Congenital Cytomegalovirus in Northern Sardinia: universal newborn neonatal hearing screening, audiological surveillance and outcomes.

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Abstract
INTRODUCTION: Cytomegalovirus (CMV) is the most common congenital infection and nongenetic cause of congenital sensorineural hearing loss and only 10% of cases are symptomatic at birth. Hearing loss is present in 50-60% of cases in symptomatic infants and up to 15% in asymptomatic cases. Late-onset cases, whose time of development cannot be predicted, are also reported (Marsico 2017). The study objective was to present outcomes of hearing-targeted CMV screening in North Sardinia. MATERIALS AND METHODS: Audiological findings and history of 44 children affected by congenital CMV, born between 2012 and 2023, and referred to the Audiovestibology Service of the University Hospital of Sassari were systematically analysed. All cases were evaluated through evoked otoacoustic emissions (OAE), diagnostic Auditory Brainstem Responses (ABR) and impedance analysis early in the phase of neonatal hearing screening. Pathological cases were identifyed cases and further diagnostic investigations and rehabilitation were undertaken when indicated. Subsequent audiological surveillance of non-pathological cases at screening was also carried out. RESULTS Of the 44 babies born by mothers positive to cytomegalovirus during pregnancy we report seven cases with hearing loss. In two cases the hearing loss has been bilateral and profound, three babies reported a unilateral hearing loss, one profound the others of moderate entity. Other two cases resulted affected by moderate bilateral SNHL. No cases of late-onset hearing loss were found. CONCLUSION This systematic collection provides an overview of the trend of HL related to congenital CMV in Northern Sardinia and is therefore aimed to stimulate a more frequent and steady program of neonatal screening and audiological long term follow up for this disease.

Keywords: congenital CMV, Universal newborn hearing screening, Congenital hearing loss

Introduction

Human Cytomegalovirus (CMV), also known as Human Herpesvirus 5 (HHV-5), is a member of the family Herpesviridae, reported to be globally highly common. Transmission can occur through contact with biological fluids (blood, saliva, urine, tears, vaginal secretions) and sexual intercourses.

Congenital CMV (cCMV) is the main cause of congenital infection in developed countries, with an incidence between 0.2% and 2.3% (Boppana, 2010).

The most frequent modality of vertical transmission occurs from mother to child during pregnancy (prenatal infection), during childbirth (perinatal infection) or through breastfeeding (postnatal infection).

Perinatal or postnatal transmission mostly occurs as asymptomatic infection and without late onset complications, except in rare exceptions.

Maternal infection can be primary or non-primary. Primary infection is contracted by the mother for the first time during pre-

gnancy, while non-primary is a condition of reactivation of the virus that was previously in the latent phase or a reinfection with a new strain in a woman who had previously already contracted CMV.

The risk vertical transmission is based on the type of maternal infection: for primary infections it varies between 30% and 40% in the first two trimesters of pregnancy and between 40% and 70% in the third trimester. However, the risk of transmission following non-primary infection is much lower (1-2%) (Revello 2002). In general, 10% of children with cCMV infection is expected to develop serious permanent injuries. Anyway, the timing of maternal infection is greatly correlated with the risk of sequalae in the children.

In fact, maternal primary infections during first trimester are considered at much higher risk of well-known early sequelae such as congenital malformations, premature birth, miscarriage and fetal death as well delayed undesired outcomes.

Usually 85-90% of newborns with congenital infection are asymptomatic but within the first years of life, approximately 10% of these may develop late sequelae, including in 5% of cases a late onset sensorineural hearing loss of varying degrees, which is in turn present at birth in only about 1% of cCMV cases (Marsico 2017).

As for infants with symptomatic cCMV infections, approximately 40% to 60% of them will manifest permanent sequelae, with sensorineural hearing loss (SNHL) being the most common, followed by cognitive impairment, retinitis, and cerebral palsy.

