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Puhakka, Laura

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Title page

The burden of congenital cytomegalovirus infection: a prospective cohort study of 20 000 infants in Finland

Laura Puhakka, Department of Pediatric Infectious Diseases, Children's Hospital, University of Helsinki and Helsinki University Hospital, Helsinki, Finland

Maija Lappalainen, Laboratory Services (HUSLAB), Department of Virology and Immunology, University of Helsinki and Helsinki University Hospital, Finland

Tuula Lönnqvist, Department of Child Neurology, Children's Hospital, University of Helsinki and Helsinki University Hospital, Helsinki, Finland

Riina Niemensivu, Department of Otorhinolaryngology-Head and Neck Surgery, University of Helsinki and Helsinki University Hospital, Helsinki, Finland

Päivi Lindahl, Department of Ophthalmology, University of Helsinki and Helsinki University Hospital, Helsinki, Finland

Tea Nieminen, Department of Pediatric Infectious Diseases, Children's Hospital, University of Helsinki and Helsinki University Hospital, Helsinki, Finland

Raija Seuri, Department of Pediatric Radiology, HUS Medical Imaging Center, University of Helsinki and Helsinki University Hospital, Helsinki, Finland

Irmeli Nupponen, Department of Neonatology, Children's Hospital, University of Helsinki and Helsinki University Hospital, Helsinki, Finland

Sunil Pati, Pediatrics, University of Alabama at Birmingham, Birmingham, AL, USA

Suresh Boppana, Pediatrics and microbiology, University of Alabama at Birmingham, Birmingham, AL, USA

Harri Saxen, Department of Pediatric Infectious Diseases, Children's Hospital, University of Helsinki and Helsinki University Hospital, Helsinki, Finland

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Correspondence:

Laura Puhakka (corresponding author)

Children's Hospital,

Helsinki University Central Hospital,

Stenbäckinkatu 11, PL 281,

00029 HUS, Helsinki, Finland

tel. +358 40 7064839

laura.puhakka@hus.fi

Alternate corresponding author:

Harri Saxen

tel. +358 50 4274967

Helsinki University Central Hospital,

Stenbäckinkatu 11, PL 281,

00029 HUS, Helsinki, Finland

harri.saxen@hus.fi

Summary of the article's main point

In our population-based screening study, the burden of cCMV infection was low: the prevalence was only 0.2% and the CMV positive infants did not differ from matched healthy controls at 18 months of age in neurodevelopmental, ophthalmological or hearing outcomes.

Abstract

Background

Congenital cytomegalovirus infection (cCMV) is the most common congenital infection causing significant morbidity. This study was undertaken to evaluate the benefits of screening newborns for cCMV and to understand the disease burden in Finland.

Methods

Infants born in the Helsinki area hospitals were screened for CMV by a real-time polymerase chain reaction assay of saliva. The CMV-positive infants and matched controls were monitored to determine neurodevelopmental, audiological, and ophthalmological outcome at 18 months of age. Griffiths Mental Development Scales, otoacoustic emission and sound field audiometry, and ophthalmologic examination were performed.

Results

Of the 19,868 infants screened, 40 had confirmed cCMV for a prevalence of 2/1000 (95% CI 1.4–2.6/1000). Four infants (10%) had symptomatic cCMV. Griffiths general quotient did not differ between CMV positives (mean 101.0) and controls (mean 101.6) ($p=0.557$), nor did quotients in any of the Griffiths subscales (locomotion, personal-social, hearing and language, eye and hand, performance) ($p=0.173–0.721$). Otoacoustic emission failed in 4/54 of CMV-positive and in 6/80 of CMV-negative ears ($p=1.000$). The mean minimal response levels over the frequencies 500Hz to 4kHz in the sound field audiometry did not differ between CMV positives (mean 34.31 dB HL) and controls (mean 32.73 dB HL) ($p=0.338$). No CMV-related ophthalmologic findings were observed.

Conclusions

The prevalence of cCMV was low, and the outcome at the age of 18 months did not differ between the infected children and the healthy controls. With such a low burden, universal newborn screening for cCMV seems unwarranted in Finland.

