Cytomegalovirus DNA detection by polymerase chain reaction in cerebrospinal fluid of infants with congenital infection: associations with clinical evaluation at birth and implications for follow-up

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Running Title: hCMV-PCR in CSF of infants with cCMV

Summary: Human Cytomegalovirus DNA detection in cerebrospinal fluid by polymerase chain reaction has been previously considered a risk factor for hearing loss or neurological sequelae in congenital infection. In the present study, it was not associated with neurological or audiological outcomes.

Abstract

Background:

DNA detection of human cytomegalovirus (hCMV) in cerebrospinal fluid (CSF) by polymerase chain reaction (PCR) is a marker of central nervous system (CNS) involvement in congenital hCMV infection (cCMV) but its prognostic value is unknown.

Methods:

A multicenter, retrospective study was performed using the Spanish Congenital Cytomegalovirus Infection Database (REDICCMV; http://www.cmvcongenito.es). Newborns with cCMV and a lumbar puncture performed were included and classified according to their hCMV-PCR in CSF result (positive/negative). Clinical characteristics, neuroimaging abnormalities, plasma viral load and audiological and neurological outcomes of both groups were compared.

Results:

A total of 136 neonates were included in the study: 21 (15.4%) with positive CSF hCMV-PCR and 115 (84.6%) with negative results. Seventeen patients (81%) in the positive group were symptomatic at birth compared with 52.2% of infants in the negative group (OR: 3.86; 95%CI: 1.28-14.1; p=0.01). Only 4 asymptomatic newborns (6.8%) had a positive CSF hCMV-PCR. There were no differences between groups regarding the rate of microcephaly, neuroimaging abnormalities, neurological sequelae at 6 months of age or plasma viral load. Sensorineural hearing loss (SNHL) at birth was associated with a positive CSF hCMV-PCR (OR: 3.49; 95%CI: 1.08-11.27; p=0.04), although no association was found at 6 months of age.

Conclusions:

A positive hCMV-PCR result in CSF is associated with symptomatic cCMV and SNHL at birth. However, no differences in neuroimaging studies, plasma viral load or outcomes at 6 months were found. These results suggest that hCMV-PCR in CSF may not be a useful prognostic marker in cCMV.

Keywords: Human cytomegalovirus, congenital infection, polymerase chain reaction, cerebrospinal fluid, pediatrics.

INTRODUCTION

Human cytomegalovirus (hCMV) is the most common cause of congenital infection in developed countries, with an estimated prevalence of 0.3 – 2.4% of all live births [1]. It is also the leading non-genetic cause of sensorineural hearing loss (SNHL) [2], and can bring about a wide spectrum of neurodevelopmental disorders [3,4]. Among congenitally-infected infants, approximately 10% have signs and symptoms of disease at birth [3], which is the most important risk factor for long-term sequelae. Other risk factors to develop sequelae are elevated blood and urine hCMV viral load, which has been associated with a higher risk of SNHL [4], and abnormal central nervous system (CNS) neuroimaging, which is currently the best predictive tool of a poor neurological outcome [5,6].

Detection of hCMV by polymerase chain reaction (PCR) in cerebrospinal fluid (CSF) holds high specificity but low sensitivity for the diagnosis of congenital hCMV infection (cCMV) [4]. Although this parameter is considered a marker of CNS involvement, its prognostic value is currently unknown [7–9]. Increased cellularity and elevated protein or beta-2-microglobulin concentrations in CSF have been associated with CNS involvement and poor neurological outcome [4,8]. However, the indication of lumbar puncture (LP) in congenitally-infected children remains controversial, especially in those who are asymptomatic at birth [10,11]. The Spanish Consensus Guidelines on cCMV management recommend performing an LP in all children with cCMV despite the poor evidence for this recommendation [4,7,8].

The aim of this study was to describe the clinical features and outcomes of patients with a positive hCMV PCR in CSF, as compared with those with a negative result.

