Journal of Clinical Virology 96 (2017) 32–36

Contents lists available at ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

Full length article

Outcomes of congenital cytomegalovirus disease following maternal primary and non-primary infection

Antonietta Giannattasio*, Pasquale Di Costanzo, Arianna De Matteis, Paola Milite, Daniela De Martino, Laura Bucci, Maria Rosaria Augurio, Carmela Bravaccio, Teresa Ferrara, Letizia Capasso, Francesco Raimondi

Department of Translational Medical Sciences-Division of Neonatology, Università "Federico II", Naples, Italy

ARTICLE INFO

Keywords:
Congenital CMV disease
Maternal serology
Neuroimaging
Prognosis

ABSTRACT

Background: Natural history and long term prognosis of congenital cytomegalovirus (CMV) disease according to maternal primary versus non-primary infection are not clearly documented.
Objective: To investigate clinical, laboratory and neuroimaging features at onset and long term outcome of congenitally CMV-infected patients born to mothers with non-primary infection compared with a group of patients born to mothers with primary infection.
Study design: Consecutive neonates born from 2002 to 2015 were considered eligible for the study. Patients underwent clinical, laboratory and instrumental investigation, and audiologic and neurodevelopmental evaluation at diagnosis and during the follow up.
Results: A cohort of 158 congenitally infected children was analyzed. Ninety-three were born to mothers with primary CMV infection (Group 1) and 65 to mothers with a non-primary infection (Group 2). Eighty-eight infants had a symptomatic congenital CMV disease: 49 (46.2%) in Group 1 and 39 (60%) in Group 2. Maternal and demographic characteristics of patients of Group 1 and Group 2 were comparable, with the exception of prematurity and a 1-min Apgar score less than 7, which were more frequent in Group 2 compared to Group 1. Prevalence of neuroimaging findings did not significantly differ between the two groups. An impaired neurodevelopmental outcome was observed in 23.7% of patients of Group 1 and in 24.6% cases of Group 2. Similarly, the frequency of hearing loss did not differ between the two groups (25.8% versus 26.2%, respectively).
Conclusions: Neurodevelopmental and hearing sequelae are not affected by the type of maternal CMV infection. Preventing strategies should be developed for both primary and non-primary infections.

1. Background

Congenital cytomegalovirus (cCMV) infection is a common cause of neurodevelopmental disabilities [1]. Unlike other perinatal infections as congenital rubella or toxoplasmosis, CMV maternal immunity acquired prior to conception does not ensure a complete protection of fetus from infection [2–6]. Approximately 40% of women experiencing a CMV primary infection during pregnancy will transmit virus to their fetus. Of the infants infected in utero, about 10% will exhibit some symptoms at birth that are consistent with cCMV symptomatic infection [6]. In case of maternal non-primary infection, the risk for fetus to be infected by CMV is around 1% [5,7–9]. Earlier studies showed that maternal immunity to CMV prior to pregnancy can prevent CMV-related fetal damage [10,11]. More recent data have indicated that a preconceptional maternal immunity cannot be viewed as protective in terms of CMV fetal damage and hearing loss [8,12–15]. However, differences in natural history and long term prognosis of cCMV disease according to maternal primary versus non-primary CMV infection are not clearly documented.

2. Objectives

To compare clinical, laboratory and neuroimaging features at onset and long term outcome of patients with cCMV born to mothers with non-primary infection and those born to mothers with primary infection.

Abbreviations: cCMV, congenital cytomegalovirus; HUS, head ultrasound; LSV, lenticulostriated vasculopathy; CT, computed tomography; MRI, magnetic resonance imaging

* Corresponding author at: Department of Translational Medical Sciences-Division of Neonatology, Università "Federico II", Via Pansini 5,80131, Naples, Italy.
E-mail address: antogianna@libero.it (A. Giannattasio).

http://dx.doi.org/10.1016/j.jcv.2017.09.006
Received 20 April 2017; Received in revised form 5 September 2017; Accepted 10 September 2017