In general, hearing loss can be unilateral or bilateral (asymmetric or symmetric); in approximately half of the cases it presents a progressive course or with peri-verbal onset, ranging in extent from mild to profound.

Aim of the present study is to evaluate the impact of cCMV infection on hearing in the referral hospital of North Sardinia, the University Hospital of Sassari (AOUSS), with a Universal National Hearing Screening (UNHS) program in place and running since 2012.

Materials and methods

Since 2012, Audiovestibology Service of AOUSS has been in charge of the UNHS program, as a referral center for diagnosis and early treatment of childhood hearing loss for all the babies born at our facility and at the other hospitals in Northern Sardinia

NICU (Neonatal Intensive Care Unit) and non-NICU babies, born at AOU Sassari or referred from nearby centers lacking an onsite screening program, are screened with a multi-step protocol (De Luca, 2022).

The newborn babies population with audiological risk factor of CMV contracted prenatally, perinatally and postnatally is included in the UNHS and in the audiological surveillance program.

All cases are recorded in an anonymized database which has been systematically updated since 2012. Over the years, this has allowed us to have constant monitoring of the hearing effects of cCMV in our population.

The audiological screening protocol for CMV babies is summarized in Figure 1.

All newborns with known maternal infection or with isolated CMV on salivary, urinary or blood tests are directed to our Centre after the execution of transient evoked otoacoustic emissions (TEOAEs) before discharge (within 3 days) and independently of the outcomes of the first stage are referred to the second stage with a new TEOAEs and a diagnostic Auditory Brainstem Response (ABR) within the second month of life. Thus, infants with cCMV, regardless of hearing screening status, receive a further diagnostic audiological evaluation between 3 and 6 weeks of age.

Before testing, babies are evaluated by an otolaryngologist and parents are counseled again also on the practical execution and rationale of the diagnostic test.

Both asymptomatic and symptomatic cCMV cases were then subjected to subsequent diagnostic ABR on a quarterly basis within the second year of life. During the second year of life and based on the patient's cooperation and general cognitive conditions, they are directed to audiological surveillance with tympanometry and acoustic reflexes test and Performance/Play Audiometry and subsequentlyPure tone audiometry every six months up to the third year of life and then annually up to school age. In symptomatic cases with reported hearing loss, audiological monitoring is continuous on an annual audiological follow up.

Any baby with a hearing threshold higher than 25 dB in at least one ear at ABR is considered affected by hearing loss. Babies with a confirmed hearing loss are then referred to audiological evaluation that includes a tympanometry and acoustic reflexes test, Auditory Steady – State Responses (ASSR), neuropsychiatric and speech therapy evaluation within the 6th month of life to obtain the most accurate audiological diagnosis and properly recommend early rehabilitation or follow-up.

Results

Of the entire population of 15,357 newborns undergoing UHNS from January 2012 to December 2023, forty-four cases of cCMV were recorded. Out of them four cases reported between could not be systematically analyzed because of incomplete documentation or because they did not complete surveillance and where therefore lost at follow up. Of the entire newborn population screened for hearing during this period, cCMV had therefore a prevalence of 0.3%.

Of these cases 69% had a mother who tested IgM positive for CMV during third trimester of pregnancy.

Cytomegalovirus has been detected in 25 cases through blood test, 10 cases through urine testing, and in 9 cases it is not detected despite the mother's positivity during pregnancy.

No cases of non-primary maternal infection were recorded.

In our register we have recorded one case of cCMV infection contracted in the third trimester of monochorionic monoamniotic twins pregnancy with urine test positivity in both newborns without hearing impairment at either neonatal screening or audiological surveillance. Another recorded unusual finding is the conifection of CMV and Sars- cov2 during pregnancy with urine test positivity for CMV in a newborn and one more in a mother during the third trimester with resulting normal hearing level in both cases.