PATIENTS AND METHODS

Study population and Data collection

A multicenter, retrospective, observational study of infants with cCMV was performed within the Spanish Registry of Congenital Cytomegalovirus Infection (REDICCMV; http://www.cmvcongenito.es). This is a prospective longitudinal cohort started in January 2011 that includes children diagnosed with cCMV in 34 hospitals in Spain, followed since

birth. Recorded data include: maternal information and prenatal diagnostic testing (serology, amniocentesis, ultrasound), symptoms and diagnostic tests at birth (including neuroimaging and baseline hearing assessment), antiviral treatment and outcomes (clinical, neurodevelopmental and hearing evaluation every 6 months). At the time of this study (November, 2015), 311 children had been included in the registry, all of them with confirmed cCMV, defined as positive hCMV culture or identification of viral DNA by PCR in urine, blood or saliva during the first two weeks of life [12]. Patients diagnosed beyond the neonatal period by PCR in dried blood spots on Güthrie cards were also included in the Spanish Registry, but were excluded from this study.

Study data were collected and managed using REDCap (Research Electronic Data Capture) application tools hosted at Hospital Universitario 12 de Octubre, Madrid, Spain [13], which is a secure, web-based application designed to support data capture for research studies [13]. Data collection was done after obtaining parents' informed consent. The study was approved by the Institutional Review Board of Hospital Universitario 12 de Octubre.

Inclusion Criteria and Definitions

Children included in the study had to be diagnosed of cCMV at birth and a sample of CSF had to be obtained and tested for hCMV-PCR within 30 days of life and before antiviral treatment. A follow-up of at least 6 months was also required. Patients with other congenital infections besides hCMV, primary or secondary immunodeficiency, born to mothers with primary or secondary immunodeficiency and those with traumatic LP (defined as CSF specimens with ≥500 red blood cells/mm^3) [14–16], were excluded.

Symptoms of cCMV were evaluated in the cohort at birth and at 6 months of age.

Symptomatic infection at birth was defined by the presence of thrombocytopenia (platelet count <$100x10^3$/µL), petechiae, jaundice, hyperbilirrubinemia (direct bilirubin level >2 mg/dL), elevated alanine aminotransferase levels (>80 IU/L), hepatomegaly, splenomegaly, neurological symptoms (hypotonia, seizures, paresis or weak suck), choriorretinitis, small for

gestational age (SGA), microcephaly, SNHL or neuroimaging abnormalities in cranial ultrasound or magnetic resonance imaging (MRI) (excluding isolated lenticulostriate vasculopathy) [4]. Asymptomatic cCMV at birth was considered when none of the aforementioned signs or symptoms, laboratory or neuroimaging abnormalities were present after a complete evaluation. Asymptomatic infants were most often identified upon previous confirmed hCMV infection during pregnancy, a positive hCMV-PCR in donated or stored cord blood or when investigating causes of prematurity. Screening for congenital hCMV infection is not routinely performed in Spain. SGA was defined as a birth weight and/or length below a standard deviation (SD) of -2 for age and gender according to the Spanish Growth Charts updated in 2008 [17]. *Microcephaly* was defined as a head circumference below a SD of -2 for age and gender per the same Spanish Growth Charts [17]. As for *cerebral ultrasound and MRI abnormalities*, only those previously described in cCMV were considered, including periventricular calcifications, ventricular dilatation, periventricular and subependymal cysts, ventricular adhesions, cortical atrophy, cerebellar hypoplasia, polymicrogyria, lisencephaly and white matter abnormalities [6,18–20].

SNHL was defined as a hearing threshold >25 dB tested by brainstem-evoked response (BAER) in any ear. SNHL was evaluated at birth and after 6 months of follow-up.

Neurological abnormalities at 6 months of age were defined as the presence of epilepsy in treatment, motor impairment (spasticity and or paresis), chorioretinitis, microcephaly or neurodevelopmental delay; and were evaluated by the responsible physician with the help of a neuropediatrician depending on each center's availability. All newborns with SNHL or CNS involvement, including positive hCMV-PCR in CSF as an isolated finding, received antiviral (ganciclovir and/or valganciclovir) treatment for at least 6 weeks, in accordance with 2009 consensus document from the Spanish Society for Pediatric Infectious Diseases (SEIP) [4].