3. Study design

3.1. Study population

The study was conducted at the Perinatal Infection Unit of the University Federico II (Naples, Italy), a center with a dedicated multidisciplinary team. Neonates born from 2002 to 2015 with cCMV infection were considered eligible for the study. Infants were referred to our unit because of the presence of cCMV-related symptoms at birth or because of evidence of maternal infection on serologic screening during pregnancy. Diagnosis of cCMV infection was based on virus detection by polymerase-chain reaction assay in neonatal urine samples collected within 2 weeks. Symptomatic cCMV infection was defined in the presence of microcephaly (head circumference < 2 SD below the mean for age and birth weight), seizures, chorioretinitis, hepatosplenomegaly, petechiae, elevated serum transaminase levels, cholestasis, thrombocytopenia (< 10,0000 platelets/mm^3), hearing impairment, and abnormal findings on central nervous system (CNS) imaging evaluation (single or multiple calcifications, ventricolomegaly, cerebral atrophy, white matter or neuronal migration abnormalities) [16]. Isolated lenticulostriated vasculopathy (LSV) detected at Head Ultrasound (HUS) in absence of other abnormalities was not considered as a sign of symptomatic cCMV infection [17]. The study protocol matched the standard care applied in our center to all infants with cCMV infection [18]. Neuroimaging study included HUS and/or brain Computed Tomography (CT) and/or brain Magnetic Resonance Imaging (MRI).

Maternal CMV infections were categorized by analyzing maternal and newborn hospital records. Maternal primary infection was defined in the presence of serocoversion from negative to positive CMV-specific immunoglobulin G (IgG) antibodies during pregnancy; if prior CMV IgG were not available, diagnosis of presumed primary infection was based on presence of CMV–specific immunoglobulin M (IgM) antibodies and a low CMV IgG-avidity. A non-primary infection was defined in the presence of detectable CMV-specific IgG antibodies before pregnancy; if pre-conceptional IgG were not available, a presumed non-primary infection was based on presence of IgG at first antenatal blood sample taken within the first 12 weeks of gestation in absence of specific IgM-antibodies.

Infants were included in the study in the presence of certain classification of maternal CMV infection, complete data at diagnosis and during the observation period, and if the follow up was > 1 year. Patients with other perinatal infections or other chronic concomitant diseases were excluded.

The study was approved by the Ethical Committee of our Institution (protocol number 274/16).

3.2. Hearing and neurodevelopmental assessment

Audiological evaluation was performed every 3–6 months until the age of three years, and every 6–12 months later. Hearing function was evaluated at birth by auditory brainstem evoked responses (BAERs) test and during the routinely follow up by age-specific tests. Hearing thresholds were: 21–40 dB for mild hearing loss, 41–70 dB for moderate hearing loss, and > 70 dB for severe hearing loss [19].

Hearing loss was considered as sensorineural if the air-bone gap was less than 10 dB. Tympanometry was routinely performed in all cases to exclude middle ear disorders.

Neurodevelopmental examination was performed by using the Griffiths Mental Developmental Scales (version extended revised GMDS-ER 0–2 and 2–8) and by Denver test. The ages of posture-motor control milestone acquisition were carefully recorded for each child. Cognitive impairment in children aged ≥30 months was assessed by the Wescheler Scale, while behavioral problems in children aged ≥18 months were investigated by the Child Behavior Checklist (CBCL). An impaired neurological outcome was defined in the presence of developmental/cognitive impairment (DQ/IG < 70), motor delay requiring rehabilitation, epilepsy and behavioral/emotional problems (affective problems, attention deficit/hyperactivity and oppositional behavior). Severe mental retardation was defined in the presence of global IQ < 50. Sensorineural hearing loss and visual impairment were not included in the neurodevelopmental outcome but they were independently analyzed.

3.3. Statistical analysis

All data were recorded on a standardized case report form. Statistical analysis was performed using the Statistical Package for Social Science (SPSS). The chi square and the Fisher exact test were used to assess statistical significance of demographic characteristics, clinical data, and outcomes. P values < 0.05 were deemed as statistical significant.

