Hearing Screening-Driven Investigation of Newborns for Congenital Cytomegalovirus Infection at a German University Hospital

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Posted Date: 7 May 2026

doi: 10.20944/preprints202605.0404.v1

Keywords: congenital CMV; newborn hearing screening; hearing impairment; cCMV-screening

 doi:10.20944/preprints202605.0404.v1

Article

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Abstract

Congenital Cytomegalovirus (cCMV) infection is the leading non-genetic cause of sensorineural hearing loss in newborns. Systematic nationwide screening programs are lacking. Antiviral valganciclovir therapy could improve auditory outcomes if initiated within the first 30 days of life, making timely diagnosis crucial. To address this, we investigated whether a hearing screening-based protocol is suitable. Between 2015 and 2019, newborns ≤21 days of age with repeated abnormal Newborn Hearing Screening (NHS) were prospectively enrolled at University Hospital Frankfurt. Oral mucosal swabs were tested for CMV DNA by real-time PCR, with confirmatory urine and blood-diagnostics in positive cases. Of 2,741 infants presenting for repeat NHS, 2,059 (75.1%) showed normal bilateral findings. Of the 682 (24.9%) with abnormal results, 575 (84.3%) were >21 days and thus ineligible. 107 infants (3.9%) met both criteria—abnormal NHS and age ≤21 days—of whom 100 entered per-protocol analysis. Two (2%) were confirmed cCMV-positive and received valganciclovir. Among the 48 infants who additionally underwent DBS testing, diagnostic sensitivity and specificity were 100%. The presented NHS–driven cCMV protocol reliably identified cCMV-infected newborns timely to offer antiviral therapy. In the absence of universal cCMV screening, this targeted approach offers a challenging, but WHO-screening-criteria-compliant strategy to enable timely antiviral intervention.

Keywords: congenital CMV; newborn hearing screening; hearing impairment; cCMV-screening

1. Introduction

Human cytomegalovirus (CMV), also known as Human Herpesvirus 5, is an enveloped double-stranded DNA virus that was independently discovered in 1956 and 1957 [1,2]. It is the most common congenital infection in developed countries, affecting 0.2–0.6% of live births in Germany and approximately 7 per 1,000 births worldwide [3,4]. CMV persists lifelong in lymphocytes and other

 doi:10.20944/preprints202605.0404.v1

cell populations, with seroprevalence rates of up to 60% in industrialized nations and nearly 100% in developing countries, mostly dependent on socioeconomic status [5,6].

Congenital CMV (cCMV) infection results from vertical transmission during pregnancy, with 14-52% of newborns from mothers with primary infection during pregnancy becoming intrauterine infected, depending on the time of primary infection, age and socio-economic status of the women [7,8]. In Germany, the CMV transmission rate is 40% relating to the whole pregnancy [9]. Reinfections with different strains or reactivations (10-20% of CMV-positive mothers) show much lower transmission rates of up to 2% [10,11]. Although 90% of congenitally infected children are initially clinically asymptomatic, CMV causes significant morbidity and mortality [6]. Severe manifestations include microcephaly, intracranial calcifications, hepatosplenomegaly, and intrauterine growth restriction. Most notably, sensorineural hearing loss (SNHL) is the most common sequela, occurring in 5–20% of asymptomatically infected infants and 30–65% of symptomatic cases at birth [12–15].

CCMV diagnosis relies primarily on polymerase chain reaction (PCR) amplification of CMV DNA from urine within the first 21 days of life. Oral secretions are easier to take and can help to identify newborns with CMV-infection, but congenital has to be distinguished from postnatal infection, e.g., transmitted through breast-milk [16,17]. Beyond three weeks of life, congenital infection may still be diagnosed retrospectively via CMV PCR from dried blood spot (DBS) cards. Serological testing for CMV-specific IgG and IgM antibodies and viral culture on fibroblasts are alternative diagnostic methods. Ganciclovir and its oral prodrug valganciclovir are the mainstay antivirals for symptomatic congenital CMV disease, with evidence of improved long-term hearing preservation and neurodevelopmental outcomes, and could be started as off-label therapy within the first month of life [18–20].

Despite the substantial clinical burden of congenital CMV-associated hearing loss, no CMV active vaccine is currently available for prevention. Notably, the United States has implemented hearing-screen driven cCMV screening programs since 2013 (Utah) and 2016 (Connecticut), identifying affected infants who may benefit from early antiviral intervention [21]. Since July 1, 2025, DBS testing for cCMV has become mandatory for all newborns born in Connecticut [22]. Germany currently lacks such a systematic screening program.

This study aims to detect cCMV infection in infants with repeated newborn hearing screening (NHS) failures and to evaluate whether protocol-based screening – using transiently evoked otoacoustic emissions (TEOAE) and automated auditory brainstem response (AABR), followed by laboratory cCMV confirmation – can identify symptomatically infected newborns eligible for antiviral therapy in a timely manner.

2. Patients and Methods

2.1. Patients

Eligible participants included newborns aged ≤3 weeks who attended the Department of Paediatric Audiology at University Hospital Frankfurt between 3rd August 2015 and 31st December 2019 due to an initial failed hearing screening via TEOAE or AABR, and who demonstrated a further abnormal result on repeat hearing screening. Written informed consent was obtained from parents or legal guardians. Exclusion criteria were age >3 weeks, normal findings at hearing screening, and a known or previously excluded cCMV infection.

For enrolled infants, a buccal swab for CMV DNA testing was obtained by rotating a sterile cotton swab in each buccal pouch for approximately 3 seconds. To minimise contamination with CMV-containing breast milk, sampling was performed at least 30 minutes after the last milk feeding [23]. Swabs were analysed by CMV PCR at the Institute for Medical Virology, University Hospital Frankfurt.

Newborns with a positive CMV PCR result underwent confirmatory diagnostics, including clinical examination (length, weight, head circumference), cranial ultrasound, CMV PCR and CMV

 doi:10.20944/preprints202605.0404.v1

virus isolation and culture from urine, and blood tests (complete blood count, clinical chemistry [AST, ALT, GLDH, total and direct bilirubin, creatinine, urea], CMV serology, and CMV PCR).

With parental consent, the DBS card was requested from the regional screening centre. If still available (i.e., not destroyed according to national regulations), the dried blood spots (DBS) were tested for CMV DNA by PCR at the German consultation laboratory for congenital CMV infection in the virology department of University Hospital Tuebingen, Germany.

The following data were collected for all enrolled infants: name, date of birth, dates and results of hearing screening and buccal swab, any known CMV status, CMV PCR result from the buccal swab, results of confirmatory diagnostics, and CMV PCR result from the DBS card.