Lastly, it is important to remark that there was variability in the techniques employed for PCR and laboratory exam performance at different institutions. Seven different CMV-PCR kits

were used in 15 laboratories. All of them applied a quantitative real-time PCR technique coupled with automated nucleic acid extraction systems.

Statistical Analysis

Data analysis was performed using SPSS statistical software, version 22.0 (IBM Corp., Armonk, NY, USA). Continuous variables were described with mean and standard deviation (SD) or median and interquartile range (IQR). Categorical variables were described with absolute and relative frequencies. Comparisons between categorical variables in the two groups were performed using Chi-square or Fisher's exact test. Odds ratio (OR) with 95% confidence intervals (95%CI) were calculated for hCMV-PCR in CSF result and each variable. For SNHL and neurological abnormalities at 6 months, OR was adjusted for antiviral treatment length (in days) using a logistic regression model. Plasma hCMV viral load at diagnosis was transformed into the base-10 logarithmic scale, after confirming that our sample did not follow a normal distribution using the Shapiro-Wilk test. Contrast between groups for this variable was done using Student's t-test on log10 mean values. Antiviral treatment duration in symptomatic patients was compared in according with hCMV-PCR in CSF result using Mann-Whitney U test. A two-tailed *p* value <0.05 was considered statistically significant.

RESULTS

Study Population

LP was performed in 173 (55.6%) out of 311 children with cCMV registered in our database at the time of the study, 96 (55.5%) with symptomatic cCMV and 77 (44.5%) with asymptomatic infection. After the application of the inclusion and exclusion criteria, 136 children (45.7% males) were eligible for the analysis (Figure 1). Mean gestational age at birth was 37.2 (SD:3) weeks. Symptomatic cCMV at birth was detected in 77 cases (56.6%). Most frequent signs were petechiae/purpura (35%) and visceromegaly (24.7% hepatomegaly, 23.4% splenomegaly). LP was performed at a median age of 5 (IQR:1.9-

10.4) days. hCMV-PCR in CSF was positive in 21 children (15.4%) and negative in 115 (84.6%).

Comparisons according to the result of hCMV-PCR in CSF

Clinical and neuroimaging data of all patients and the comparison between those with positive and negative hCMV-PCR in CSF are shown in Table 1. Blood mean hCMV viremia was similar in both groups, 3.9 log10 copies/ml (SD:0.9) in positive group versus 3.6 (SD:1.1) in the negative group (p=0.98). A total of 118 patients had a hCMV-PCR performed in both blood and CSF. Of 22 patients with a negative blood hCMV-PCR, only 1 had a positive hCMV-PCR in CSF, but no association was found between a positive PCR in blood and CSF (p=0.12; OR: 4.8 [CI 95%: 0.6-38.4]). hCMV viral load in CSF was available only in 7 patients (median: 332 copies/ml; IQR:150-21000).

Positive hCMV-PCR in CSF was associated with a high rate of symptoms at birth and SNHL in the neonatal period, while no associations were observed between it and the other variables, including SNHL at 6 months of age (Table 1). Only 4 out of 59 (6.8%) asymptomatic patients had a positive hCMV-PCR in CSF. Their characteristics are detailed in Table 2. None of these children developed neurological abnormalities nor SNHL after 6 months of follow-up.

Antiviral Treatment

One hundred and five (77.2%) patients received antiviral therapy, including 28 patients with asymptomatic hCMV infection at birth and all the patients with symptomatic infection. All 21 patients with positive hCMV-PCR in CSF were treated in accordance with Spanish guidelines [4]. The asymptomatic patients that were treated showed laboratory test results close to the reference limit to define symptomatic infection, as per physician decision. There were non-significant differences in antiviral treatment duration between patients with positive and negative hCMV-PCR in CSF, 181 days (IQR:82-185.5) versus 82 days (IQR:16.5-194.5), respectively (p=0.97).

