Hearing loss in children with congenital cytomegalovirus infection: an 11-year retrospective study based on laboratory database of a tertiary paediatric hospital

**SUMMARY**

Congenital cytomegalovirus infection is considered the main cause of infantile non-genetic neurosensory hearing loss. Although this correlation was described more than 50 years ago, the natural history of internal ear involvement has not yet been fully defined. Hearing loss is the most frequent sequela and is seen in a variable percentage up to 30%; the hearing threshold is characterised by fluctuations or progressive deterioration. The purpose of this study was to evaluate the prevalence of hearing loss in cases of congenital CMV infection from Modena county, starting from the database of the microbiology and virology reference laboratory. All children undergoing urine testing for suspected CMV infection or viral DNA testing on Guthrie Card in the period between January 2004 and December 2014 were enrolled in the study. Family paediatricians were contacted and asked about clinical information on the possible presence at birth or subsequent occurrence of hearing loss, excluding cases where this was not possible. The results showed an annual prevalence of congenital cytomegalovirus infection among suspected cases that was stable over time despite the progressive increase in subjects tested. The prevalence of hearing loss was in line with the literature, whereas in long-term follow-up cases of moderate, medium-to-severe hearing loss with late onset were not detected. The introduction of newborn hearing screening in the county has allowed early diagnosis of hearing loss at birth as non-TEOAE-born births underwent a urine virus test. Moreover, despite all the limitations of the study, we can conclude that European epidemiological studies are needed to better define the relationship between congenital CMV infection and internal ear disease as the impact of environmental and genetic factors is still not entirely clarified.

**KEY WORDS:** Cytomegalovirus • Congenital infection • Hearing loss • Newborn screening • Late sequelae

**RIASSUNTO**

Il citomegalovirus è la più comune causa di infezione congenita nei paesi industrializzati, con una prevalenza stimata intorno allo 0,3-2% dei nati vivi. Di questi, il 10-15% presenta sequelae tardive tra cui deficit visivi, uditivi, ritardo mentale. La infezione congenita da citomegalovirus, ad oggi, è ancora ritenuta la principale causa di ipoacusia neurosensoriale infantile su base non genetica. Sebbene tale correlazione sia stata descritta più di 50 anni fa, la storia naturale del coinvolgimento dell’orecchio interno non è stata ancora completamente definita. La ipoacusia neurosensoriale è la sequela più frequente ed in una percentuale variabile, a seconda delle casistiche fino al 30%, la soglia è caratterizzata da fluttuazioni o deterioramento progressivo. Lo scopo di questo studio è stato quello di valutare la prevalenza della ipoacusia nei casi di infezione congenita da CMV della provincia di Modena, partendo dal database del laboratorio di Microbiologia e Virologia, centro di riferimento per la intera provincia. Inoltre, ci si è proposti di valutare l’entità della ipoacusia come sequela tardiva. Sono stati arruolati tutti i bambini sottoposti ad esame delle urine per sospetta infezione da CMV o mediante ricerca del DNA virale su Guthrie Card, nel periodo compreso tra gennaio 2004 e dicembre 2014. Mediante il contributo dei pediatri di famiglia e del servizio di Audiologia di Modena sono state raccolte le informazioni cliniche sulla eventuale presenza alla nascita o insorgenza successiva di ipoacusia, escludendo dallo studio i casi in cui ciò non è stato possibile. I risultati hanno dimostrato una prevalenza annuale di infezione congenita da citomegalovirus stabile nel tempo nonostante l’aumento progressivo dei nati sottoposti al test. La prevalenza della ipoacusia nei casi di infezione congenita è in linea con la letteratura mentre non sono emersi, nel lungo periodo, casi con ipoacusia media/severa bilaterale ad insorgenza tardiva. La introduzione dello screening audiolinguale neonatale nella nostra regione (2012) ha permesso una diagnosi precoce della ipoacusia in quanto i nati con esito fai alle TEOAE venivano sottoposti anche al test per la ricerca del virus nelle urine. Pur con tutti i limiti di uno studio basato su revisione di casistica, possiamo concludere che permane la necessità di studi epidemiologici europei che consentano di definire meglio la relazione tra infezione congenita da CMV e patologia dell’orecchio interno in quanto l’impatto dei fattori ambientali e genetici non è ancora del tutto chiarito. La sinergia tra screening audiolinguale neonatale ed esecuzione del test per la ricerca del virus nelle urine si è dimostrata molto proficua.