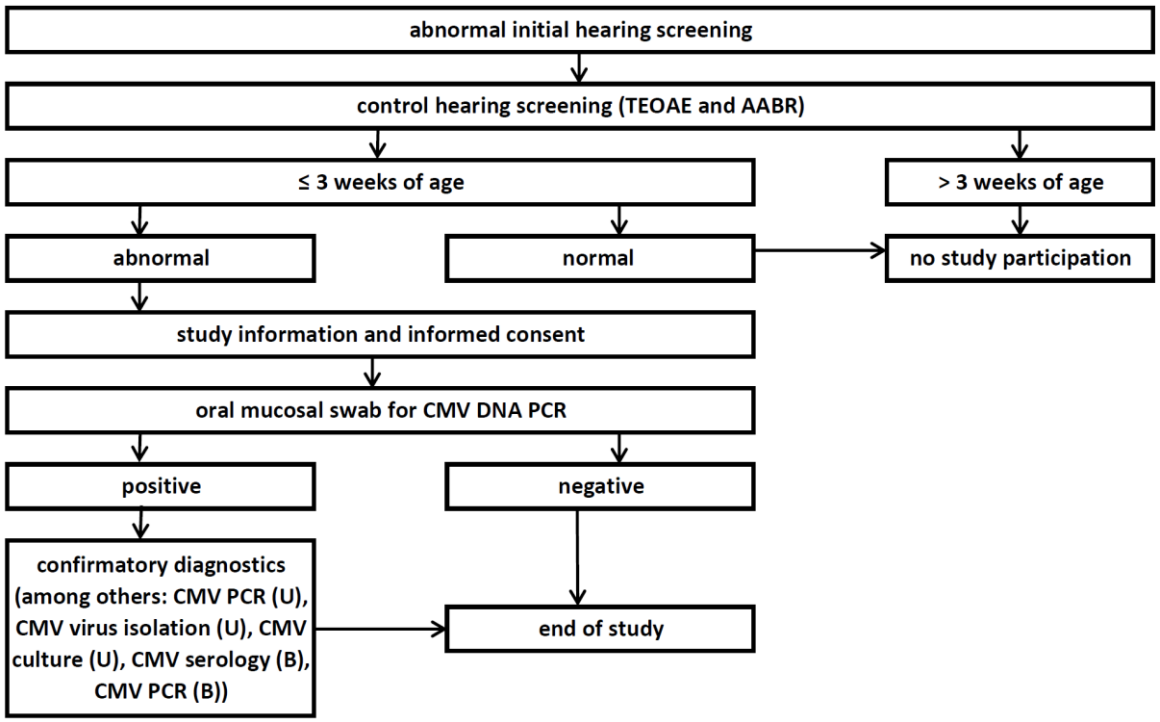

Figure 1. Study flow-chart (U = Urine; B = Blood).

2.2. Transiently Evoked Otoacoustic Emissions (TEOAE)

TEOAEs are low-level sounds emitted by outer hair cells in the cochlea in response to acoustic stimuli. These emissions are captured by a sensitive microphone in the ear canal. They typically cover the main speech frequency range from 0.5 to 4 kHz. The presence of TEOAEs indicates that the outer hair cells of the cochlea are functioning correctly and that the middle ear is clear, which is essential for normal sound amplification. Since these emissions generally disappear when a hearing loss exceeds 30 to 35 dB HL, their positive detection suggests that hearing thresholds are within a normal or near-normal range.

TEOAE were recorded with the Echo-Screen TDA system (Fischer-Zoth Diagnosesysteme GmbH; distribution: Mack Medizintechnik GmbH, Pfaffenhofen/Ilm, Germany) at the Department of Paediatric Audiology at University Hospital Frankfurt. Each ear was tested sequentially using an ear-canal probe integrating a click transducer and microphone, coupled to the external auditory canal via a silicone adapter to ensure an airtight seal. The device delivered a nonlinear click sequence at 70–85 dB SPL (self-calibrated depending on ear-canal volume) at an approximate repetition rate of 60 Hz. Cochlear responses were evaluated in the 6–12 ms post-stimulus interval. Response detection and pass/refer classification were performed automatically by the device using its built-in binomial statistical algorithm to test for the presence of a stimulus-related response, based on eight test points and parallel analysis across eight quality groups. A response was considered present when the probability for a random distribution fell below 0.3% (significance 99.7%); otherwise, the device

 doi:10.20944/preprints202605.0404.v1

returned a "refer" result if the criterion was not met after 200 scored stimuli. Measurements were conducted in a low-noise setting.

2.3. Automated Auditory Brainstem Response (AABR)

AABR screening is based on the auditory brainstem response (ABR), as described by Jewett and Williston in 1971 [24]. The ABR comprises a series of far-field electrophysiological waveforms generated along the auditory pathway within the first approximately 10 ms following acoustic stimulation. Typically, five to seven waves (I–VII) can be identified, with wave V being the most robust component in neonates and therefore primarily used for automated detection. The detection of Wave V in the AABR proves a proper transmission of auditory information to the brainstem and indicates regular retrocochlear function.

Bilateral AABR screening was performed using the Echo-Screen TDA device (Fischer-Zoth Diagnosesysteme GmbH; distribution: Mack Medizintechnik GmbH, Pfaffenhofen an der Ilm, Germany). Each ear was tested sequentially using an ear-canal probe. Disposable gel electrodes were positioned with the non-inverting (active) electrode on the high forehead and electrodes on both mastoid processes. During monaural testing, responses were recorded differentially between the forehead and the ipsilateral mastoid (ears tested sequentially). The electrode on the mastoid of the contralateral (non-tested) ear is used as the reference. Electrode impedance was verified by the device and maintained below 12 kΩ. Acoustic stimulation consisted of a 35 dB nHL broadband click presented at an approximate repetition rate of 55 Hz. Responses were sampled at 10.2 kHz and evaluated using an analysis interval of 170 samples (17 ms) with the device's built-in binomial statistical detection algorithm; a "pass" was assigned when the predefined 99.5% significance criterion was reached.

2.4. CMV PCR

Oral mucosa swabs were analysed at the Institute for Medical Virology, University Hospital Frankfurt, using an in-house real-time PCR assay (accredited according to DIN EN ISO 15189). Nucleic acids were extracted with the QIAsymphony system and DSP virus/pathogen midi kit (Qiagen GmbH, Hilden, Germany). CMV DNA concentration was quantified on the ABI PRISM® 7500 analyser (Applied Biosystems, Waltham, MA, USA) with TaqMan Gene Expression Master Mix (Thermo Fisher Scientific, Darmstadt, Germany), targeting the UL89 gene. Oral mucosa swabs were evaluated semi-quantitatively using Ct-values, whereas CMV DNA concentrations were quantified in urine, serum, and plasma samples. As an internal control, murine CMV virions (strain Smith; ATCC VR-1399) were used [25]. Viral concentrations were calibrated to the CMV World Health Organization (WHO) International Standard and reported as IU/ml (limit of detection: 200 IU/ml). For confirmation diagnostics, CMV DNA PCR testing was additionally performed on urine (minimum 0.8 ml) and EDTA plasma (minimum 1.6 ml). Prior testing, swabs and urine were diluted with an equal volume of phosphate-buffered saline (PBS). This dilution step was omitted for plasma testing.

The DBS for CMV DNA were sent to the German consultation laboratory for congenital CMV infection in the virology department of University Hospital Tuebingen, Germany. There, an entire dried blood spot (13 mm) was analysed using quantitative real-time PCR (values in copies/ml) and additionally nested PCR (qualitative) according to previously published methods [26].