4. Results

During the study period, 224 patients with cCMV were identified. Sixty-six children were excluded from the analysis because of the unavailability of preconceptional and/or prenatal maternal CMV exams. Of the remaining 158 patients, 93 (59%) were born to mothers with a primary CMV infection (Group 1) and 65 (41%) to mothers with a non-primary infection (Group 2) (Table 1). The reasons of cCMV-screening in newborns of non-primary infection group were presence of symptoms at birth in the majority of cases (39/65, 60%), abnormal findings on fetal US in 3 (4.6%) cases (intrauterine growth restriction in all cases), history of maternal immunosuppression in 2 (3.1%) cases. In the remaining 21 (32.3%) patients the diagnosis of cCMV was performed because of newborn CMV screening. No mothers in Group 1 and two (3.1%) mothers in Group 2 had immunosuppression during the pregnancy (p = 0.08). Immunosuppression was due to the need of high dose of steroids because of maternal multiple sclerosis in both cases. Eighty-eight infants were classified as having a symptomatic cCMV infection: 49/93 (46.2%) patients of Group 1 and 39/65 (60%) of Group 2. Signs and symptoms at diagnosis in the two groups of patients are presented in Table 2.

The majority of patients (n = 140, 88.6%) received more than one neuroimaging study (three neuroimaging studies in 116 cases). The prevalence of abnormal findings differed according to the type of neuroimaging exam, being LSV the most frequent finding detected by HUS, calcifications by CT, and white matter disease, callosal and

Table 1
Characteristics of patients with cCMV born to mothers with CMV primary and non-primary infection.

Features	Group 1: maternal primary infection (n = 93)	Group 2: maternal non-primary infection (n = 65)	P
Male, n	46 (49.5)	31 (47.7)	0.8
Age at last observation (years)	3.8 ± 2.6	3.6 ± 2.4	0.5
Abnormal findings on fetal US[a]	9/70 (12.8)	12/54 (22.2)	0.2
Mean gestational age at delivery	38 ± 2.6	35.9 ± 4.2	0.002
Preterm infants[b]	13 (14)	24 (37)	0.0008
Infant with a 1-min Apgar score < 7	4 (4.3)	12 (18.5)	0.008
Patients small for gestational age[c]	15 (16.1)	15 (23.1)	0.2

Values are expressed as numbers and percentages or mean and standard deviation (SD), as appropriated.

[a] Not available in all cases.

[b] Prematurity was defined in case of gestational age less than 37 weeks.

[c] Patients small for gestational age were classified in case of a birth weight below the 10th percentile conditional on gestational age and sex.

Table 2
Signs and symptoms of cCMV infection at onset in 88 symptomatic patients divided according to the type of maternal CMV infection.

Features	Group 1: maternal primary infection (n = 49)	Group 2: maternal non-primary infection (n = 39)	P
Severe onset	42 (85.7)	35 (89.7)	0.5
Microcephaly	6 (12.2)	11 (28.2)	0.06
Small for gestational age	12 (24.5)	11 (28.2)	0.7
Neurologic signs	7 (14.2)	13 (33.3)	0.03
Chorioretinitis	4 (8.2)	12 (30.8)	0.006
Liver involvement with cholestasis	11 (22.4)	8 (20.5)	0.8
Skin signs/petechiae	5 (10.2)	8 (20.5)	0.8
Thrombocitopenia	5 (10.2)	11 (28.2)	0.02
Pathological newborn hearing screening test	14 (28.6)	15 (38.5)	0.3
Abnormal HUS[a]	34/48 (70.8)	30/38 (78.9)	0.4
Abnormal brain CT[a]	28/46 (60.9)	21/33 (38.7)	0.8
Abnormal brain MRI[a]	27/44 (61.4)	29/35 (82.8)	0.06

Values are expressed as numbers and percentages.
[a] Not available in all cases.

Table 3
Abnormal findings at head ultrasound in 86 symptomatic cCMV infected patients divided according to the type of maternal CMV infection.