Introduction

Cytomegalovirus (CMV) is the most common cause of congenital infection and an important cause of sensorineural hearing loss (SNHL) and neurodevelopmental disabilities. The global prevalence of congenital CMV infection (cCMV) is approximately 7/1000 of all newborns [1, 2]. The prevalence of cCMV is associated with the seroprevalence of CMV in the population, the higher the seroprevalence among pregnant women, the higher the prevalence of cCMV [3]. In a recent cross-sectional study, the CMV seroprevalence in Finnish pregnant women was shown to be 71.5 % [4].

Only about 10% of the infected infants have clinical findings at birth (symptomatic cCMV), and about half of these symptomatic infants will have long term sequelae [1, 2]. Antiviral treatment of symptomatic infants has been shown to improve outcomes [5]. However, most CMV-positive infants do not have clinical findings at birth (asymptomatic cCMV) and therefore are not recognized at birth. About 10-15% of infants with asymptomatic cCMV will develop long-term sequelae, mostly SNHL [2, 6]. Universal newborn CMV screening has been advocated to identify all infants with CMV. Although antiviral therapy is currently not recommended for asymptomatic infants [7], identifying these infants at birth allows monitoring for early detection of hearing loss and developmental disabilities so that early intervention can be instituted to improve outcomes. However, precise estimates of prevalence and disease burden are needed to determine the cost-effectiveness of newborn CMV screening.

This population-based screening study was undertaken to investigate the prevalence and outcome of cCMV infection in Finland, and to evaluate the benefits of universal newborn CMV screening.

Methods

The ethics committee for women, children, and psychiatry in the Hospital District of Helsinki and Uusimaa approved the study protocol.

CMV screening was offered to babies born at four Helsinki area hospitals from September 2012 to January 2015 including Kätilöopisto, Jorvi, and Lohja Hospitals providing care to low-risk pregnancies, and the Women's tertiary care Hospital providing care to both low-risk and all high-risk pregnancies in the region. The screening hospitals cover about 25% of all deliveries in Finland. The parents of a newborn baby were provided with the study information attached to the established package for all newborns. After obtaining informed consent from both parents, dried saliva samples were collected during the first week of life as described [8]. The nurse staff taking care of the newborn in the well-baby nursery or neonatal ward collected the samples. The samples were stored in -20°C until they were transported to the University of Alabama at Birmingham (UAB) for testing by real-time polymerase chain reaction (PCR) assay to detect CMV DNA [8]. The sample was considered positive if one or more genomic equivalents (ge) of CMV DNA per reaction were detected in the PCR assay. Screening positive results were confirmed by testing urine samples for CMV culture and saliva samples by PCR at 3 months of age. Serum samples obtained at ages 3 months and 12-18 months were tested for CMV IgG and IgM antibodies (Architect CMV IgG, CMV IgM, Abbott Diagnostics). For each CMV-positive infant, one CMV-negative healthy control was matched, based on the timing of birth, sex, gestational age, and ward.

Outcome

Outcome was assessed at 18 months of age. Neurodevelopmental assessment was carried out using the Griffiths Mental Development Scales (1996 revision) from birth to 2 years, by an experienced pediatrician (LP) [9, 10]. The Griffiths scales evaluate five subscales: locomotor, personal-social, hearing and language, eye-hand coordination, and performance. General quotient and sub-quotients in every subscale were determined according to the test manual.

All the newborn babies, also the study babies, are screened for hearing in the maternity hospitals. The universal newborn hearing screening protocol in Helsinki area includes otoacoustic emission

(OAE) performed before discharge. Only one ear is tested, as unilateral hearing is regarded sufficient for language and social development. The study children underwent hearing evaluation at 18 months of age using transient-evoked otoacoustic emission (TEOAE) of both ears, and sound field audiometry (SF, Interacoustics, ISO 389-7). TEOAE measures an acoustic response from individual ears produced by the inner ear to a sound stimulus. The SF measures behavioral response to frequency-modulated tones of both ears simultaneously. SF testing is reliable in detecting functionally relevant hearing losses that would interfere with speech and language development. However, SF does not detect unilateral hearing losses. A mean threshold of 35dB between 500Hz and 4kHz in children with normal TEOAEs were considered as normal hearing.

