EDITORIAL

Newborn Dried Blood Spot Testing for Congenital Cytomegalovirus Screening
The Little Engine That Could

Gail J. Demmler-Harrison, MD

In the article by Dollard et al,[1] the preliminary results of a sentinel newborn screening program for congenital cytomegalovirus (CMV) comparing detection of CMV DNA in newborn dried blood spots (NBDBS) using enhanced, more sensitive DNA extraction and polymerase chain reaction (PCR) methods are reported. They compared NBDBS testing with CMV DNA detection in saliva swabs in a newborn screening study involving more than 12 000 newborns of a projected 25 000 enrolled in a multicenter Minnesota study. In this study, Dollard et al[1] demonstrate a prevalence of 0.45% of congenital CMV and show high analytical sensitivity (combined: sensitivity, 85.7%; 95% CI, 74.3%-92.6%; University of Minnesota laboratory: sensitivity, 73.2%; 95% CI, 60.4%-83.0%; US Centers for Disease Control and Prevention laboratory: sensitivity, 76.8%; 95% CI, 64.2%-85.9%) for NBDBS CMV DNA detection. Prior studies involving retrospective diagnosis of symptomatic newborns have shown a similar sensitivity of 62% to 95%, but when NBDBS were used for large-scale, prospective newborn screening for congenital CMV, similar to the study by Dollard et al,[1] sensitivities as low as 37% were reported.[2,3] By using enhanced PCR methods, Dollard et al[1] have rekindled the hope that NBDBS testing may be a viable method for large-scale, universal newborn screening for congenital CMV.

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Congenital CMV is a common congenital infection, infecting a mean 0.4% to 0.6% (range, 0.2%-2.2%) of all live births, making it a global public health issue.[4] The prevalence of this congenital infection varies with maternal age, demographic characteristics of the population, and geographical location of the births. However, accurate prevalence numbers elude public health officials because not all newborns are tested for congenital CMV.

The diagnosis of congenital CMV involves 3 aspects: timing, sample, and methodology. The time to diagnose congenital CMV is the first 21 to 28 days of life. When CMV tests are done past this critical window of opportunity, CMV acquisition from other sources, such as maternal breast milk, transfusions, and person-to-person transmission, come into play. The samples used to detect CMV in newborns may be urine, which is the recognized reference standard; saliva, which is a more easily obtained sample; and blood, including NBDBS testing. Contemporary methods for CMV detection in these samples involves a variety of constantly evolving DNA detection methods, and this is where the article by Dollard et al[1] shows us the devil is in the details when it comes to successful CMV DNA detection methodology.[4]

Testing for congenital CMV also involves 3 strategies: diagnostic testing, targeted testing, and universal newborn screening. Diagnostic testing of newborns who exhibit 1 or more signs or symptoms associated with congenital CMV is the here and now. However, even some of these newborns elude diagnosis and experience diagnostic odysseys before they are identified. Healthy newborns who failed on their newborn hearing screening or require further testing may have congenital CMV as a cause or contributor to their congenital hearing loss. Targeted testing is an evolving process, and many large birthing hospitals and a few states in the US and Canada as well as several European countries routinely test these newborns for congenital CMV before they leave their birth hospitals.[5-7] Universal newborn screening for congenital CMV involves testing all newborns across the board for congenital CMV and is a lofty goal for the future, and the future is now.[8]

Universal newborn screening will allow more accurate definition of the burden of congenital CMV infection and disease, allow us to monitor trends over time, and allow us to monitor the impact of future preventive measures, such as a CMV vaccine, and currently available health education measures, also known as the CMV knowledge vaccine, which seeks to raise CMV awareness and asks women to modify behaviors to reduce CMV acquisition and transmission during pregnancy.[8] In addition, universal screening would detect all symptomatic and asymptomatic newborns with congenital CMV, allow the accurate and timely identification of these newborns, allow appropriate monitoring for late-onset sequelae, especially sensorineural hearing loss, and allow effective antiviral and functional therapy interventions, which will improve outcomes in these children. However, opponents of universal newborn screening raise concerns of identifying a large number of newborns who are asymptomatic and who will never experience sequelae, which is actually most newborns with congenital CMV, worrying parents unnecessarily, incorrectly identifying vulnerable children, and burdening the health care system.