Of 90 patients with available results of hearing evaluations at birth and at 6 months of age, 67 (74.4%) received antiviral treatment (Table 1). There was no association between hCMV-PCR result in CSF and SNHL at 6 months in these children (Table 1). When adjusted for antiviral treatment length, no significant association was found between hCMV-PCR result in CSF at birth and SNHL or neurological abnormalities at 6 months (Table 1). Remarkably, in the group with positive hCMV-PCR in CSF, none of the patients with normal hearing at birth developed SNHL at 6 months, whereas this sequela developed in 3 untreated patients in the negative group. Their characteristics are detailed in Table 3.

DISCUSSION

The significance of positive hCMV-DNA detected by PCR in CSF in the prognosis of infants with cCMV is not well established. In the cohort presented here with 136 patients, positive PCR results were more common among infants with symptoms and SNHL at birth, similar to the findings described by Halwachs-Baumann *and cols,* who reported CSF PCR results in 27 symptomatic children [9]. However, this was not a significant prognostic factor in this study as it did not correlate with neuroimaging abnormalities at birth, or with hearing loss or neurological abnormalities at 6 months of age. Literature on this topic is scarce and yields conflicting results. In a small case series including 13 infants, a positive CSF CMV-PCR at birth correlated with poor neurodevelopmental outcomes [7]. On the other hand, a more recent study of 22 children showed that hCMV detection in CSF was not associated with increased rate of SNHL or neuroimaging abnormalities [21]. In the present study, 4 asymptomatic newborns with a positive hCMV-PCR in CSF showed neither hearing loss nor neurologic impairment at 6 months of age. However, all children with a positive CSF result received antiviral treatment, regardless of the symptoms at birth, which may have biased our analysis. No association was found between a positive hCMV-PCR in CSF at birth and SNHL or neurological abnormalities at 6 months after adjusting for antiviral treatment length. However, antiviral treatment sure acts as a confounder and these results should be taken with caution.

We found an association between a positive CSF hCMV-PCR result and SNHL in the neonatal period, which was not reproduced at 6 months of follow-up. None of those newborns with normal hearing at birth in the positive CSF hCMV-PCR group developed SNHL after 6 months of follow-up, but all of them received antiviral treatment. Three patients in the negative CSF hCMV-PCR group developed SNHL at 6 months. Those 3 infants were untreated since they were asymptomatic at birth. Therefore, a negative result in CSF in asymptomatic children may not preclude the development of hearing loss in the future.

In cCMV, CSF parameters have been reported only rarely, and their role as a marker of brain damage remains unclear. CSF protein greater than 120 mg/dl is noted in approximately half of the cases [6,22,23]. In one study, increased protein levels were associated with neurological abnormalities as well as hearing loss [23]. Other studies found no association with imaging abnormalities on brain CT or poorer neurological outcomes [6,22]. Besides, CSF protein concentrations depend on serum protein concentrations and on the permeability of the blood-CSF barrier. Immaturity of the blood-CSF barrier is thought to result in higher CSF protein concentrations in neonates and young infants compared with older children and adults [24]. In recent years, the prognostic value of other biochemical markers in CSF has been investigated. In a series of 26 patients with symptomatic cCMV, elevated beta$_2$-microglobulin (β_2-m) levels in CSF at birth were the best independent biomarker of moderate-severe adverse prognosis. The combination of raised β_2–m and neuroimaging abnormalities further improved their predictive value [8]. Data regarding CSF protein and β_2-m levels were not consistently recorded in the Spanish Registry of Congenital Cytomegalovirus Infection, and were not included in our analysis.

Neurological symptoms due to hCMV are almost exclusive to cCMV. There has been an improved understanding of the neuropathogenesis of cCMV [25]. A prominent infiltration of fetal activated $CD8^+$ T-cells has been observed in fetuses with severe brain damage, probably reflecting the occurrence of hCMV-specific immune responses [25]. In these

fetuses, lesions suggestive of superimposed hypoxic damage have also been described in the context of severe placental infection [25]. These findings suggest that CNS anomalies induced by cCMV are likely to result from a direct effect of viral replication in the brain and from an indirect effect occurring at two different levels: in the brain, where the infection can induce immune-mediated damage, and in the placenta, where the infection can cause placental insufficiency and, consequently, hypoxic brain damage [25]. Therefore, newborns with significant neurological involvement but who were infected early in pregnancy may not have active encephalitis at birth.