Ophthalmologic examination is described in detail in Supplemental Table 1.

Imaging

Cranial ultrasound (US) was performed in infected infants and healthy controls at three months of age. Brain MRI was performed only based on clinical indications.

Maternal CMV infection

CMV antibodies were measured from archival maternal sera obtained at the end of the first trimester. A commercial assay (Architect CMV IgG, CMV IgM, and CMV IgG Avidity assay, Abbott Diagnostics) was used, according to the manufacturer's instructions [11, 12]. Based on the CMV antibody status the maternal infection was classified as non-primary infection (high avidity CMV-IgG in the early pregnancy), primary infection in the first trimester (low avidity CMV-IgG and positive CMV-IgM in the early pregnancy), or primary infection during the 2nd or 3rd trimester (no CMV-IgG antibodies in the early pregnancy)

Statistical methods

Analyses were performed with IBM SPSS Statistics version 22. Chi-Square or Fisher's exact test were used to compare categorical variables. Parametric linear variables were compared with T-test and non-parametric linear variables with Mann-Whitney U test.

Results

Newborn CMV screening

Saliva specimens were collected from 19,868 infants, mainly in well-baby nurseries (98.3%) (Figure 1). Approximately 54% of all infants born in the screening hospitals were screened. The characteristics of the screened population are shown in Table 1. The mothers of the screened children were representative of the women aged 20–39 years living in Finland, considering the country of origin, number of children, proportion of primipara, and marital status [13].

Screening saliva samples from 56 infants tested positive for CMV using the real-time PCR. Based on the results of urine CMV culture and saliva PCR obtained at 3 months of age, 40/56 of the screening positive infants were considered to have confirmed cCMV for a prevalence of 2/1000 infants (95% CI 1.4–2.6 / 1000). Urine CMV culture at 3 months of age was negative in 14 infants. One infant with unavailable urine control sample had no CMV antibodies at one year of age (Supplemental table 2). These 15 infants were considered to have false positive screening tests. One screening positive infant did not enroll in the follow-up study for confirmation. The screening saliva samples of the 15 infants considered false positives contained significantly lower CMV load than the samples of the 40 infants with confirmed cCMV (Figure 2).

All CMV positive children were positive for CMV IgG at 3 months of age. Of the fifteen children with false positive screening results, 14 were tested for antibodies at 3 months age. All were negative for CMV IgM, ten had CMV IgG and four had no antibodies for CMV. Nine of the ten CMV IgG positive children were retested for CMV antibodies at 12-18 months of age when

maternal antibodies had faded and only one child had antibodies at this stage. She was regarded as having possible postnatal infection. See Supplemental Table 2.

Evaluation of infected infants

The demographic factors and clinical findings of CMV-positive infants and matched healthy controls are presented in Table 2. Only one positive infant had clinical symptom of CMV infection, microcephaly (-3.3 SD) [14]. None of the remaining 39 CMV positive infants had any clinical findings suggesting CMV infection in the newborn period, including neonatal hearing screening, and pediatric examination performed to all newborns at approximately 1-3 days age before discharge from the hospital. Three infants had calcifications on cranial ultrasound at 3 months of age. These four infants (10%); one with microcephaly and three with calcifications were considered to have symptomatic cCMV. None of the infants were treated with antivirals.

The neurodevelopmental outcome measured by Griffiths Mental Development Scales was ascertained at 18 months in 37/40 patients and 51/54 controls. The outcome at 18 months did not differ between patients and controls in general quotient ($p=0.557$) or any of the subscales ($p=0.173-0.721$) (Table 3 and Figure 3).

Hearing function was evaluated at 18 months of age in 35 patients and 46 controls. Of the infants who had technically successful testing, the TEOAE failed in 4/54 CMV positive and 6/80 control ears ($P=1.000$). No difference in the mean thresholds on sound field audiometry was observed (34.31 dB HL for patients and 32.73dB HL for controls) ($p=0.133$) (Table 3).