For newborns to be screened universally for congenital CMV in the US, a good and probably essential first move is to have it included on the recommended universal screening panel (RUSP). To be included on the RUSP, a condition must meet the following criteria: (1) it can be identified at birth, (2) a test with appropriate sensitivity and specificity is available, and (3) there are demonstrated benefits of early detection that

include timely interventions and effective treatment for the condition.[9] Most CMV experts believe congenital CMV currently meets these criteria and supported its resubmission for reconsideration in March 2019, which is under evaluation and review at this time.[10] We must be ready if CMV passes the RUSP inspection.

Congenital CMV can be, and ideally must be, identified at birth or within the first days to weeks of life, and there are available interventions that, while not curing congenital CMV, have been shown in randomized clinical trials to improve outcomes.[11] However, consensus is still lacking on the best sample to use for universal newborn screening for congenital CMV. Newborns with congenital CMV often, but not always, have CMV viremia at a level detectable by current methods. In some studies, symptomatic and asymptomatic newborns at risk of sequelae, such as hearing loss, may have higher levels of viremia than those who do not experience sequelae.[12,13] These findings beg the question, then, of how sensitive must the NBDBS process be to be acceptable for universal screening. Is the current sensitivity adequate if it detects at-risk newborns and reprieves the ones who are destined to escape sequelae? Long-term follow-up studies of newborns identified with congenital CMV infection by prospective NBDBS testing may be able to answer this question.

Urine and saliva samples usually contain large quantities of virus and are totally appropriate for diagnostic testing for symptomatic newborns suspected of having congenital CMV and for targeted testing of healthy newborns who fail on their newborn hearing screening or require further testing. And because they contain large quantities of virus, they have also been proposed as samples appropriate for universal screening.[2,14] However, the use of these samples for universal newborn screening would entail a totally new sample collection and testing platform for universal screening. The routine of collecting the NBDBS samples on all newborns and the logistics of routing them to central laboratories and then reporting results to caregivers is already in place and are strengths of NBDBS samples for universal newborn screening. However, proponents of the use of NBDBS samples have suffered until recently from a relatively insensitive platform compared with saliva and urine testing. The results in the study by Dollard et al[1] may be a total game changer for the NBDBS proponents. Furthermore, scientists who have adapted even more sensitive DNA detection assays, such as the loop-mediated isothermal assay for detection of DNA in clinical samples from newborns, may be able to adapt loop-mediated isothermal assay methodology to detect CMV DNA in NBDBS.[14]

Sampling using NBDBS was first introduced by Robert Guthrie, MD, PhD, in 1963 to detect and treat phenylketonuria, and in 1994, the first CMV research scientists adapted NBDBS to diagnose congenital CMV.[3,4] The research scientists who continue to improve and perfect the methodology to detect CMV DNA in NBDBS are akin to the "I think I can" little blue engine in the children's story, "The Little Engine That Could," which teaches the value of optimism and hard work.[15] By adapting the collection methods, by using optimal filter paper to enhance DNA adherence, by improving DNA elution procedures, and by developing novel amplification and detection methods, NBDBS may soon meet the challenge and reach the sensitivity and specificity necessary for universal screening for congenital CMV. The little engine that could would then reach the top of the hill and slide down the other side of the hill of success, positively stating "I thought I could," delivering the goods and benefitting all the children born with congenital CMV as well as the parents and health care workers who care for them.

ARTICLE INFORMATION

Author Affiliation: Department of Pediatrics, Section of Infectious Diseases, Baylor College of Medicine and Infectious Diseases Service, Texas Children's Hospital, Houston.

Corresponding Author: Gail J. Demmler-Harrison, MD, Department of Pediatrics, Section of Infectious Diseases, Baylor College of Medicine and Infectious Diseases Service, Texas Children's Hospital, 1102 Bates St, Feigin Tower, Ste 1150, Houston, TX 77030 (gdemmler@bcm.edu).

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