Antiviral therapy is currently recommended in all newborns with CNS involvement, including those with SNHL, and it should be considered in symptomatic cCMV newborns, especially in those with serious end-organ disease [4,10,11,26]. On the other hand, there is a lack of data regarding the benefits of antiviral treatment on asymptomatic newborns and those with mild symptoms (such as isolated visceromegaly or mild thrombocytopenia). In the latter, the identification of prognostic factors at the time of diagnosis would be of great interest. However, several concerns arise regarding the potential prognostic utility of hCMV-PCR in CSF: there are no studies showing that a positive hCMV-PCR in CSF in asymptomatic children is associated with higher risk of long-term sequelae, although the rate of positive results is very low in asymptomatic children (6.8% in our series), which could make it difficult to obtain conclusive results. Besides, traumatic LP occurs frequently in newborns (30-46%), which could lead to false positive hCMV-PCR results [15]. We excluded patients with traumatic LP with more than 500 red blood cells/mm^3 in CSF, but it is unclear if lower RBC counts could lead to false positive results as well. Finally, a negative PCR in CSF at birth seems not to preclude late-onset hearing loss in asymptomatic children, at least in this cohort.

The main limitations of our study are its retrospective design, lack of CSF hCMV viral load, cell count, protein and β_2–m analysis recording, and the differences in each participating center regarding the performance of LP, antiviral therapy and patient follow-up (which

resulted in not all patients being tested for all variables). As antiviral treatment length may influence the prognosis in symptomatic patients [26], a prospective design would have been more appropriate to evaluate this variable. Antiviral treatment of asymptomatic newborns with positive PCR results may have influenced the good outcome of those patients. Children's development was not evaluated using standardized screening instruments/tools, so minor delays could have passed unnoticed. Another important limitation is the short follow-up period for SNHL and neurological abnormalities. Therefore, we intend to analyze the outcomes of these patients at the age of 3 to 4 years to better evaluate minor speech difficulties and delays.

However, this is the largest study published to date evaluating possible associations between CSF PCR in infants with cCMV and clinical outcomes. We have only included children with confirmed congenital infection and LP performed as newborns, and excluded those with traumatic LP to avoid false positive results. In this study, a positive CSF PCR was associated with symptomatic cCMV at birth but not with worse neurological or audiological outcomes at 6 months of age. LP in children with asymptomatic congenital infection does not seem useful as positive CSF PCR results occurred only rarely. There were asymptomatic children with negative CSF PCR result with hearing loss at 6 months of age, and the value of other biochemical or virological markers in CSF is not well known. Further prospective studies with longer follow-up periods are needed to fully determine if is there is an association of CSF PCR results with SNHL and neurological abnormalities.

NOTES

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Potential conflicts of interest

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Figure 1. Patient inclusion flowchart

[a]2 children with HIV infection (mother to child transmission) and 1 child with primary immunodeficiency (not yet classified).
[b]6 children born to mothers with HIV infection and 1 child born to a mother with common variable immunodeficiency.
Abbreviations: hCMV, human cytomegalovirus; HIV, human immunodeficiency virus.

Table 1. Clinical and neuroimaging characteristics and outcomes of patients included in the study. Comparison according to hCMV-PCR result in CSF.