Findings	Group 1: maternal primary infection (n = 48)	Group 2: maternal non-primary infection (n = 39)	P
Lentriculostriated vasculopathy	23 (47.9)	15 (39.5)	0.4
Calcifications (single or multiple)	9 (18.7)	6 (15.8)	0.8
Pseudocysts/cysts	8 (16.7)	5 (13.2)	0.6
Ventriculomegaly	3 (6.2)	4 (10.5)	0.5
Cerebellar abnormalities	1 (2.1)	0	0.4
Germinolysis	4 (8.3)	0	0.06
Migration abnormalities	0	2 (5.3)	0.1

Values are expressed as numbers and percentages.

cerebellar malformations by MRI scans. Genetic disorders were ruled out in case of major CNS abnormalities as migration abnormalities or cerebellar malformations. Prevalence of neuroimaging findings at HUS, CT and MRI did not significantly differ between the two groups. HUS features in symptomatic patients are reported in Table 3. HUS revealed abnormal neuroimaging findings in 54.7% of asymptomatic patients, being LSV the most common detected feature.

Overall, 25 of 49 (51%) symptomatic patients of Group 1 received antiviral treatment (ganciclovir in 7 cases, valganciclovir in 11 and both treatments in 7 patients) versus 26/39 (66.6%) symptomatic patients of Group 2 (ganciclovir in 3 cases, valganciclovir in 10 and both treatments in 13 infants) (p = 0.5).

4.1. Neurodevelopmental and hearing outcomes according to maternal CMV immunity

No difference in the occurrence of sequelae between the two groups was found. Overall, an impaired neurodevelopmental outcome was observed in 22 (23.7%) patients of Group 1 and in 16 (24.6%) cases of Group 2 (p = 0.9). Neurodevelopmental sequelae in 22 patients of Group 1 were developmental/cognitive impairment in 10 (45.4%) cases (it was severe in 5 patients), language impairment in 5 (22.7%) patients, behavioral/emotional problems in 4 (18.2%) cases, motor delay in 2 (9.1%) subjects, epilepsy in one (4.5%) child. Sequelae in 16 patients belonging to Group 2 were developmental/cognitive impairment in 11 (68.7%) cases (it was severe in 5 cases), language impairment in 2 (12.5%) patients, behavioral/emotional problems in one (6.2%) child, motor delay in 2 (12.5%) cases (p > 0.05 for all type of neurodevelopmental sequalae compared to Group 1). Hearing loss was present in 24/93 (25.8%) patients belonging to Group 1 and in 17/65 (26.2%) patients of Group 2 (p = 0.8).

Table 4
Outcome in 88 symptomatic cCMV infected patients divided according to the type of maternal CMV infection.

Outcome	Group 1: maternal primary infection (n = 49)	Group 2: maternal non-primary infection (n = 39)	P
Neurodevelopmental impairment	16 (32.6)	16 (41)	0.4
Sensorineural hearing impairment	21 (42.9)	14 (35.9)	0.5

Values are expressed as numbers and percentages.

Then, we separately analyzed the outcomes according to the presence or not of symptoms at birth. In the group of 88 symptomatic patients, neurodevelopmental and hearing outcomes did not differ according to the type of maternal CMV infection, as reported in Table 4. As for the type of hearing impairment, it was severe in 9/21 (42.9%) cases of Group 1 and in 5/14 (35.7%) patients of Group 2 (p = 0.7). Furthermore, the occurrence of bilateral versus monolateral hearing loss did not differ between the two groups (47.6% of bilateral impairment in Group 1 versus 42.8% in Group 2; p = 0.8). Considering that a proportion of symptomatic patients had received antiviral treatment, we also compared outcomes in treated and untreated patients divided according to maternal infection. No significant difference in the rates of patients with neurodevelopmental and hearing impairments between treated and untreated patients was observed, as presented in Table 5.

As for the group of 70 asymptomatic patients, an impaired neurodevelopmental outcome was observed in a higher percentage of cases of patients of Group 1 compared to Group 2 (Table 6). Neurodevelopmental sequelae were mild motor impairment in 2 cases, language impairment in 2 cases, epilepsy in one patient and behavioral/emotional problems in one case. Overall, 6/70 (8.6%) asymptomatic patients had a sensorineural hearing loss at the end of observation period (Table 6).

5. Discussion

This study addresses the impact of a preconceptional maternal CMV immunity on clinical, laboratory and neuroimaging data in the newborn period and on long term prognosis of cCMV disease in a large number of infected patients. We found a maternal non-primary CMV infection in about 40% of cCMV patients. Previous reports showed a significant percentage of maternal non-primary cCMV infection in populations with medium-high seroprevalence of CMV [2,4,20,21].