Ophthalmologic follow-up at 18 months old was completed for 35 patients and 47 controls. None of the children had abnormal findings. One CMV-positive child had significant hyperopia, and one control child had intermittent esotropia. The hyperopia is a physiological finding at 3-18 months of age and normally resolves with age. The change in spherical equivalents or cylinder between 3-month and 18-month measurements did not differ between groups in either eye ($p=0.35-0.91$).

Cranial US at three months of age was performed in all CMV-positive infants and all but two healthy controls. Brain MRI was performed in five children with cCMV based on clinical indications: calcifications seen on US in three children, microcephaly in one child, and delayed motor development in one child. Imaging findings are presented in Supplemental Table 3.

Type of maternal CMV infection

The maternal CMV infection during pregnancy was categorized as non-primary in 53% (20/38), primary infection in the early pregnancy in 16% (6/38) and primary infection during later gestation in 32% (12/38) of mothers. In two cases the timing of maternal primary infection could not be defined because the antenatal serum samples were obtained later in gestation (at gestational age 22+4 and 33 + 1). Two symptomatic infants were born to mothers with non-primary infection, and the other two were born to women with primary infection during pregnancy. The antenatal CMV antibody data are presented in Supplemental Table 4.

Discussion

In our population-based screening study, the prevalence of cCMV infection was 2/1000. The prospective follow up for neurodevelopmental, hearing, and ophthalmological outcome did not reveal differences between infected infants and matched controls at 18 months of age.

Previously reported prevalence of cCMV from studies in different geographic regions was about 6-7/1000 [1, 2]. Prevalence for cCMV has ranged from 6/1000 to as high as 11.9/1000 in developing countries [15, 16]. In a study from Slovenia, a cCMV prevalence of 1.4/1000 was reported [17]. As the prevalence of cCMV has been higher in populations with high seroprevalence [3], we expected higher prevalence in Finland with CMV seroprevalence rate of ~70% among pregnant women [4, 18].

One explanation for the low prevalence of cCMV in Finland is the avoidance of saliva contact between mothers and their toddlers, which could decrease the number of primary infections as well

as reinfections during pregnancy. In Finland, it is a standard practice to counsel parents of young children to avoid saliva contact with their children to prevent transmission of *Streptococcus mutans* infection from parents to offspring. These measures include not sharing toothbrushes or utensils, not inserting a child's pacifier in their own mouth, and not kissing the child on the mouth [19, 20]. Counselling pregnant women to avoid contact with toddlers' saliva has shown to decrease significantly the number of CMV infections acquired during pregnancy [21-23].

The low prevalence could also be due to missing some real cases because the samples were stored and transported to UAB for testing. The real time PCR assay of dried saliva samples has been shown to be highly sensitive and specific in a large multi-center newborn CMV screening study [8]. The viral DNA is very stable and we don't think the storage and transport would have affected the sensitivity of the assay.

A strength of our study is that the screened population was unselected. All infants born in the area were eligible for CMV screening. The screening hospitals took care of 25% of all deliveries in Finland. Therefore, our study provides an unbiased estimate of the cCMV prevalence in healthy infants. However, only 54 % of children born in the area during the study period were enrolled in the study and screened for CMV. This may have caused selection bias. However, the screened population did not differ from the general Finnish women aged 20 to 39 years of age based on the country of origin, proportion of primipara, number of children, or marital status. Therefore, we think that it is unlikely that the low prevalence of congenital CMV infection in our study is due to a selection bias.

A review of the long-term outcome data from newborn screening studies showed that about half of the symptomatic infants and 13.5% of asymptomatic infants had sequelae [2]. Only one infant in our cohort was noted to be symptomatic at birth. Three additional infants had abnormal neuroimaging findings. In a large cohort of almost 11,000 infants screened in China, none of the 75 CMV-positive infants had clinical abnormalities at birth [24]. In a study from Japan, only one of 60

infants with cCMV identified on screening had microcephaly, and all other children were clinically asymptomatic [25]. In that study, imaging revealed calcifications in one infant, and five infants had sensorineural hearing loss.