Study Variables		Total of patients with or without study variable[a]	hCMV-PCR in CSF result		*p*-value	OR (95% CI)
			Positive	Negative		
Symptomatic hCMV infection at birth	**Yes**	77 (56.6%)	17 (81%)	60 (52.2%)	0.01	3.86 (1.28-14.1)
	No	59 (43.4%)	4 (19%)	55 (47.8%)		
Prematurity	**Yes**	39 (29.5%)	6 (28.6%)	33 (29.7%)	0.92	0.95 (0.31-2.62)
	No	93 (70.5%)	15 (71.4%)	78 (70.3%)		
Microcephaly at birth	**Yes**	12 (8.8%)	2 (9.5%)	10 (8.7%)	1	1.10 (0.15-5)
	No	124 (91.2%)	19 (90.5%)	105 (91.3%)		
Cerebral ultrasound abnormalities at birth	**Yes**	50 (37.6%)	11 (52.4%)	39 (34.8%)	0.14	2.05 (0.79-5.39)
	No	83 (62.4%)	10 (47.6%)	73 (65.2%)		
MRI abnormalities at birth	**Yes**	45 (65.2%)	11 (73.3%)	34 (63%)	0.46	1.61 (0.46-6.53)
	No	24 (34.8%)	4 (26.7%)	20 (37%)		
Sensorineural hearing loss at birth	**Yes**	29 (32.2%)	8 (57.1%)	21 (27.6%)	0.04	3.49 (1.08-11.27)
	No	61 (67.8%)	6 (42.9%)	55 (72.4%)		
Sensorineural hearing loss at 6 months of age	**Yes**	32 (35.6%)	8 (57.1%)	24 (31.6%)	0.07	2.89 (0.90-9.25)
	No	58 (64.4%)	6 (42.9%)	52 (68.4%)		
Sensorineural hearing loss at 6 months of age adjusted for antiviral treatment length	**Yes**	32 (35.6%)	8 (57.1%)	24 (31.6%)	0.48	0.63 (0.17-2.30)
	No	58 (64.4%)	6 (42.9%)	52 (68.4%)		
Sensorineural hearing loss at 6 months of age (only patients treated at birth)	**Yes**	29 (43.3%)	8 (57.1%)	21 (39.6%)	0.24	2.03 (0.62-6.70)
	No	38 (56.7%)	6 (42.9%)	32 (60.4%)		
Neurological abnormalities at 6 months of age	**Yes**	26 (25.7%)	6 (42.9%)	20 (23%)	0.18	2.49 (0.73-8.22)
	No	75 (74.3%)	8 (57.1%)	67 (77%)		
Neurological abnormalities at 6 months of age adjusted for antiviral treatment length	**Yes**	26 (25.7%)	6 (42.9%)	20 (23%)	0.40	0.63 (0.11-1.50)
	No	75 (74.3%)	8 (57.1%)	67 (77%)		

[a]Not all patients have been tested for all variables.

Abbreviations: CSF, cerebrospinal fluid; hCMV, human cytomegalovirus; PCR, polymerase chain reaction.

Table 2. Characteristics of asymptomatic patients with positive CSF hCMV-PCR result.

	Patient 1	Patient 2	Patient 3	Patient 4
hCMV infection diagnosis				
- **Age**	4 DOL	Fetal	7 DOL	Fetal
- **Test indication**	Preterm birth	Maternal seroconversion at 1st trimester	Preterm birth	Maternal CMV hepatitis (1st trimester)
- **Amniocentesis**	NP	27 WOG; PCR (-)	NP	31 WOG; PCR (+)
Fetal Neuroimaging				
- **Fetal US**	Normal	Normal	Normal	Normal
- **Fetal MRI**	NP	NP	NP	Normal (31 WOG)
Treatment during pregnancy	No	No	No	hCMV-IGIV 1 dose
Gestational age at birth	32 WOG	38 WOG	34 WOG	40 WOG
Weight at birth in gr.	1280 (-1.35 SD)	3.33 (0.38 SD)	1590 (-1.35 SD)	3025 (-0.75 SD)
Length at birth in cm.	38 (-1.58 SD)	51 (0.73 SD)	40 (-1.51 SD)	50 (0.13 SD)
HC at birth in cm.	28 (-0.94 SD)	35 (0.72 SD)	29 (-1.10 SD)	34.7 (-0.10 SD)
Sex	Male	Male	Female	Male
Neuroimaging at birth				
- **Cerebral US**	Normal	Normal	Normal	Normal
- **Cerebral MRI**	NP	NP	Normal	Normal
Laboratory data at birth				
- **ALT (U/L) / DB (mg/dL)**	32 / 2	50 / NP	50 / NP	69 / 0.8
- **Platelet count ($x10^3$/µL)**	453	228	169	277
CSF data at birth				
- **CC (cell/mm^3) / BC(cell/mm^3)**	NP	50 / 0	10 / 240	23 / 0
- **Prot (mg/dL)**	NP	84	85	112
Antiviral Treatment				
- **Antiviral and duration**	GCV 45 days	GCV 16 days VGC 37 days	GCV 21 days	VGC 180 days
6 months follow up assessment				
- **SNHL**	No	No	No	No
- **Neurological abnormalities**	No	No	No	No

