabolic disease using blood samples collected in hospital nurseries. Drops of newborn blood, typically collected from a heel stick, are blotted within circles marked on filter paper. These dried blood spots (DBSs) are submitted to central laboratories for testing. Although the diagnosis of congenital cytomegalovirus (CMV) infection requires detection of virus in body fluids collected during the first 3 weeks of life, retrospective diagnosis has been made years after birth using polymerase chain reaction (PCR) to detect CMV DNA in stored newborn DBSs [1, 2]. The ability to make a diagnosis of congenital CMV infection from DBSs and the fact that the logistics of collecting these samples, routing them to central laboratories, and reporting results to caregivers are already in place suggested the possibility of implementing universal newborn screening for congenital CMV infection.

The American College of Medical Genetics (ACMG) established basic principles for defining criteria for newborn screening and selecting conditions for which screening should be mandatory or at least considered [3]. The ACMG reviewed 84 conditions including 3 infectious diseases (human immunodeficiency virus infection, congenital toxoplasmosis, and congenital CMV infection) and scored them on the basis of clinical characteristics, including potential for treatment, analytical characteristics of the screening test, and availability of resources to diagnose, treat, and provide care for screen-positive patients. Congenital CMV infection was ranked 72nd, with a score that indicated deficiencies in meeting several of the evaluation criteria. However, the ACMG did state, “Because of the limited involvement of infectious disease experts, the expert group chose to defer decision-making on infectious diseases” (page S298, reference 3). In their report, it was stated that, “to be included as a primary target condition in a newborn screening program, a condition should meet the following minimal criteria: it can be identified at a time (24–48 hours after birth) at which it would not ordinarily be detected clinically; a test with appropriate sensitivity and specificity is available for it; and there are demonstrated benefits of early detection, timely intervention and efficacious treatment of the condition” (page S298, reference 3).

The paper by Leruez-Ville et al [4] in this issue of Clinical Infectious Diseases proposes a screening test for congenital CMV infection that could use DBS and possibly be incorporated into the routine newborn screening program. An in-house real-time PCR assay based on amplification of conserved sequences from the CMV major immediate early co-transactivator (UL123) and a commercial PCR assay were used in parallel to test 10-mm dried blood spots from a group of newborns who had symptoms of congenital infection or were born to mothers with primary CMV infection—clearly a study population with a much higher pretest probability of a positive result than one would encounter in screening unselected newborns. The DBS PCR results compared very favorably with detection of virus in urine specimens using the same PCR assays, achieving sensitivity of 95%–100%, specificity of 98%–99%, positive predictive value of 94%–97%, and negative predictive value of 99%–100%. PCR testing of DBSs worked well as a diagnostic tool for symptomatic newborns or those born to mothers with primary CMV infection—clearly a study population with a much higher pretest probability of a positive result than one would encounter in screening unselected newborns. The DBS PCR results compared very favorably with detection of virus in urine specimens using the same PCR assays, achieving sensitivity of 95%–100%, specificity of 98%–99%, positive predictive value of 94%–97%, and negative predictive value of 99%–100%.

PCR testing of DBSs worked well as a diagnostic tool for symptomatic newborns or those born to mothers with primary CMV infection. However, it is not clear from these results that the same approach would be satisfactory for newborn screening. With the in-house assay, 4 of 207 DBS samples obtained from
uninfected newborns had positive results, yielding a rate of false-positive results of 1.9% (95% confidence interval [CI], 0%–3.8%); the commercial assay had a similar rate of false-positive results: 3 (1.5%) of 205 (95% CI, 0%–3.1%). A reasonable estimate of the prevalence of congenital CMV infection in newborns in Europe is 0.5% of live births [5]. Applying the results obtained by Leruez-Ville and colleagues to screening newborns in Europe for congenital CMV infection would yield 3–4 false-positive results for every true-positive result detected. All of the positive results would require confirmatory testing and counseling.

Only 1 study has attempted to screen newborns for CMV infection using DBS on a scale that approaches that required for universal newborn screening. Boppana et al [6] tested 20,442 newborns, comparing results with PCR of 6-mm DBS to rapid virus culture of saliva samples (fluorescent focus assay using monoclonal antibody to immediate early antigen) as the reference standard, and reported sensitivity 28.3% and 34.4%, respectively, for single- and double-primer PCR assays. Even considering differences in study populations and size, it is clear that both the in-house and commercial assays performed in the Leruez-Ville et al [4] laboratory were much better in terms of sensitivity. The study by Boppana and colleagues reported 92 confirmed CMV infections, leaving 20,350 uninfected newborns. If one extrapolates from the results of Leruez-Ville and colleagues with a single positive duplicate on the in-house assay (sensitivity, 100%; specificity, 98.1%), all 92 confirmed cases would have been identified, and 388 uninfected newborns would have screened positive.

If untreated congenital CMV infection inevitably led to disability or death, and if a highly effective treatment was available, a relatively high ratio of false-positive/true-positive newborn screens might be acceptable. However, that is not the case. Approximately 80% of newborns with congenital CMV infection do not have symptoms at birth and have no sequelae. At the present time, treatment is reserved for newborns who have symptomatic congenital CMV infection; they are detected clinically and can be confirmed by available diagnostic tests. The symptomatic newborns appear to benefit from antiviral treatment with better hearing and developmental outcomes, but treatment is not curative and does not prevent all disability [7, 8].

In addition to these concerns, adapting the diagnostic PCR assays used to detect CMV in DBS samples for screening of newborns will be a significant obstacle. State laboratories now use tandem mass spectrometry to test for all 29 disorders that the ACMG has recommended for universal newborn screening. These laboratories typically use integrated systems of robotic equipment which sample DBS (usually a 3-mm punch), process samples, perform tests, and report results. Although PCR is certainly amenable to automation, it is not the platform being used routinely by laboratories to test newborn DBSs. Some of the specific procedures used by Leruez-Ville et al [4], such as cutting each 10-mm DBS into 10 pieces, would likely have to be modified to adapt to available high-throughput equipment.

It is not clear that any test for congenital CMV infection of suitable sensitivity and specificity is currently available for DBS. However, it is quite possible that the assays that have shown good sensitivity could be adapted for mass screening using DBS, with the caveat that near perfect specificity is desired. Transitioning to mass screening will require technical modifications in test procedures which could affect sensitivity and specificity. Other body fluids, such as saliva or urine samples, may be superior to blood samples for screening, because the amount of virus in these fluids is consistently higher. Collecting urine or saliva specimens on filter paper could ultimately be a more effective approach to screening for congenital CMV infection than use of DBSs. In addition, the benefit of early detection, intervention for and treatment of cases that are not clinically apparent needs to be better defined if arguments for universal newborn screening are to be persuasive.

Although the case for screening all newborns for congenital CMV infection is not convincing at this time, newborn screening could be a valuable public health tool right now. Sentinel screening of newborns for congenital CMV infection in selected hospitals or geographic areas would allow more accurate definition of the burden of disease and would provide the ability to monitor the impact of large scale preventive measures, such as a vaccine or health education of young women on steps to reduce exposure risk. A sentinel screening program would also provide an excellent opportunity to evaluate and optimize screening methods, whether they use DBS or other samples.

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**References**


