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Neonates with congenital Cytomegalovirus and hearing loss identified via the universal newborn hearing screening program☆

William D. Rawlinson[a,b,1,*], Pamela Palasanthiran[c,1], Beverly Hall[a], Laila Al Yazidi[c], Michael J. Cannon[e], Carolyn Cottier[d], Wendy J. van Zuylen[a,f], Monica Wilkinson[g]

[a] Serology and Virology Division, NSW Health Pathology, Prince of Wales Hospital, Sydney, NSW, Australia
[b] School of Medical Sciences & School of Biotechnology and Biomolecular Sciences, University of New South Wales, Sydney, NSW, Australia
[c] Department of Immunology and Infectious Diseases, Sydney Children's Hospital & School of Women's and Child Health, University of New South Wales, Sydney, NSW, Australia
[d] Hearing Support Services, Sydney Children's Hospital, Sydney, NSW, Australia
[e] National Center on Birth Defects and Developmental Disabilities, US Centers for Disease Control and Prevention, Centers for Disease Control and Prevention, GA, USA
[f] School of Medical Sciences, University of New South Wales, Sydney, NSW, Australia
[g] Audiology Department, Sydney Children's Hospital, Sydney, NSW, Australia

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ABSTRACT

Background: Congenital cytomegalovirus (CMV) is the most common non-genetic cause of sensorineural hearing loss. Currently, there are no universal CMV screening programs for newborns or routine CMV testing of neonates with hearing loss in Australia, or elsewhere.

Objectives: This study was undertaken to determine the prevalence of congenital CMV infection in infants with hearing loss identified using routine resources via the Australian universal neonatal hearing screening (UNHS) program.

Study design: Infants who failed UNHS, referred for audiological testing and found to have permanent hearing loss were screened for CMV via PCR of urine and saliva. Congenital CMV was diagnosed if CMV was detected in infants ≤ 30 days of age, or using retrospective testing on stored new born screening cards, retrospective testing, or using clinical criteria if > 30 days of age. The cohort was analyzed for time of testing and prevalence of congenital CMV determined.

Results: The Audiology Department reviewed 1669 infants who failed UNHS between 2009 and 2016. Thirty percent (502/1669) had permanent hearing loss confirmed, of whom 336/502 were offered CMV testing. A definite (n = 11) or probable (n = 8) diagnosis of congenital CMV occurred in 19/323 (5.9%), of whom definite diagnoses were made in 4/19 on tests positive prior to 21 days of life, in 5/19 who were positive on neonatal blood screening card (NBSC) testing, in 2/19 who were positive on placental testing. In 8/19 probable diagnoses were made based on positive testing between ages 23–42 days and a consistent clinical syndrome in the absence of another cause for hearing loss after genetic and other testing. CMV testing mirrored the timing of audiological testing, with ~40% completing audiology and CMV testing by 21 days, and 64% by 30 days.

Conclusion: This program, utilizing existing clinical services identified probable congenital CMV in ~6% of a large cohort failing UNHS with permanent hearing loss, of whom more than half were definite diagnoses. No additional assets were required to those already existing in this tertiary referral pediatric centre, whilst providing useful and timely data for clinical and audiological management.

1. Background

Congenital CMV is the most common non-genetic cause of fetal malformation in the developed world [1–3]. It is estimated that each year ~380 neonates in Australia and ~3600 neonates in the USA, develop disease due to congenital CMV infection. The complications of congenital CMV infection usually extend into adulthood, causing life-long disabilities, including sensorineural hearing loss (SNHL) and

Abbreviations: CMV, Cytomegalovirus; PCR, Polymerase Chain Reaction; UNHS, Universal Neonatal Hearing Screening; NBSC, newborn screening card

☆ The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

* Corresponding author at: Serology and Virology Division, NSW Health Pathology, Level 4 Campus Centre, Prince of Wales Hospital, Randwick, NSW, 2031, Australia.
E-mail address: w.rawlinson@unsw.edu.au (W.D. Rawlinson).

[1] Joint first authors.

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neurodevelopmental disability [4–6]. Approximately 10% of congenital CMV-infected neonates are born with SNHL, whereas many (~15%) initially asymptomatic CMV-infected neonates develop hearing loss postnatally before the age of five years [1,7–10].

There are no universal CMV screening programs for newborns or routine CMV testing programs of neonates with hearing loss in Australia. This means a diagnosis of congenital CMV infection currently depends largely on clinical suspicion, leading to diagnosis of only a small proportion of symptomatic congenital CMV infected neonates, and essentially no asymptomatically infected newborns [1,11]. Although universal neonatal hearing screening (UNHS) is offered in Australia, achieving an estimated national coverage rate of ~96%, there is no national standard for the etiological investigation of neonates with confirmed hearing loss. However, detecting congenital CMV in the newborn period could avoid unnecessary diagnostic tests, provide parents with anticipatory guidance for care of their neonate, and provide a cause for the hearing loss [12]. Congenitally infected neonates with moderate to severe CMV disease may further benefit from antiviral therapy, as treatment with the antiviral drug valganciclovir (ValGCV) for six months results in improved hearing and developmental outcomes, as reported in a recently completed double blinded randomized controlled trial [13].