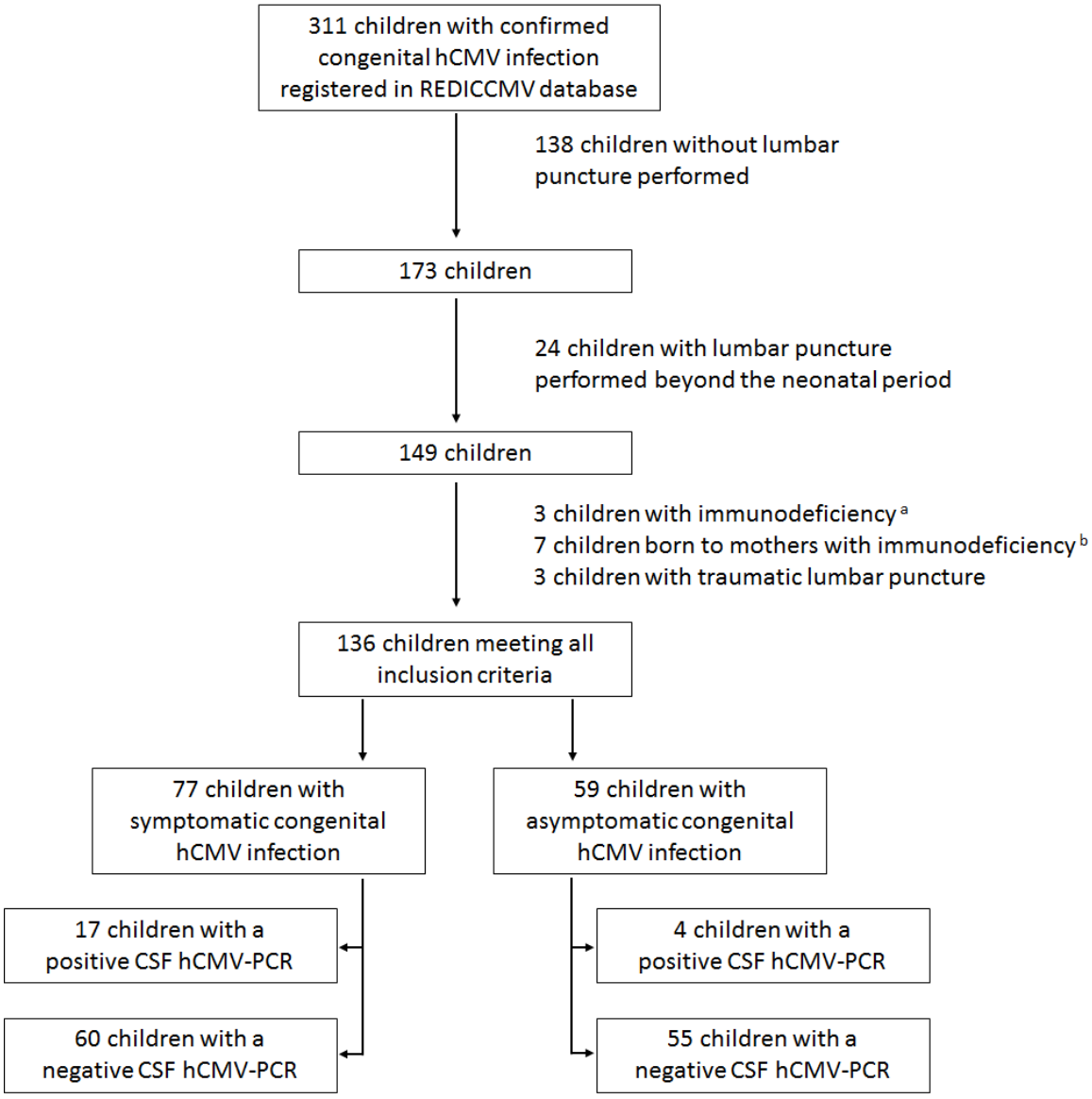
Abbreviations: ALT, alanine aminotransferase; BC, cerebrospinal fluid blood cells; CC, cerebrospinal fluid cell count; CSF, cerebrospinal fluid; DB, Direct bilirubin; DOL, days of life; GCV, ganciclovir; HC, head circumference; hCMV, human cytomegalovirus; hCMV-IGIV, human cytomegalovirus intravenous immune globulin; MRI, magnetic resonance imaging; NP, Not performed; PCR, polymerase chain reaction; Prot, cerebrospinal fluid proteins; SD, standard deviations; SNHL, sensorineural hearing loss; US, Ultrasound; VGC, Valganciclovir; WOG, Weeks of gestation.