None of the infected infants in our cohort failed the newborn hearing screening. The hearing outcome at 18 months was not inferior to healthy controls. This is in contrast to the findings from a meta-analysis of 14 screening studies in developed countries that reported SNHL in 32.8% of symptomatic and 9.9% of asymptomatic children [6]. None of the children in our cohort required hearing amplification by 18 months of age. In a longitudinal study from cCMV register in Belgium, 1.6% of children with asymptomatic cCMV infection needed hearing rehabilitation compared to 29% of symptomatic infants [26]. In our study, only behavioral hearing testing in the SF was carried out to evaluate hearing function. TEOAE was measured from both ears separately, but the measurements were unsuccessful due to insufficient co-operation in many cases. The testing (TEOAE and SF) was conducted by an experienced audiologists in the same environment and with the same equipment for both cases and controls. It is possible, however, that unilateral mild hearing losses, and high frequency losses were not identified. Since the CMV-associated hearing loss can emerge during early childhood, some of the infected children could develop hearing loss after the end of this follow-up [6].

Consistent with previous reports, none of the children in our cohort had any ophthalmological findings related to cCMV infection [27]. One positive child had significant hyperopia. In the cohort of 94 children, one hyperopia case is expected and the finding is likely not related to cCMV.

The neurodevelopmental assessment at 18 months did not differ between infected infants and controls in any of the five Griffiths subscales. This finding is similar to that reported by Pearl et al, where the 36 CMV-positive asymptomatic infants had similar results in Griffiths Developmental scales, compared to 74 healthy controls, at two years of age [28]. In another cohort, 9% (3/35) of asymptomatic infants had neurologic abnormalities by one year of age [29]. However, no new

developmental delays were observed at seven years of age among the children who had developed normally at one year of age [30]. The recent review by Bartlett et al highlights the importance of control group in evaluating the effect of CMV infection in outcome [31]. Although the prevalence of neurodevelopmental abnormalities have earlier been reported to be 6.5%, a more recent systematic review found no difference in neurodevelopmental assessments between asymptomatic cCMV positive and controls in the 11 studies involving control groups [2, 31]. Another recently published prospective cohort from Texas reported that the academic performance among teenagers with asymptomatic cCMV who had normal hearing at two years age was similar to controls [32].

In our cohort the child with microcephaly, and the child with multiple abnormalities in MRI, do have an increased risk for neurodevelopmental problems at later ages. As shown in Figure 3, the Griffiths Scales of these two patients are among the lowest ones. However, lower scores were also observed in the control group. The normal development at 18 months has wide variability, and the test may not identify milder cognitive or language abnormalities. Nevertheless, these data demonstrate that severe neurodevelopmental delays are rare among infants with asymptomatic cCMV.

Maternal non-primary CMV infections accounted for 53 55 % of the congenital CMV cases in our cohort. None of the six children, whose mother had acquired primary infection in the first trimester, had clinical symptoms of cCMV. One of these children had calcifications in the cranial ultrasound but no apparent developmental delays. The child with microcephaly and the child with multiple calcifications and polymicrogyria on the MRI were born to mothers with non-primary infection. Interestingly, a similar proportion of non-primary infections (54%) was observed in our earlier retrospective register based report of symptomatic cCMV infants [33].

A limitation of our study is that in 15 infants, the positive saliva screening result was considered a false positive which is higher than previously reported [8]. In this study any positive signal on real-time PCR was considered positive so that none of the infected infants will not be missed. This is in