2.5. Anti-CMV IgM and IgG Testing

Serum samples were analysed for anti-CMV IgM and IgG using the Enzygnost® anti-CMV IgM and IgG assays (Dade Behring, Marburg, Germany) on the Behring ELISA Processor BEP 2000 (Siemens Healthineers AG, Erlangen, Germany) according to manufacturer's instructions. The results were calculated using the alpha method, subtracting the control antigen value in the Behring ELISA

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Processor. CMV IgM and IgG results are reported semi-quantitatively as arbitrary units per millilitre (AU/mL).

2.6. CMV Isolation

The Shell-Vial assay was used for rapid CMV detection from fresh urine (<16h) on foreskin fibroblasts according to an already established in-house protocol [27,28]. Infected cells were microscopically counted and averaged. Results were valid only if negative controls showed no staining and positive controls showed specific red-brown nuclei.

3. Results

3.1. Study Population and Screening Characteristics

From August 3rd, 2015, to December 31st, 2019, the Department of Paediatric Audiology at University Hospital Frankfurt recorded 3,032 appointments for control of a primarily failed hearing screening. Of these, 291 (9.6%) were for non-study indications (e.g., post-ototoxic therapy), while 2,741 (90.4%) patients presented for initial repeat screening after a failed first test—the core group analysed in our study (Figure 2).

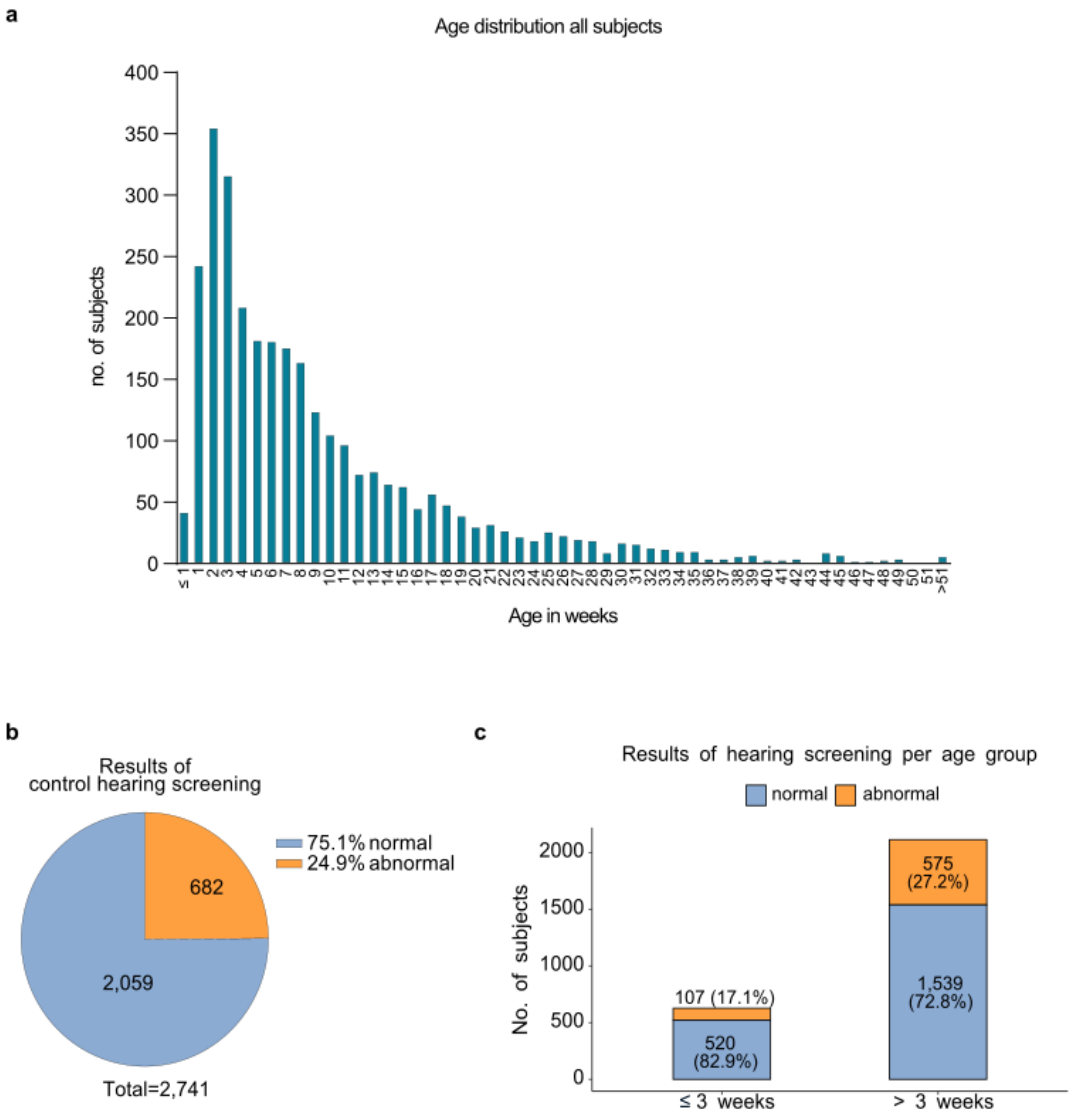

Figure 2. Study Population and Hearing Screening Results: a) Age distribution of patients at the time of the control hearing screening; b) Results of control hearing screening; c) Results of hearing screening per age group.

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Mean age at repeat screening was 70 days (median 46); 2,114 children (77.1%) were >3 weeks old. Thus, 627 infants (22.9%) remained within the 3-week diagnostic window for cCMV if repeat screening failed. Age distribution is shown in Figure 2a.

2,059 of all 2,741 analysed infants (75.1%) showed normal results bilaterally in the control hearing-screening, both in the TEOAE and AABR tests. Meanwhile, 682 patients (24.9%) had at least one abnormal finding in the control hearing screening (Figure 2b).

Of the 682 patients with abnormal control hearing screening, 575 (84.3%) were older than 21 days and thus ineligible for study participation. Only 107 of the 2,741 infants (3.9%) showed abnormal confirmatory screening while remaining within the eligible age window of ≤21 days (Figure 2c).

3.2. Study Enrollment and CMV Sample Collection

Six of these 107 eligible infants did not receive study enrollment. Two had a previously documented cCMV status (exclusion criterion met). Two infants presented with unilateral ear canal stenosis, which was incorrectly classified as an exclusion criterion. Two additional candidates could not be adequately enrolled due to language barriers in parental counseling.