A definitive diagnosis of congenital CMV infection in neonates is made using real-time PCR of saliva and/or urine during the first three weeks of life [14]. If CMV is detected later in life, then postnatal CMV infection cannot be excluded, except by testing blood spots from retrieved neonatal blood screening cards (NBSC or Guthrie's cards), where these are available. However, a diagnosis of possible or probable congenital CMV can be made later if appropriate symptoms and signs are present, with suggestive laboratory findings. This allows appropriate clinical followup in infants with possible/probable disease, negative NBSC card tests (possibly resulting from low sensitivity of testing), and late presentation [7]. This study aimed to use existing resources to determine the prevalence of congenital CMV infection in infants with hearing loss identified via the Australian UNHS program, and report clinical features in the subset of infected infants.

2. Methods

2.1. Study design and population

Between October 2009 and October 2016, infants were prospectively accrued in order of the date of their presentation for audiological testing at the Audiology Department at the Sydney Children's Hospital (SCH), following failed newborn hearing screen using automated auditory brainstem response testing (AABR). The audiologists assessed children using diagnostic auditory brainstem response testing (ABR) to assess hearing level, as well as otoacoustic emissions (OAE) to assess the cochleae, and tympanometry to assess middle ear function. SNHL was determined by a threshold of hearing greater than 20 dB for both air and bone conduction using ABR testing. Children with any degree of permanent hearing loss were tested for CMV and other aetiologies of hearing loss using standard protocols. The SCH is a tertiary referral pediatric hospital in Sydney, Australia and the Audiology Department at SCH is responsible for approximately one-third of all universal hearing screening undertaken in the South-Eastern region of New South Wales (NSW) (~32,000 children annually) and conducts all testing on infants born who fail initial UNHS. In order to improve diagnostic services appropriate to the needs of the Audiology Department at SCH, the South Eastern Area Laboratory Services (SEALS) commenced testing urine, saliva and blood spots from NBSC samples using cytomegalovirus polymerase chain reaction (CMV PCR) in 2009. A collaborative multidisciplinary team of audiologists, hearing specialists, infectious disease physicians and the pathology service developed the enhanced diagnostic care clinical algorithm for infants with hearing loss to be tested for CMV in a timely manner, to determine congenitally infected infants and clinical follow-up. This algorithm was reviewed annually. According to the algorithm, infants ≤30 days who did not pass the Auditory Brainstem Response (ABR) test, were recommended CMV PCR testing of urine and saliva. Infants > 30 days were recommended urine and saliva testing for CMV and, if positive, retrospective testing for CMV was performed on stored NBSC samples, where informed consent and access to NBSC were available. Clinical features and/or testing of relevant samples for diagnosis of congenital CMV, e.g. placental CMV studies as requested by attending physicians.

Audiologists informed the team whenever a child had CMV testing done, allowing the Infectious Diseases Service to follow-up. Urine and saliva CMV PCR test results were available within 72 h and checked in that time period. Parents were also given written information about the CMV testing and follow-up, at the time of Audiology appointment. Infants ≤30 days in whom CMV was identified were seen urgently by the Infectious Disease service. All infants were reviewed subsequently by the Hearing Support Service (HSS) for counselling and management, including testing for other etiologies for congenital hearing loss that included brain Magnetic Resonance Imaging (MRI) with petrous temporal views.

All infants who failed UNHS and had audiological confirmation of neurological hearing loss were included in this study. Newborns identified as congenitally infected with CMV prior to attending hearing screening were not included in this study, in order to establish the contribution of CMV in the etiology of hearing loss among infants not previously diagnosed as congenitally infected, and via our system of identification and testing. This study also excluded infants already known or suspected to have congenital CMV prior to UNHS, such as newborns diagnosed *in utero* after known primary maternal CMV infection.

2.2. Samples and CMV testing

Between October 2009 and October 2011 only urine samples were tested for CMV. Between October 2011 and October 2016, urine and saliva swabs were tested following refinement of saliva swab testing methods [15]. Saliva samples were taken at least an hour after last breast feed, to avoid possible contamination with residual CMV breast milk. Specimens were processed using established, accredited diagnostic laboratory protocols [16]. In brief, extraction of DNA was performed using MagNA Pure semi-automated machines (Roche, Australia), with qualitative testing for CMV using PCR of urine and saliva [11]. Testing of NBSC samples was conducted according to developed protocols [17,18].

2.3. Analysis

Infants were analyzed by age groups ≤30 days and > 30 days at CMV testing, to enable the distinction between the congenitally infected infants who would have been identified in a timely manner for urgent clinical follow-up and counselling as per the window of treatment opportunity.[14] The age cut off of ≤30 days was also used in this study as this is the age limit where antiviral therapy has been previously investigated and used for treatment [19,20].

Infants ≤30 days of age with SNHL were diagnosed with congenital CMV, if CMV was detected in urine and/or saliva within the first 3 weeks of life, or through newborn screen testing, and diagnosed with probable congenital CMV infection if the CMV PCR detected CMV between 22 and 30 days of life.

Infants > 30 days of age with SNHL were deemed to have congenital CMV if i) CMV was detected via NBSC testing, or ii) CMV was detected via NBSC testing and in urine and/or saliva, or ii) CMV was detected in urine and/or saliva with clinically consistent features of congenital CMV or another laboratory test supporting congenital CMV, e.g. detection of CMV in the placenta. It is acknowledged this allowed sensitive detection of congenital CMV, with loss of some specificity, due