Two cases with cCMV polymorbidity and severe encephalopathy underwent antiviral therapy (ganciclovir and valganciclovir), in

Figure 1

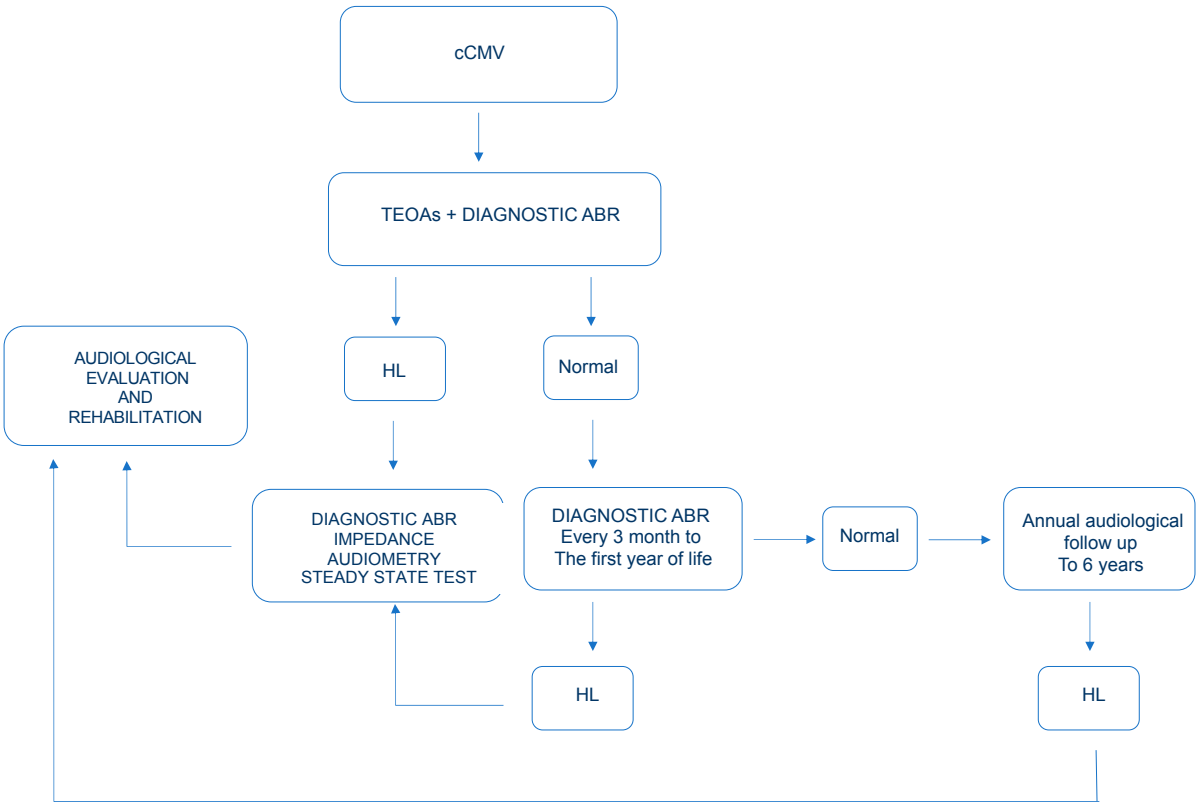

Table 1

AN	ABR	ABR	ABR	ABR	ABR	ABR	ABR	ABR
Patients	Right ear	Left ear	Right ear	Left ear	Right ear	Leftt ear	Right ear	Left ear
	0-3 month	0-3 month	3- 6 months	3-6 month	6- 9 months	6-9 months	9-12 monts	9-12 monts
1	10	10	10	10	10	10	10	10
2	10	10	10	10	10	10	10	10
3	20	20	20	20	20	20	10	10
4	10	10	10	10	10	10	10	10
5	10	10	10	10	10	10	10	10
6	20	20	10	10	10	10	10	10
7	10	15	10	10	10	10	10	10
8	20	60	10	50	10	50	10	50
9	10	10	10	10	10	10	10	10
10	10	10	10	10	10	10	10	10
11	10	10	10	10	10	10	10	10
12	10	10	10	10	10	10	10	10
13	10	10	10	10	10	10	10	10
14	10	10	10	10	10	10	10	10
15	10	10	10	10	10	10	10	10
16	10	10	10	10	10	10	10	10
17	10	10	10	10	10	10	10	10
18	10	40	10	50	10	40	10	40
19	10	10	10	10	10	10	10	10
20	10	10	10	10	10	10	10	10
21	60	70	50	60	60	70	60	70
22	10	10	10	10	10	10	10	10
23	10	10	10	10	10	10	10	10
24	10	10	10	10	10	10	10	10
25	10	10	10	10	10	10	10	10
26	70	70	70	60	60	60	60	60
27	10	10	10	10	10	10	10	10
28	10	10	10	10	10	20	10	10
29	10	10	10	10	10	10	10	10
30	10	10	10	10	10	10	10	10
31	10	10	10	10	10	10	10	10
32	10	10	10	10	10	10	10	10
33	10	10	10	10	10	10	10	10
34	10	10	10	10	10	10	10	10
35	10	10	10	10	10	10	10	10
36	40	40	40	40	40	40	40	40
37	10	10	10	10	10	10	10	10
38	10	10	10	10	10	10	10	10
39	10	10	10	10	10	10	10	10
40	90	80	90	80	80	80	80	80
41	10	10	10	10	10	10	10	10
42	10	10	10	10	10	10	10	10
43	10	10	10	10	10	10	10	10
44	10	10	10	10	10	10	10	10

one more case antiviral therapy was administered to the mother during pregnancy.

The results of the diagnostic ABR performed systematically as per protocol at quarterly intervals within the year are shown in table 1. At our institution, diagnostic ABRs are recorded in a silent room cabin by experienced audiological technicians.

In seven cases (out of the 44 cCMV infections) a sensorineural hearing loss has been detected. Five/7 cases with sensorineural hearing loss had NICU admission of which two were preterm newborns (before 33 weeks of gestation).