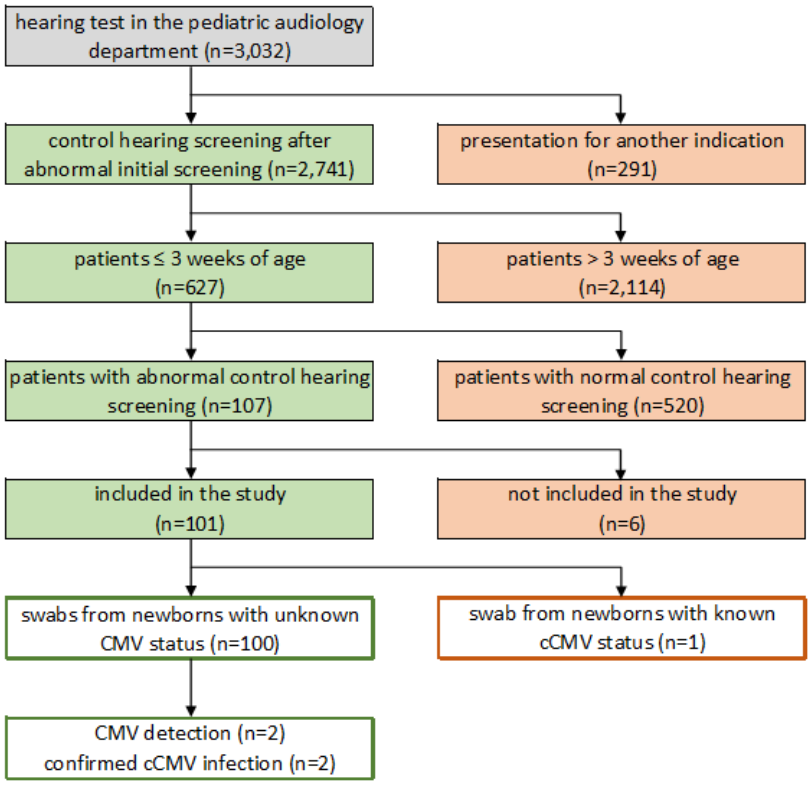

Figure 3. Overview of patient presentations in paediatric audiology. Six infants received CMV swabs despite meeting exclusion criteria and were therefore excluded from per-protocol analysis: two were older than 21 days, one had an unaffected control screening, one had a previously excluded cCMV infection status, one presented for control screening after an unaffected initial screening with known cCMV, and one underwent initial screening at home birth not captured in the 2,741-patient cohort.

3.3. Patient Characteristics and Hearing Screening Results

The per-protocol cohort comprised 100 infants who met all inclusion criteria. Participants had a mean age of 14 days at enrollment, with the youngest participant 4 days old and the oldest 21 days old. Age distribution is shown in Figure 4a.

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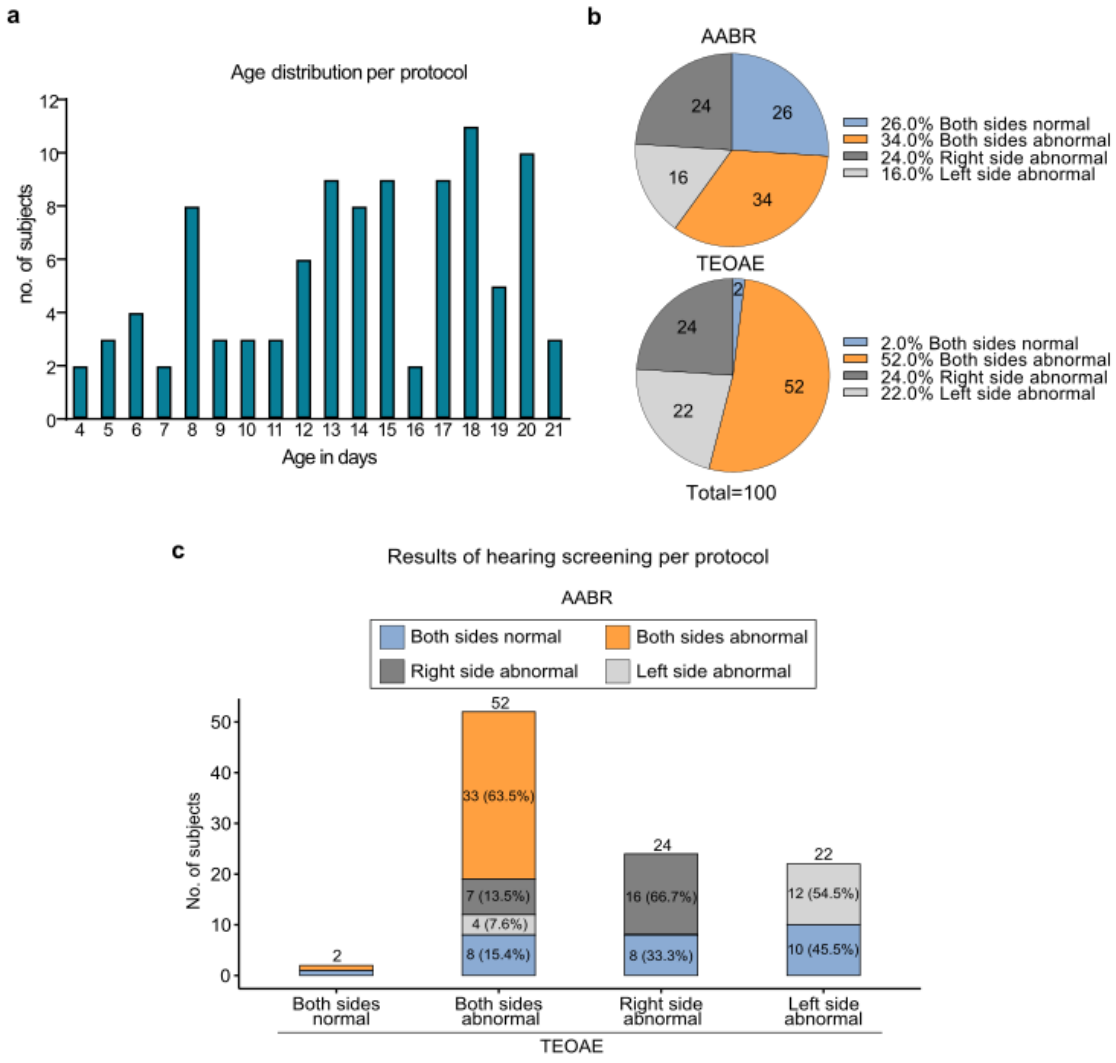

Figure 4. Patient Characteristics and Hearing Screening Results: a) Age distribution of patients; b) Hearing screening results according to AABR and TEOAE; c) Overall hearing screening results.

Hearing screening results are presented in Figure 4b-c. Most infants showed bilateral abnormalities in both TEOAE (52%) and AABR (34%) testing (overall: 63.5%), whereas a minority exhibited abnormalities solely in AABR.

3.4. CMV Oral Mucosal Swabs Results

Of the 100 oral mucosal swab samples analyzed, 98 (98%) yielded negative CMV PCR results, while 2 (2%) were CMV PCR positive. The Ct values for CMV-positive samples averaged 21,2.

3.5. Confirmatory Diagnostics

Both CMV PCR positive patients in oral mucosal swabs underwent confirmatory diagnostics with concordant positive results (100% concordance). Detailed laboratory parameters including CMV DNA in oral mucosal swabs (Ct values), CMV IgG and CMV IgM serology, CMV isolation from urine, CMV DNA in plasma, and CMV DNA in urine as well as CMV DNA in DBS and the results of the hearing tests are presented in Table 1.

 doi:10.20944/preprints202605.0404.v1

Table 1. Results of hearing tests and laboratory confirmation diagnostics in per-protocol analysis.