Table 3. Characteristics of asymptomatic patients with a negative CSF hCMV-PCR result without receiving antiviral treatment who developed sensorineural hearing loss after 6 months.

	Patient 1	Patient 2	Patient 3
hCMV infection diagnosis			
- **Age**	Fetal	Fetal	4 DOL
- **Test indication**	Maternal seroconversion at 1st trimester	Maternal symptomatic CMV infection (mononucleosis-like syndrome) at 2nd trimester	Preterm birth
- **Amniocentesis**	20 WOG; PCR (+)	NP	NP
Fetal Neuroimaging			
- **Fetal US**	Normal	Normal	Normal
- **Fetal MRI**	29 WOG: Normal	NP	NP
Treatment during pregnancy	No	No	No
Gestational age at birth	38 WOG	38 WOG	34 WOG
Weight at birth in gr.	2280 (-0.62 SD)	2875 (-0.63 SD)	1680 (-1.39 SD)
Length at birth in cm.	51 (+0.73 SD)	47 (-0.97 SD)	43 (-0.69 SD)
HC at birth in cm.	33 (-0.64 SD)	34 (+0.04 SD)	30 (-0.75 SD)
Sex	Male	Male	Male
Neuroimaging at birth			
- **Cerebral US**	Normal	Normal	Normal
- **Cerebral MRI**	NP	NP	NP
Laboratory data at birth			
- **ALT (U/L) / DB (mg/dL)**	57 / 0.2	41 / 0.3	9 / NP
- **Platelet count (x10^3/µL)**	226	NP	190
CSF data at birth			
- **CC (cell/mm^3) / BC(cell/mm^3)**	28 / 0	20 / 180	60 / 477
- **Prot (mg/dL)**	105	81	110
6 months follow up assessment			
- **Neurological abnormalities**	No	No	No

Abbreviations: ALT, alanine aminotransferase; BC, cerebrospinal fluid blood cells; CC, cerebrospinal fluid cell count; CSF, cerebrospinal fluid; DB, Direct bilirubin; DOL, days of life; HC, head circumference; hCMV, human cytomegalovirus; MRI, magnetic resonance imaging; NP, not performed; PCR, polymerase chain reaction; Prot, cerebrospinal fluid proteins; SD, standard deviations; US, Ultrasound; WOG, Weeks of gestation.

Figure 1


311 children with confirmed congenital hCMV infection registered in REDICCMV database
138 children without lumbar puncture performed
173 children
24 children with lumbar puncture performed beyond the neonatal period
149 children
3 children with immunodeficiency a
7 children born to mothers with immunodeficiency b
3 children with traumatic lumbar puncture
136 children meeting all inclusion criteria
77 children with symptomatic congenital hCMV infection
59 children with asymptomatic congenital hCMV infection
17 children with a positive CSF hCMV-PCR
60 children with a negative CSF hCMV-PCR
4 children with a positive CSF hCMV-PCR
55 children with a negative CSF hCMV-PCR