In two out of seven cases the hearing loss has been bilateral and profound, one of them progressive and both were associated with encephalopathy and died by the fourth year of life due to long-term systemic complications. Three cases resulted affected by left unilateral hearing loss, one profound the others of moderate entity, that did not change or worsen over the years. The last two cases resulted affected by moderate bilateral

sensorineural hearing loss, that required rehabilitation by hearing aids and speech therapy but did not show worsening of hearing levels over the years.

Discussion

Our screening program follows a fairly common protocol that involves TEOAEs as the test of choice for the first and second stage of screening and ABR for the definitive diagnosis (De Luca 2022). TEOAEs and aABRs are the most employed technologies in newborn hearing screening, as they are fast, reliable, and validated. (Papacharalampous 2011).

With regard to congenital CMV, early diagnosis of infection during pregnancy is crucial for the planning and subsequent audiological monitoring of the newborn. Numerous studies emphasise and agree on the relevance of frequent screening tests during pregnancy and in the prenatal period (Lazzarotto, 2008) (Boppana, 2022).

Maternal serological IgM and IgG Cmv screening test is offered in Italy to all pregnant women with unknown or negative serological status, at the first visit and in any case within the first trimester and repeated every 4-6 weeks up to 24 weeks of pregnancy (Guideline 1/2023 SNLG). This procedure is essential for early diagnosis of CMV infection and according to the literature the most definitive diagnosis of primary CMV infection in a pregnant woman is by detection of seroconversion (Revello, 2002).

As suggested by previous studies fetal CMV disease is preferentially associated with maternal infection occurring in the first part of pregnancy (Liesnard,2000).

In our population, seroconversion occurred in 69% of cases during the third trimester of pregnancy, which may explain the low incidence of HL.