Laboratory parameters	Patient 1	Patient 2
CMV DNA oral mucosal swab [Ct value]	24.97	17.48
Urine CMV DNA [IE/ml]	60,871,272	34,387,553
Plasma CMV DNA [IE/ml]	< 200	2,270
CMV DNA in DBS [copies/ml]	2,430	negative
CMV DNA in DBS [qualitative]	positive	positive
CMV isolated from urine [nuclei]	500	1,000
Serum CMV IgG [AE/ml]	1,200	1,700
Serum CMV IgM [AE/ml]	negative	negative
Hearing-tests **TEOAE: right/left** **AABR: right/left**	 abnormal / normal normal / normal	 abnormal / abnormal normal / abnormal

3.6. DBS Card Results

DBS cards were obtained and tested for CMV DNA in 48 of the 100 per-protocol participants (48%). Of these, 2 cards (4.2%) tested CMV-positive and 46 (95.8%) tested CMV DNA negative. Concordance between DBS card results and oral mucosal swab results was achieved in 46 cases (95.8%). One positive CMV PCR result from a DBS card was classified as false-positive, as both confirmatory diagnostics and the corresponding oral mucosal swab provided no evidence of cCMV infection. One DBS card initially tested negative in automated real-time PCR; however, upon testing using the nested PCR, CMV DNA was successfully detected. One DBS card test had to be excluded from the per-protocol analysis because the corresponding newborn presented for the initial screening rather than for a repeat hearing screening in the Department of Paediatric audiology.

3.7. Diagnostic Accuracy: Sensitivity and Specificity

Among the 48 per-protocol participants who underwent multiple independent CMV tests, all metrics yielded 100%: sensitivity (2/2), specificity (46/46), positive predictive value (2/2), and negative predictive value (46/46).

3.8. Clinical Outcome

In summary, our study protocol successfully identified symptomatic cCMV infection in two infants within the critical diagnostic window of 21 days or fewer. This was achieved through targeted screening prompted by abnormal newborn hearing screening results in a preselected cohort of infants with undetermined hearing status and unknown cCMV infection status. Both patients received off-label antiviral therapy with valganciclovir. In patient 1, hearing impairment remained stable below the threshold for hearing aid fitting, whilst in patient 2, hearing normalised completely. No further clinical findings could be observed in these children.

4. Discussion

This study demonstrates that a protocol-based, hearing screening–driven approach to cCMV diagnosis is highly effective in identifying newborns with cCMV-associated hearing loss within the critical diagnostic window of the first 21 days of life. The presented protocol thereby affords the opportunity to complete the recommended cCMV diagnostic workup in affected neonates and to offer antiviral therapy against cCMV within the recommended first 30 days of life. Among 100 protocol-eligible infants with repeated abnormal NHS, 2% were confirmed to have cCMV infection. This cCMV detection rate exceeds the prevalence of congenital hypothyroidism (~0.35‰)—the most common disorder in standard neonatal metabolic screening panels—by far, approximately 60-fold. This highlights the substantial clinical burden of cCMV associated hearing loss in the investigated

 doi:10.20944/preprints202605.0404.v1

targeted population. Due to the strict inclusion criteria of our study, the presented results do not reflect the incidence of cCMV in newborns with hearing impairment, which is even higher [29].

The diagnosis of cCMV infection was made in the identified two children by CMV DNA detection in their buccal swab with Ct values of 25 and 17, confirmed by CMV DNA concentrations of 61 and 34 x 10^6 IE/ml in their urine. The CMV DNA concentrations in blood were quite low, at 200 and 2.270 IU/ml respectively. Interestingly, the CMV DNA results in the DBS samples were inversely proportional, with 2.430 copies/ml and non-quantifiable, as shown in Table 1. The serological findings in both children were not suggestive for cCMV infection: both newborns had positive CMV IgG and negative CMV IgM levels. These findings underscore the high importance of diagnosing cCMV infection by using urine and mucosal swab samples [30,31].

The hearing impairments detected in the two children with cCMV infection during NHS were confirmed in subsequent paediatric audiological follow-up examinations as unilateral hearing loss in each case. Both patients received antiviral therapy with valganciclovir. The hearing impairment in patient one remained stable below the threshold for hearing aid fitting, and in patient two, hearing function normalised completely over time during antiviral therapy. As part of the follow-up examinations, patient one was last presented for the Bayley-III test at the age of one year. Here, he demonstrated normal cognitive, gross and fine motor development, as well as a developmental lead of 4 months in the linguistic domain. Patient two was last seen for a follow-up examination at the age of 6 years and presented with somatic, cognitive and neurological development entirely appropriate for age, including normal hearing function.

A central strength of the presented protocol is its alignment with the WHO screening criteria as defined by Wilson and Jungner [32]. cCMV infection represents a well-defined and clinically important condition. It can be reliably detected through validated molecular diagnostics (CMV-PCR from oral mucosal swabs and urine), and an accepted, effective treatment exists in the form of parenteral ganciclovir or oral valganciclovir, which has been shown to improve long-term hearing and neurodevelopmental outcomes when initiated within the first 30 days of life [18,19,33]. The natural history of the disease is well understood, and the primary diagnostic test itself is simple, non-invasive, and accurate. In our small per protocol cohort for example, the CMV diagnostic achieved 100% sensitivity and specificity, which is in accordance to the exsive studies on cCMV diagnosis by mucosal swabs published by Boppana et al. 2010 and 2011 [34,35]. Meanwhile it had been shown that mucosal swabs taken with respect to diagnose cCMV infection can result in false positive findings due to CMV shed in breast milk [36]. Therefore, it is crucial to adhere to the post-feed sampling interval as well as to the demanded cCMV confirmation diagnostics of our protocol, especially to CMV DNA analysis in urine.

Taken together the presented full investigation plan fulfils the essential requirements for a justified screening intervention: the illness is important, detectable at a clinically pre-symptomatic or early symptomatic stage, and treatable with meaningful clinical benefit.