contrast to the larger multi-center newborn CMV screening study where a cut-off of 5 copies per reaction was considered as a positive result [8]. In addition, the saliva samples were collected and stored at -20°C at the study site and periodically transported to the testing laboratory. The viral loads in screening samples from infants with false-positive results were significantly lower than from infants with confirmed infection (Figure 2). In another recent large screening study from France utilizing saliva PCR, 36/87 (41%) of screening positive infants were considered to be false positive [34]. The findings from our study and the French study highlight the importance of confirming positive screening results based on saliva PCR by testing repeat samples. Another limitation of our study is that the follow-up urine and saliva samples for confirmation were collected at 3 months of age and therefore, perinatal or postnatal CMV infection cannot be excluded. Of the 15 false positive children, 14 infants were tested for CMV antibodies. Twelve (12/14) infants tested negative for CMV antibodies either at 3 months of age, or later at 12-18 months age when maternal antibodies would have faded. This finding confirms that these children could be regarded as false positives. One child with positive antibodies at 3 months of age was not re-tested after maternal antibodies had faded. One child with negative urine CMV culture and negative saliva PCR at 3 months of age had positive CMV urine culture and CMV antibodies at 18 months of age. She had most probably acquired the CMV infection postnatally (Supplemental Table 2). Even though breast milk is a possible source of contamination, most of the saliva samples were collected during the 3 days of life when CMV is usually not detected in colostrum.

The low cost and availability of PCR tests are prompting a discussion of the benefits of screening for cCMV [35, 36]. Early diagnosis would benefit the symptomatic infants, allowing early antiviral treatment. Although antiviral therapy is not recommended for infants with asymptomatic cCMV, newborn screening will allow optimal follow-up and enable early intervention to improve outcomes. The cost-effectiveness of CMV screening will likely depend on the prevalence and disease burden of cCMV.

Findings of this prospective newborn CMV screening study showed low prevalence of cCMV (2/1000) in Finland. The outcome in 40 congenitally infected infants did not differ from the healthy controls at 18 months of age. Our findings demonstrate that the burden of cCMV varies significantly between different populations and it is important to assess the local burden of disease when considering newborn CMV screening programs.

Contributors

HS, TL, ML, TN, and LP designed the study with contributions from all other authors. LP coordinated the screening with help from IN. LP performed the medical examination including neurodevelopmental evaluation to all subjects, and collected and analyzed the data with support from HS, TL, RN, TN, and SB. ML was responsible for the laboratory analysis of serum and urine samples and handling of saliva samples and coordination of the logistics of saliva samples before shipment to Alabama. SP and SB developed the real-time PCR protocol for the analysis of the saliva samples, and SP conducted the real-time PCR with support from SB. RN was responsible for the hearing evaluations, PL for the ophthalmological examinations and RS for the 3-months ultrasounds. LP, HS, TL, and SB drafted the initial manuscript. All authors critically reviewed the manuscript and approved the final version.

Declaration of interest

All authors declare no competing interests.

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Table 1: Demographic factors of screened population ^a

Characteristics	Percentage
Mother born in Finland	88.1%
Mother born abroad	11.9%
Europe, not Finland	7.3%
America	0.6%
Africa	1.4%
Asia	2.6%
Australia and New Zealand	0.03%
Number of children (mean / SD)	1.858 / 0.9543
Primipara	38%
≥3 previous deliveries	4.7%
Married	66%

^a Based on a cohort of 9167 mothers of screened infants (screened Oct. 1, 2012–Oct. 31, 2013)

Table 2: Demographic findings and clinical characteristics of patients and controls

	CMV positive n=40	Controls n=54	p^a
Male / Female	16 / 24	25 / 29	0.543
Mother's age (mean)	31.08	32.76	0.114
Father's age (mean)	33.78	33.92	0.887
Mother's education ^b (mean)	5.78	5.94	0.436
Father's education ^b (mean)	5.67	5.42	0.805
Married (%)	75	73.1	0.835
Mother born outside of Finland (%)	15	1.9	0.039
Father born outside of Finland (%)	17.5	5.6	0.06
Parity (mean)	1.7	1.76	0.890
Older siblings in day care (%)	30 %	21 %	0.331
Gestational weeks (mean)	39+4	39+5	0.515
Placental weight (g) (mean)	562	605	0.348
Birth weight (kg) (mean)	3.315	3.474	0.264
Birth height (cm) (mean)	49.7	49.9	0.527
Head circumference (cm) (mean)	34.5	34.9	0.306
Apgar 1 min (mean)	8.9	8.83	0.649
Age at discharge from hospital, days (mean)	2.85	2.63	0.259

^a Chi-Square of Fisher's exact was used to compare categorical variables. Independent samples T-test was used to compare parametric linear variables and Mann Whitney U test to compare nonparametric linear and ordinal variables.