For the diagnosis in the fetus the gold standard is detection in amniotic fluid (Donner 1994), whose sampling is an invasive procedure and therefore not frequently practiced.

A fascinating aspect is the vertical trasmission of CMV in twin pregnancy. Only few documented cases of congenital HCMV infection in twins (Schneeberger 1994) have been described in literature: in monozygotic twins with a monochorionic placenta, congenital CMV infection has been observed to occur in both children, while in dizygotic twins with a dichorionic placenta, only one of the twins was generally infected. Moreover Revello and Zavanotti (2002) reported absence of signs or clinical symptoms in both twins whit monochorionic-diamniotic placenta with CMV urine test positive at birth. Similarly, our cases of monochorionic-diamniotic twins with normal HL could makes us confident that over the years these cases will not develop hearing loss.

Another important marker is the presence of CMV DNA in the newborn's blood, where there is a correlation between viremia levels and encephalic abnormalities and SNHL (Donner 1994). In our records, CMV has been detected in 25 cases through blood tests, and in six out of seven cases reporting HL, viremia was high, particularly in the two cases with encephalic abnormalities and bilateral deep HL.

During the first three weeks of life, the detection of CMV in urine and saliva also proves to be a good indicator (Prosser 2021), so much so that it is used in several UNHS programmes in OAE first-step fail cases (Berrettini, 2017). Timing in performing these tests certainly played a crucial role in the detection of infection in the 9 cases that whit negative

on serology or salivary tests, despite mother's sieroconversion.

Regarding asymptomatic cases, in our population they represents about 84% of cases in accordance to the literature that reports a rate of asymptomatic cCMV infections at birth of 85-90% (Marsico 2017). These cases have a lower probability than symptomatic cases of developing SNHL and a high chance of passing the screening. This finding is further reinforced by the absence of late onset cases (6-7 years of surveillance is the protocol adopted at our center). Concurrently we are unable to predict with certainty whether HL may occur at a later stage, so audiological surveillance is strongly recommended in agreement with the literature that describes that besides maternal seroconversion, presence of symptoms at birth, "fail/refer" at newborn hearing screening, and the extent of viral blood load could are all predictors for the early or delayed SNHL (Lo, 2022).

CMV-related hearing loss may present in infants with symptomatic infection as bilateral, whereas in infants with asymptomatic cCMV, HL is often unilateral and postnatal, late-onset and/or progressive (Puhakka 2022). This is confirmed in our series where we recorded two symptomatic cases with bilateral moderate-severe and profound SNHL and five other asymptomatic cases of which 3 with unilateral hearing loss and 2 others with mild-moderate bilateral NSHL (of which one progressive).

While it is not still clear what factors correlate with the progression of hearing loss, several authors hypothesize that the natural history of a hearing loss from cCMV is in fact characterized by progression and, that this is also reflected at the sensorineural hearing threshold better at birth is more easily tested later and thus allows identification of possible progression than in those who already have profound hearing loss at the outset. These findings lead to long-term follow-up of children with cCMV to detect progression of hearing loss (Madden, 2005) as well as in cases with unilateral SNHL where a late-onset SNHL of contralateral ear in literature has been demonstrated 50% of cases (Rohren 2024).

Because of awareness of such a natural history of hearing loss in CMV, in agreement with the literature and international guidelines (JEHDI 2019), the audiological surveillance program at our Center is extended until school age even on cases with normal hearing thresolds at birth.

Conclusions

The results we have reported on the audiological effects of CMV in the population of newborns in North Sardinia over more than 10 years of systematic collection is therefore aimed to stimulate a more frequent and steady program of neonatal screening and audiological surveillance. Early diagnosis and treatment will indeed significantly affect the development of cognitive-linguistic skills and thus the entire life of the individual apporting benefits that would go far beyond just preventing or curing deafness.

Conflicts of interest

The authors declare no conflict of interest.

Informed consent statement

Patient consent was waived due to the retrospective design and anonymous nature of data.

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