CCMV-infection represents a causally treatable aetiology of congenital sensorineural hearing loss, whereas in the most other causes only rehabilitative approaches such as hearing aids or cochlear implants could be offered. Antiviral off-label therapy with enteral valganciclovir offers an opportunity to address the underlying infectious process and preserve or improve auditory function. For affected families, early identification and timely treatment initiation are therefore of high value—both medically and personally. Preventing hearing loss is highly cost-effective. It minimizes expenditures for auditory rehabilitation (e.g., expensive medical devices like CIs, hearing aids), specialized support services, specialized schooling, while facilitating long-term socioeconomic integration. Unaddressed hearing loss causes annual costs of nearly one trillion US dollars. The WHO postulates a return on investment (ROI) of 1:16 for investments in hearing care [37,38]. Due to its critical importance, the universal newborn hearing screening (UNHS) was established in Germany by an amendment to the Paediatric Guidelines (Kinder-Richtlinien) by the Federal Joint Committee (G-BA) in June 2008 [39]. Because approximately 21% of all permanent bilateral hearing loss in children at the age of four is attributable to cCMV infection [40] especially investment in the

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prevention, early detection, and treatment of cCMV mitigates long-term socioeconomic burdens by reducing reliance on specialized education and medical devices, while fostering economic productivity through lifelong contributions to social security. Beyond hearing loss, cCMV causes neurodevelopmental sequelae such as cerebral palsy, microcephaly, and visual impairment, necessitating lifelong care and costly interventions. These comorbidities further escalate the socioeconomic burden by requiring extensive specialized support and long-term assistance. Several studies state that the costs for screening and early intervention are marginal compared to the gains in cognitive capacity and economic productivity over the entire lifespan [21,41–43]. This makes the secondary screening protocol presented here scientifically and clinically very precious although a universal screening would be more cost-effective in prevention and early treatment [44]. Shahar-Nissan et al. (2020) showed that early treatment after CMV primary infection in the first trimester of pregnancy can reduce the risk of vertical CMV transmission by approximately 70% [45].

An inevitable limitation of the study is the strict inclusion criterion of an age at repeated failed NHS ≤ 3 weeks. Since infants at age > 3 weeks were not tested, hearing loss caused by cCMV may not have been captured. Consequently, this leads to an underestimation of the proportion of cCMV-related hearing loss within the overall population. In our study 575 newborns with repeated failed NHS (84.3%) were older than 21 days and thus ineligible for study participation. Only 107 of the 2,741 infants (3.9%) showed abnormal confirmatory screening while remaining within the eligible age window of ≤21 days.

Therefore, the presented data also highlight a significant systemic challenge: 77.1% of infants presenting for repeat NHS were older than 21 days at the time of their appointment—the upper age limit for reliable cCMV diagnosis from fresh samples. Thus, the vast majority of children with potential cCMV-related hearing loss are referred too late for the diagnostic and therapeutic window to be exploited. This finding underscores an urgent need for structural improvements in NHS referral pathways, including clear communication to primary care providers and parents about the time-critical nature of NHS follow-up appointments with respect to cCMV.

A further limitation of hearing screening-driven investigations is that infants with asymptomatic cCMV and normal hearing at birth remain undetected. Consequently, these children do not receive the same clinical follow-up as symptomatic neonates, leading to a significant risk of missing or delaying the diagnosis of late-onset hearing loss. Furthermore, the underlying aetiology of cCMV can no longer be verified once the narrow diagnostic window for neonatal testing has passed. Consequently, many hearing impairments developing later in childhood remains classified as hearing loss of unknown etiology.

Implementing this protocol in routine clinical care would present organizational challenges: timely parental counselling, rapid sample collection coordinated with feeding schedules, and swift referral to confirmatory diagnostics. All require coordinated, multidisciplinary efforts and compliant parents. These demands are challenging, particularly in busy neonatal and paediatric audiology settings. Five improperly obtained buccal swabs for CMV-DNA analysis in our study underline the struggles of following study protocol criteria in real-world clinical practice. However, since cCMV is a causally treatable aetiology of congenital hearing loss and timely identification opens a small but crucial window of opportunity for antiviral intervention, these challenges are well worth addressing.

In conclusion, the hearing screening-driven cCMV protocol described herein is well-suited to the systematic and timely identification of newborns with cCMV-associated hearing impairment who may benefit from antiviral therapy. In the absence of universal cCMV screening, this targeted approach represents a pragmatic, evidence-based and WHO criteria-compliant strategy to extend clinical benefit to affected infants and their families.

 doi:10.20944/preprints202605.0404.v1

Author Contributions: Conceptualization: H.B.; Methodology: H.B., L.M., A.W., S.K. & H.R.; Investigation: H.B., L.M., S.K., A.W., N.D., A.B., U.R., H.R., N.K.; Data curation/Formal Analysis: L.M. & H.B.; Visualization: L.M., T.M & N.K.; Writing—original draft: N.K., L.M., H.B. & H.R.; Review and editing: all authors; All authors reviewed the results and approved the final version of the article.

Funding information: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Ethical approval: The study was conducted in accordance with the Declaration of Helsinki in the latest version. The protocol for the study was reviewed and approved by the Ethics Committee of the Johann Wolfgang Goethe-University Hospital, Frankfurt am Main (198/15).

Informed consent: Written informed consent was obtained from all parents, or single mothers prior to study related sample taking.

Conflicts of Interest: The authors have no conflicts of interest to declare.

References

1. M.G. SMITH, Propagation in tissue cultures of a cytopathogenic virus from human salivary gland virus (SGV) disease, Proc. Soc. Exp. Biol. Med. 92 (1956) 424–430. https://doi.org/10.3181/00379727-92-22498.
2. J.M. CRAIG, J.C. MACAULEY, T.H. WELLER, P. WIRTH, Isolation of intranuclear inclusion producing agents from infants with illnesses resembling cytomegalic inclusion disease, Proc. Soc. Exp. Biol. Med. 94 (1957) 4–12. https://doi.org/10.3181/00379727-94-22841.
3. S.C. Dollard, S.D. Grosse, D.S. Ross, New estimates of the prevalence of neurological and sensory sequelae and mortality associated with congenital cytomegalovirus infection, Rev. Med. Virol. 17 (2007) 355–363. https://doi.org/10.1002/rmv.544.
4. H. Buxmann, K. Hamprecht, M. Meyer-Wittkopf, K. Friese, Primary Human Cytomegalovirus (HCMV) Infection in Pregnancy, Dtsch. Arztebl. Int. 114 (2017) 45–52. https://doi.org/10.3238/arztebl.2017.0045.
5. S. Hoehl, A. Berger, S. Ciesek, H.F. Rabenau, Thirty years of CMV seroprevalence-a longitudinal analysis in a German university hospital, Eur. J. Clin. Microbiol. Infect. Dis. 39 (2020) 1095–1102. https://doi.org/10.1007/s10096-020-03814-x.
6. S. Manicklal, V.C. Emery, T. Lazzarotto, S.B. Boppana, R.K. Gupta, The "silent" global burden of congenital cytomegalovirus, Clin. Microbiol. Rev. 26 (2013) 86–102. https://doi.org/10.1128/CMR.00062-12.
7. S. Stagno, Primary Cytomegalovirus Infection in Pregnancy, JAMA 256 (1986) 1904. https://doi.org/10.1001/jama.1986.03380140074025.
8. A. Kenneson, M.J. Cannon, Review and meta-analysis of the epidemiology of congenital cytomegalovirus (CMV) infection, Rev. Med. Virol. 17 (2007) 253–276. https://doi.org/10.1002/rmv.535.
9. A. Daiminger, U. Bäder, G. Enders, Pre- and periconceptional primary cytomegalovirus infection: risk of vertical transmission and congenital disease, BJOG 112 (2005) 166–172. https://doi.org/10.1111/j.1471-0528.2004.00328.x.
10. C. Wang, X. Zhang, S. Bialek, M.J. Cannon, Attribution of congenital cytomegalovirus infection to primary versus non-primary maternal infection, Clin. Infect. Dis. 52 (2011) e11-3. https://doi.org/10.1093/cid/ciq085.
11. B.L. Hughes, C. Gyamfi-Bannerman, Diagnosis and antenatal management of congenital cytomegalovirus infection, Am. J. Obstet. Gynecol. 214 (2016) B5-B11. https://doi.org/10.1016/j.ajog.2016.02.042.
12. I. Foulon, Y. de Brucker, R. Buyl, E. Lichtert, K. Verbruggen, D. Piérard, F.A. Camfferman, L. Gucciardo, F. Gordts, Hearing Loss With Congenital Cytomegalovirus Infection, Pediatrics 144 (2019). https://doi.org/10.1542/peds.2018-3095.
13. K.B. Fowler, S.B. Boppana, Congenital cytomegalovirus (CMV) infection and hearing deficit, J. Clin. Virol. 35 (2006) 226–231. https://doi.org/10.1016/j.jcv.2005.09.016.
14. J. Goderis, A. Keymeulen, K. Smets, H. van Hoecke, E. de Leenheer, an Boudewyns, C. Desloovere, R. Kuhweide, M. Muylle, L. Royackers, I. Schatteman, I. Dhooge, Hearing in Children with Congenital Cytomegalovirus Infection: Results of a Longitudinal Study, J. Pediatr. 172 (2016) 110-115.e2. https://doi.org/10.1016/j.jpeds.2016.01.024.