^b ISCED education classification 0-8: 0=early childhood education. 1=primary education. 2=lower secondary education. 3=upper secondary education. 4=post-secondary non-tertiary education. 5=short-cycle tertiary education. 6=bachelor or equivalent level. 7=master or equivalent level. 8=doctoral or equivalent level

Table 3: Outcome of patients and controls

Neurodevelopmental outcome	CMV positives n 37	Controls n 51	p^a
Griffiths General Quotient (mean)	100.97	101.59	0.557
Griffiths Locomotor (mean)	100.8	100.5	0.715
Griffiths Personal-Social (mean)	105.5	104.6	0.721
Griffiths Hearing and Language (mean)	101.2	102.0	0.472
Griffiths Eye and Hand Coordination (mean)	103.2	101.7	0.650
Griffiths Performance (mean)	96.3	99.8	0.173
Hearing outcome	CMV positives n 35	Controls n 46	
TEOAE both ears pass	23	35	0.572
TEOAE right ear pass	25	36	
TEOAE right ear fail	2	4	
TEOAE right ear technically unsuccessful ^b	8	6	
TEOAE left ear pass	25	38	
TEOAE left ear fail	2	2	
TEOAE left ear technically unsuccessful ^b	8	6	
TEOAE failed / technically successful measurements all ears	4 / 54	6 / 80	1.000
SF hearing thresholds (mean (min-max))	34.31 (25-45)	32.73 (20-40)	0.338

TEOAE: transient-evoked otoacoustic emission, SF: sound field audiometry

^a Chi-Square of Fisher's exact was used to compare categorical variables, and Mann Whitney U test to compare nonparametric linear and ordinal variables.

^b Unsuccessful measurement because lack of co-operation or other artefact such as noisy breathing

Figure legends:

Figure 1. Screening Flow Chart

Figure 2. Viral loads in screening saliva samples (ge per reaction of CMV DNA). The PCR viral loads in the false positive screening saliva samples were significantly lower than in true positive screening samples $p < 0.001$ (Mann-Whitney U test)

Figure 3: Griffiths Mental Development Scales of patients and healthy controls at 18 months. No significant difference between CMV positive and controls in the Sub Quotient or General Quotient. A Locomotor: $p = 0.715$, B Personal-Social: $p = 0.721$, C Hearing and Language: $p = 0.472$, D Eye-hand Coordination: $p = 0.650$, E Performance: $p = 0.173$, General Quotient $p = 0.557$. Mann-Whitney U.

^aFalse positives. Negative urine CMV culture at 3 months (14 subjects). One subject with no urine sample at 3 months did not have CMV IgG at 18 months age. One subject with negative urine CMV culture at 3 months age had later at 18 months age positive urine CMV culture and positive CMV IgG. She is regarded as having postnatal infection. Additional data in Supplemental Table 2.

Figure 1: Screening Flow Chart

Figure 2. Viral loads in screening saliva samples (ge per reaction of CMV DNA). The PCR viral loads in the false positive screening saliva samples were significantly lower than in true positive screening samples $p < 0.001$ (Mann-Whitney U test)

ge = genomic equivalent

Figure 3: Griffiths Mental Development Scales of patients and healthy controls at 18 months

No significant difference between CMV positive and controls in the Sub Quotient or General Quotient. A Locomotor: $p=0.715$, B Personal-Social: $p=0.721$, C Hearing and Language: $p=0.472$, D Eye-hand Coordination: $p=0.650$, E Performance: $p=0.173$, General Quotient $p=0.557$. Mann-Whitney U.

Symptomatic patients are presented with colored dots:

- child with small calcifications on US and intensified signal in periventricular space and deep white matter on MRI
- child with abnormalities in imaging: large germinolytic cysts, multiple small calcifications, intensified signal, polymicrogyria in frontal lobes on MRI
- child with significant microcephaly
- child with small calcifications in right basal ganglion on US