**PAROLE CHIAVE:** Citomegalovirus • Infezione congenita • Ipoacusia • Screening neonatale • Sorveglianza audiologica
Introduction

Congenital cytomegalovirus (cCMV) infection is currently estimated to be the main cause of non-inherited sensorineural hearing loss. Considered the most common congenital infection in humans, cCMV has a prevalence in the hearing-impaired children population between 2% and 18%. The virus can be transmitted to the foetus through placental blood, contact with infected vaginal secretions during delivery, breast milk, or blood transfusions after birth. The risk of transmission to the foetus is estimated to be the main cause of non-inherited sensorineural hearing loss.

Although the association between cCMV and hearing loss was first noted 50-60 years ago, the natural history of the inner ear involvement is still unclear. Even if 85-90% of cCMV are asymptomatic at birth, 10-15% of these infants may develop hearing, visual, or neurodevelopmental impairment. Moreover, vestibular involvement, often underestimated, may contribute to delayed motor skill acquisition in these children. In view of the fact that most newborns are asymptomatic at birth and that there are no specific screening protocols to detect infection, the actual impact of cCMV is still not completely known. Sensorineural hearing loss is the most common sequela, affecting from 33 to 65% of symptomatic newborns and from 7 to 15% of asymptomatic ones, presenting in 30% of the cases, with fluctuating auditory thresholds, characterised by deterioration or improvement over time.

As no effective prenatal therapy or vaccine exist, and since most patients with cCMV recover spontaneously, no universal screening program for cCMV has yet been implemented, although its value is an increasingly debated issue. When urine and saliva cultures obtained within the first two weeks of life are positive for cCMV, clinicians carry out complete clinical and biochemical evaluation to identify whether the infection is symptomatic or asymptomatic. The outcome of these tests affects treatment decisions, as current guidelines recommend to treat only symptomatic infants with antiviral therapy during their first 30 days of life in the presence of central nervous system disorders or in cases of focal or severe organ diseases.

Materials and methods

Data were retrospectively collected from the laboratory database of the University Medical Hospital of Modena, the reference centre for diagnosis of viral infections for the entire county. All urinary CMV tests carried out between January 2004 (the starting point was linked to the introduction of new hospital software) and December 2014 were reviewed. As a first step, all children (0-12 years) with confirmed CMV infection were enrolled in the study. Diagnosis of CMV infection was carried out by testing urine samples using the shell vial assay (SVA). Cases of cCMV infection were defined as any newborn ≤ 14 days of age with a positive CMV urine test or with a positive polymerase chain reaction (PCR)-CMV test confirmed on Guthrie cards. The main reasons for performing a cCMV test were: incidental finding of maternal CMV during pregnancy, abnormal prenatal cerebral ultrasound, preterm delivery, microcephaly (head circumference < 3rd percentile), persistent jaundice, thrombocytopenia, neutropenia. Family paediatricians were contacted and asked if their patients experienced hearing loss, and additional information regarding hearing loss was obtained from the records of the ENT/Audiology and Paediatric Departments. A new audiological evaluation was prescribed only in cases of doubt.

Since January 2012, neonates born in Modena county routinely undergo hearing testings in accordance with the screening program implemented in the Emilia Romagna region (a two stage Transitory Evoked Otoacoustic Emission-TEOAE protocol, carried out in accordance with the recommendations of the American Academy of Pediatrics). Before 2012 the Auditory Brainstem Response (ABR) test was carried out only at the time of diagnosis of cCMV. Starting from 2012, in accordance with Italian paediatric guidelines, children with cCMV infection routinely undergo ABR as part of a long-term audiological program for cCMV infected patients, which foresees assessments at 1-3 and 6 months and annual assessments up to the 6-year age for neonates with normal hearing.

The severity of sensorineural hearing loss was defined as follows: normal hearing (0-15 dB), slight (16-25 dB), mild (≥ 26 to < 40 dB); moderate (≥ 41 to < 55 dB), moderate-severe (≥ 56 to < 70 dB), severe (≥ 71 to < 90 dB) and profound (> 90 dB).

Demographic data were obtained from the Regional Health Agency. This study was approved by the Ethics Committee of Modena county (n. 166/14), informed consent was obtained through the family’s paediatricians.

Results

Urinary CMV testing was carried out in 2966 children (3.9% of total live births) between 2004 and 2014, 1954 (65%) of whom were younger than 14 days (Fig. 1). Table I shows the prevalence of children tested positive or negative for cCMV in the county. CMV infection was confirmed in 339 children and information on hearing loss was available in 250 (73.8%): 45/250 were cCMV, while
205/250 were acquired (Fig. 2). Figure 2 also details the rates of acquired and congenital infections according to the availability of hearing loss information. All these children had at least 2 years of follow-up, and 51% had at least 8 years.