 doi:10.20944/preprints202605.0404.v1

15. A. Lankadasu, S. Vengadesan, Influence of inlet shear on the 3-D flow past a square cylinder at moderate Reynolds number, Journal of Fluids and Structures 25 (2009) 889–896. https://doi.org/10.1016/j.jfluidstructs.2009.04.003.
16. S.E. Luck, J.W. Wieringa, D. Blázquez-Gamero, P. Henneke, K. Schuster, K. Butler, M.G. Capretti, M.J. Cilleruelo, N. Curtis, F. Garofoli, P. Heath, E. Iosifidis, N. Klein, G. Lombardi, H. Lyall, T. Nieminen, D. Pajkrt, V. Papaevangelou, K. Posfay-Barbe, L. Puhakka, E. Roilides, P. Rojo, J. Saavedra-Lozano, T. Shah, M. Sharland, H. Saxen, A.C.T.M. Vossen, Congenital Cytomegalovirus: A European Expert Consensus Statement on Diagnosis and Management, Pediatr. Infect. Dis. J. 36 (2017) 1205–1213. https://doi.org/10.1097/INF.0000000000001763.
17. W.D. Rawlinson, S.B. Boppana, K.B. Fowler, D.W. Kimberlin, T. Lazzarotto, S. Alain, K. Daly, S. Doutré, L. Gibson, M.L. Giles, J. Greenlee, S.T. Hamilton, G.J. Harrison, L. Hui, C.A. Jones, P. Palasanthiran, M.R. Schleiss, A.W. Shand, W.J. van Zuylen, Congenital cytomegalovirus infection in pregnancy and the neonate: consensus recommendations for prevention, diagnosis, and therapy, Lancet Infect. Dis. 17 (2017) e177-e188. https://doi.org/10.1016/S1473-3099(17)30143-3.
18. D.W. Kimberlin, C.-Y. Lin, P.J. Sánchez, G.J. Demmler, W. Dankner, M. Shelton, R.F. Jacobs, W. Vaudry, R.F. Pass, J.M. Kiell, S. Soong, R.J. Whitley, Effect of ganciclovir therapy on hearing in symptomatic congenital cytomegalovirus disease involving the central nervous system: a randomized, controlled trial, J. Pediatr. 143 (2003) 16–25. https://doi.org/10.1016/s0022-3476(03)00192-6.
19. S.E. Oliver, G.A. Cloud, P.J. Sánchez, G.J. Demmler, W. Dankner, M. Shelton, R.F. Jacobs, W. Vaudry, R.F. Pass, S. Soong, R.J. Whitley, D.W. Kimberlin, Neurodevelopmental outcomes following ganciclovir therapy in symptomatic congenital cytomegalovirus infections involving the central nervous system, J. Clin. Virol. 46 Suppl 4 (2009) S22-6. https://doi.org/10.1016/j.jcv.2009.08.012.
20. D.W. Kimberlin, P.M. Jester, P.J. Sánchez, A. Ahmed, R. Arav-Boger, M.G. Michaels, N. Ashouri, J.A. Englund, B. Estrada, R.F. Jacobs, J.R. Romero, S.K. Sood, M.S. Whitworth, M.J. Abzug, M.T. Caserta, S. Fowler, J. Lujan-Zilbermann, G.A. Storch, R.L. DeBiasi, J.-Y. Han, A. Palmer, L.B. Weiner, J.A. Bocchini, P.H. Dennehy, A. Finn, P.D. Griffiths, S. Luck, K. Gutierrez, N. Halasa, J. Homans, A.L. Shane, M. Sharland, K. Simonsen, J.A. Vanchiere, C.R. Woods, D.L. Sabo, I. Aban, H. Kuo, S.H. James, M.N. Prichard, J. Griffin, D. Giles, E.P. Acosta, R.J. Whitley, Valganciclovir for symptomatic congenital cytomegalovirus disease, N. Engl. J. Med. 372 (2015) 933–943. https://doi.org/10.1056/NEJMoa1404599.
21. Utah Department of Health and Human Services, Utah Environmental Public Health Tracking Network Web, Complete Health Indicator Report of Congenital Cytomegalovirus Infection and Hearing Loss. https://ibis.utah.gov/epht-view/indicator/complete_profile/cCMVHL.html (accessed 28 November 2025).
22. Connecticut Department of Public Health, Early Hearing Detection and Intervention Program. https://portal.ct.gov/dph/family-health/ehdi/cmv?language=en_US (accessed 28 November 2025).
23. S. Koyano, N. Inoue, T. Nagamori, H. Moriuchi, H. Azuma, Newborn screening of congenital cytomegalovirus infection using saliva can be influenced by breast feeding, Arch. Dis. Child. Fetal Neonatal Ed. 98 (2013) F182. https://doi.org/10.1136/archdischild-2012-302230.
24. D.L. Jewett, J.S. Williston, Auditory-evoked far fields averaged from the scalp of humans, Brain 94 (1971) 681–696. https://doi.org/10.1093/brain/94.4.681.
25. W. Preiser, N.S. Brink, U. Ayliffe, K.S. Peggs, S. Mackinnon, R.S. Tedder, J.A. Garson, Development and clinical application of a fully controlled quantitative PCR assay for cell-free cytomegalovirus in human plasma, J. Clin. Virol. 26 (2003) 49–59. https://doi.org/10.1016/s1386-6532(02)00022-7.
26. K. Göhring, K. Dietz, S. Hartleif, G. Jahn, K. Hamprecht, Influence of different extraction methods and PCR techniques on the sensitivity of HCMV-DNA detection in dried blood spot (DBS) filter cards, J. Clin. Virol. 48 (2010) 278–281. https://doi.org/10.1016/j.jcv.2010.04.011.
27. C.A. Gleaves, T.F. Smith, E.A. Shuster, G.R. Pearson, Comparison of standard tube and shell vial cell culture techniques for the detection of cytomegalovirus in clinical specimens, J. Clin. Microbiol. 21 (1985) 217–221. https://doi.org/10.1128/jcm.21.2.217-221.1985.
28. B. Weber, A. Hamann, B. Ritt, H. Rabenau, W. Braun, H.W. Doerr, Comparison of shell viral culture and serology for the diagnosis of human cytomegalovirus infection in neonates and immunocompromised subjects, Clin. Investig. 70 (1992) 503–507. https://doi.org/10.1007/BF00210232.