A few children (n = 6/250-13%) with cCMV infection had confirmed hearing impairment. Among these, 2 were diagnosed after 2012 through the neonatal hearing screening program, and were positive by TEOAE. The number of children presenting with hearing loss, the severity of disease and side of hearing impairment are shown in Table II. The prevalence of symptomatic cCMV after the introduction of newborn hearing screening (2/10) was 20%, while the proportion of symptomatic cCMV with hearing loss before the screening was 11%. Among the 205 children (82%) with acquired CMV, 6 (2.9%) had moderate to severe hearing impairment: in one case the Guthrie card excluded cCMV and 2 cases were associated with syndromes. The remaining 3 cases could be attributed to delayed diagnosis of cCMV (Table II). All the 6 cases with acquired CMV were born before the implementation of the newborn hearing screening.

Although the total number of samples analysed increased over the years (from 1.2% in 2004 to 4.9% in 2014), and

**Table I.** Number of newborns per year during the study period and number of children tested for suspected cCMV (age < 14 days of life). The prevalence calculated on our population are reported in brackets.

| Year | Total newborns | Children tested for suspected cCMV (prevalence %) | Children resulted negative for cCMV (prevalence %) | Children resulted positive for cCMV (prevalence %) * | Prevalence of cCMV among tested population |
|------|----------------|-----------------------------------------------|-----------------------------------------------|--------------------------------------------------|--------------------------------------------|
| 2004 | 6553           | 80 (1.2%)                                     | 72 (1%)                                       | 8 [5]                                            | 10%                                        |
| 2005 | 6770           | 163 (2.4%)                                    | 157 (2.3%)                                    | 6 [5]                                            | 3.7%                                       |
| 2006 | 6703           | 162 (2.4%)                                    | 158 (2.3%)                                    | 4                                                | 2.5%                                       |
| 2007 | 6857           | 132 (1.9%)                                    | 128 (1.8%)                                    | 4 [3]                                            | 3%                                         |
| 2008 | 7201           | 121 (1.6%)                                    | 118 (1.6%)                                    | 3                                                | 2.5%                                       |
| 2009 | 7151           | 151 (2.2%)                                    | 145 (2%)                                      | 6 [5]                                            | 4%                                         |
| 2010 | 7116           | 189 (2.6%)                                    | 184 (2.5%)                                    | 5 [4]                                            | 2.64%                                      |
| 2011 | 6949           | 224 (3.2%)                                    | 217 (3.1%)                                    | 7 [6]                                            | 3.1%                                       |
| 2012 | 6703           | 185 (2.7%)                                    | 185 (2.7%)                                    | 0                                                | -                                          |
| 2013 | 6311           | 247 (3.9%)                                    | 240 (4%)                                      | 7 [5]                                            | 2.8%                                       |
| 2014 | 6040           | 300 (4.9%)                                    | 295 (5%)                                      | 5                                                | 1.66%                                      |
| Tot  | 74354          | 1954 (2.8%)                                   | 1899 (2.5%)                                   | 55 [45]                                          | 2.8%                                       |

*: The number of newborns with information about hearing impairment are reported in squared brackets.
in particular due to the introduction of the newborn hearing screening test, the prevalence of cCMV among tested children reduced from 10% to 1.6%. Figure 3 shows the number of cCMV cases detected per year.

Discussion

This retrospective study is based on the laboratory database of a tertiary university hospital, which is the reference center for the entire country. We found that the number of tested infants increased almost four-fold during the study period, demonstrating the efforts of clinicians in identification of asymptomatic infants at least among the population at high risk (i.e. preterm infants, intrauterine growth retarded infants).

In particular, during the first years of the study period, clinical suspicion of infection mainly led to the urine CMV test. Near the end of the study period, CMV screening was increasingly addressed to asymptomatic high-risk infants in order to improve early diagnosis and secondary prevention strategies.

Large prospective studies reported a prevalence of 0.4-0.53% of cCMV(14), a recent systematic review indicates a median prevalence of 0.58% 3. In our sample, the prevalence of cCMV among tested children has showed fluctuations ranging from 1.6% to 10% that can be ascribed to the different sample size, the retrospective nature of the study and the type of population (clinical suspicion of congenital infection and/or high risk infants).

Interestingly, despite the increasing number of infants tested during the study period, the percentage of cCMV decreased, reaching the lowest percentage in the year with the larger number of infants tested. The greater and less
selected the tested population, the lower the prevalence of cCMV. In this regard, a recent review examining the disease burden in Europe suggested that more epidemiological knowledge of cCMV infection is needed to assess its actual prevalence. Epidemiological patterns over the years show fluctuations in viral congenital infection rates and differences in prevalence registered in industrialised and non-industrialised countries, suggesting that other factors, e.g. environmental or genetic, may be involved. Retrospective studies on populations of deaf children report a percentage of 2-18% of hearing loss cases due to cCMV, but its real prevalence is not yet precisely known, as in most countries CMV infection is not screened during pregnancy or in newborns. In the last 20 years, it has also been assumed that asymptomatic cCMV can be a significant cause of hearing loss. The question is still complex as childhood hearing impairment is the result of the intersection of different factors such as genetic predisposition and intrauterine, as well as perinatal and postnatal factors.

In our sample, the prevalence of hearing loss in cCMV infection was 13.3%, similar to that reported in a systematic review by Goderis, indicating an overall 12.6% prevalence of cCMV with a 76% rate of severe to profound hearing loss. In detail, the proportion of symptomatic cCMV with hearing loss is higher after the year of implementation of prevention programs such as the newborn hearing screening and Italian paediatric guidelines. It is well established that delayed identification of hearing loss can compromise a child's language acquisition and cognitive and psychosocial skills, but it is important to highlight that since hearing impairment may present after the neonatal period, a screening program at birth would fail to identify children with cCMV who will develop hearing loss later. In fact, in a recent study it was demonstrated that a cCMV screening program within the context of newborn hearing screening protocol can identify the majority of infants with CMV-related sensorineural hearing loss at birth, while missing cases at risk for late onset hearing impairment.

Currently there is a growing interest in the feasibility and benefit of linking screening for cCMV infection and neonatal audiological screening as many consider implementing a universal cCMV screening program to be an onerous solution. The only way to measure the real impact of cCMV related hearing impairment on the infant population, especially in the late onset form, would be to exclude with all certainty other comitant causes of hearing loss: for example, new hereditary sensorineural hearing loss are revealed every day, and it cannot be excluded that some genetic factors could facilitate virus-related damage to the inner ear.

None of the children of our study reported significant worsening of hearing loss after the neonatal period, and in particular none of the 51% with 8 years follow-up: this data confirms that delayed onset of hearing loss, even though possible, is not a predictable condition in cCMV infection. A recent study on a murine CMV-induced hearing loss model, which demonstrated that host derived inflammatory responses and not direct virus-mediated cytopathology may be responsible for hearing impairment, can explain the waywardness of cochlea-vestibular damage. Long-term audiological follow-up for at least 6 years is recommended, and audiological surveillance of children together with monitoring of communicative skills are essential to minimise adverse consequences of hearing loss, and require a multidisciplinary approach to the problem. This retrospective study has some limitations. Firstly, our sample accounts for only 2.6% of the infants born during the study period, and no information is added with respect to the prevalence of cCMV infection in the general population. Secondly, we cannot exclude that a few children who were scored normally hearing by paediatricians might suffer from slight/mild hearing loss or a monolateral loss whose signs were not reported by parents. Finally, due to organisational issues, we were unable to detect CMV DNA by PCR on neonatal dried blood spots when the urine sample was collected later than 14 days of life. Taking into account all these limitations, we agree on the necessity of more epidemiological European studies, mainly because the impact of environmental and genetic factors is still not fully understood. The synergy between universal newborn hearing screening program and cCMV testing for asymptomatic infants who failed the TEOAE test allows early diagnosis of congenital CMV infection and the beginning of follow-up strategies and secondary prevention programs.

Conclusions
Congenital CMV infection is still one of the main causes of hearing loss in the paediatric population and a large debate on the introduction of a universal newborn screening for cCMV infection is ongoing. The question is complex, as childhood hearing loss is the result of different factors such as genetic predisposition as well as perinatal and postnatal factors. A targeted CMV approach testing newborns who fail hearing screening is useful to identify most of children with CMV-related sensorineural hearing loss as the impact of environmental and genetic factors on viral infection rates is still not fully understood.
Conflict of interest statement
None declared.

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Received: November 21, 2017 - Accepted: February 18, 2018

How to cite this article: Palma S, Roversi MF, Bettini M, et al. Hearing loss in children with congenital cytomegalovirus infection: an 11-year retrospective study based on laboratory database of a tertiary paediatric hospital. Acta Otorhinolaryngol Ital 2019;39:40-45. https://doi.org/10.14639/0392-100X-2020

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