 doi:10.20944/preprints202605.0404.v1

29. C.C. Morton, W.E. Nance, Newborn hearing screening--a silent revolution, N. Engl. J. Med. 354 (2006) 2151–2164. https://doi.org/10.1056/NEJMra050700.
30. M. Leruez-Ville, C. Chatzakis, D. Lilleri, D. Blazquez-Gamero, A. Alarcon, N. Bourgon, I. Foulon, J. Fourgeaud, A. Gonce, C.E. Jones, P. Klapper, A. Krom, T. Lazzarotto, H. Lyall, P. Paixao, V. Papaevangelou, E. Puchhammer, G. Sourvinos, P. Vallely, Y. Ville, A. Vossen, Consensus recommendation for prenatal, neonatal and postnatal management of congenital cytomegalovirus infection from the European congenital infection initiative (ECCI), Lancet Reg. Health Eur. 40 (2024) 100892. https://doi.org/10.1016/j.lanepe.2024.100892.
31. Gesellschaft für Virologie, S2k-Leitlinie Prävention, Diagnostik und Therapie der CMV-Infektion bei Schwangeren und der konnatalen CMV-Infektion bei Neugeborenen und Kindern, 2024. https://register.awmf.org/de/leitlinien/detail/093-003 (accessed 28 April 2026).
32. Wilson, James Maxwell Glover, Jungner, Gunnar, Principles and practice of screening for disease: WHO Public Health Papers, 1968. https://iris.who.int/items/7a67638b-14e3-4dba-b013-dc206ff279d8 (accessed 24 March 2026).
33. D.W. Kimberlin, E.P. Acosta, P.J. Sánchez, S. Sood, V. Agrawal, J. Homans, R.F. Jacobs, D. Lang, J.R. Romero, J. Griffin, G.A. Cloud, F.D. Lakeman, R.J. Whitley, Pharmacokinetic and pharmacodynamic assessment of oral valganciclovir in the treatment of symptomatic congenital cytomegalovirus disease, J. Infect. Dis. 197 (2008) 836–845. https://doi.org/10.1086/528376.
34. S.B. Boppana, S.A. Ross, Z. Novak, M. Shimamura, R.W. Tolan, A.L. Palmer, A. Ahmed, M.G. Michaels, P.J. Sánchez, D.I. Bernstein, W.J. Britt, K.B. Fowler, Dried blood spot real-time polymerase chain reaction assays to screen newborns for congenital cytomegalovirus infection, JAMA 303 (2010) 1375–1382. https://doi.org/10.1001/jama.2010.423.
35. S.B. Boppana, S.A. Ross, M. Shimamura, A.L. Palmer, A. Ahmed, M.G. Michaels, P.J. Sánchez, D.I. Bernstein, R.W. Tolan, Z. Novak, N. Chowdhury, W.J. Britt, K.B. Fowler, Saliva polymerase-chain-reaction assay for cytomegalovirus screening in newborns, N. Engl. J. Med. 364 (2011) 2111–2118. https://doi.org/10.1056/NEJMoa1006561.
36. S.A. Ross, M.G. Michaels, A. Ahmed, A.L. Palmer, P.J. Sánchez, D.I. Bernstein, K. Feja, A. Stewart, S.B. Boppana, K.B. Fowler, Contribution of Breastfeeding to False-Positive Saliva Polymerase Chain Reaction for Newborn Congenital Cytomegalovirus Screening, J. Infect. Dis. 217 (2018) 1612–1615. https://doi.org/10.1093/infdis/jiy057.
37. World Health Organization, World report on hearing, 2021. https://iris.who.int/server/api/core/bitstreams/5c3fc19f-11c9-4488-af3f-7e636b982aca/content (accessed 17 April 2026).
38. World Health Organization, Deafness and hearing loss, 2026. https://www.who.int/news-room/fact-sheets/detail/deafness-and-hearing-loss (accessed 17 April 2026).
39. Gemeinsamer Bundesausschuss, Bekanntmachung eines Beschlusses des Gemeinsamen Bundesausschusses über eine Änderung der Kinder-Richtlinien: Einführung eines Neugeborenen-Hörscreenings, 2008. https://www.g-ba.de/downloads/39-261-681/2008-06-19-Kinder-H%C3%B6rscreening_BAnz.pdf (accessed 17 April 2026).
40. S.D. Grosse, D.S. Ross, S.C. Dollard, Congenital cytomegalovirus (CMV) infection as a cause of permanent bilateral hearing loss: a quantitative assessment, J. Clin. Virol. 41 (2008) 57–62. https://doi.org/10.1016/j.jcv.2007.09.004.
41. Cochlear Implant International Community of Action, Spend to Save 2025: Investing in Hearing Care for All, 2025.
42. P.M. Lantos, S. Gantt, M. Janko, F. Dionne, S.R. Permar, K. Fowler, A Geographically Weighted Cost-effectiveness Analysis of Newborn Cytomegalovirus Screening, Open forum infectious diseases 11 (2024) ofae311. https://doi.org/10.1093/ofid/ofae311.
43. S. Gantt, F. Dionne, F.K. Kozak, O. Goshen, D.M. Goldfarb, A.H. Park, S.B. Boppana, K. Fowler, Cost-effectiveness of Universal and Targeted Newborn Screening for Congenital Cytomegalovirus Infection, JAMA Pediatr. 170 (2016) 1173–1180. https://doi.org/10.1001/jamapediatrics.2016.2016.

 doi:10.20944/preprints202605.0404.v1

44. H. Saito, K. Sakai, M. Tanaka, K. Konomura, M. Suzuki, G. Tajima, E. Hoshino, Economic evaluation of newborn screening for congenital cytomegalovirus infection: A systematic review, European journal of pediatrics 184 (2025) 123. https://doi.org/10.1007/s00431-024-05953-1.
45. K. Shahar-Nissan, J. Pardo, O. Peled, I. Krause, E. Bilavsky, A. Wiznitzer, E. Hadar, J. Amir, Valaciclovir to prevent vertical transmission of cytomegalovirus after maternal primary infection during pregnancy: a randomised, double-blind, placebo-controlled trial, Lancet (London, England) 396 (2020) 779–785. https://doi.org/10.1016/S0140-6736(20)31868-7.