Paucity of reports exists on maternal factors at risk to deliver a newborn with a cCMV disease [22,23]. Maternal immunosuppression status may represent a factor affecting the transmission of CMV during pregnancy in women with preexisting immunity. Although we recognize that the number of immunosuppressed mothers in our study is too small to draw conclusions, maternal immunological status of women with non-primary CMV infection has not been evaluated specifically in previous studies. Studies investigating conditions that may cause interference with immune system leading to higher maternal susceptibility to latent viral reactivation are desirable.

The present study shows that clinical findings at birth and a severe cCMV disease are not affected by the type of maternal infection. Boppana et al. reported similar demographic characteristics in 8 cCMV infected children born to mothers with non-primary infection and in 35 infants born to mothers with primary or unclassified infection [14]. However, in this study the type of maternal infection could be ascertained in only 43% of cases. This is the first study that describes

Table 5
Neurodevelopmental and hearing outcomes in 88 symptomatic cCMV infected patients divided according to the antiviral treatment and to the type of maternal CMV infection.

	Group 1: maternal primary infection (n= 49)		Group2: maternal non-primary infection (n = 39)	
	Treated (n = 25)	Untreated (n = 24)	Treated (n = 26)	Untreated (n = 13)
Neurodevelopmental impairment	14 (56)	8 (33.3)	12 (46.1)	4 (30.8)
Sensorineural hearing impairment	16 (64)	5 (20.8)	10 (38.5)	4 (30.8)

Values are expressed as numbers and percentages.

Table 6
Outcome in 70 asymptomatic cCMV infected patients divided according to the type of maternal CMV infection.

Outcome	Group1: maternal primary infection (n = 44)	Group2: maternal nonprimary infection (n = 26)	P
Neurodevelopmental impairment	6 (13.6)	0	0.04
Sensorineural hearing impairment	3 (6.8)	3 (11.5)	0.4

Values are expressed as numbers and percentages.

neonatal neuroimaging findings according to maternal CMV seroimmunity. We found a similar frequency of brain abnormalities in non-primary infection group compared to primary infection group, both in symptomatic than in asymptomatic patients. In asymptomatic patients, LSV at HUS was the prevalent finding. It is to note that, although LSV seems to be a brain marker of CMV infection, its prognostic role as isolated finding is still debated [17]. Hadar et al. found higher rates of brain US abnormal findings in CMV primary infection group compared to those detected in non-primary infection group [23]. However, in this study there is no description of the type of findings detected, nor is reported if children underwent other neuroimaging studies. Furthermore, the number of symptomatic patients following a non-primary infection was too small (n = 12) to draw conclusions.

We also investigated the role of maternal CMV status on long term outcome of cCMV infection. A hearing damage was present in 26% of the whole population of our cCMV patients, without difference according to maternal serological status. Data from literature report a sensorineural hearing loss in about 30–40% of symptomatic cCMV infected patients [24,25]. Ross et al. found a less severe hearing loss in case of non-primary infection compared with children born to mothers with CMV primary infection [13]. In our study, we observed no significant difference in the occurrence of severe and/or bilateral hearing impairment according to type of maternal infection. Furthermore, in Ross et al. paper is not specified if hearing loss occurred only in symptomatic or also in asymptomatic cases. In our study, hearing impairment occurred in about 11% of asymptomatic patients belonging to the non-primary infection group. This result indicates that preconceptional CMV maternal immunity did not prevent hearing deterioration also in case of absence of symptoms at birth. As a consequence, newborn hearing screening as single test might fail to identify cCMV infected subjects which are asymptomatic at birth, but at risk to develop sensorineural hearing loss later in life. On the other hand, we found no neurodevelopmental impairment at the end of follow up in asymptomatic patients born to mothers with non-primary CMV infection, compared to about 14% of mild neurological defects in the group of maternal primary infection. Boppana et al. reported mental retardation in 4/7 children of recurrent infection group versus none of 4 children primary infection group [14]. However, in this study psychometric tests were not performed because of a very young age at least observation of patients of the second group.

Our study has several points of strength. First, we excluded from the analysis all cases with incomplete or unclear maternal serological data. This allows to clearly classified patients in the group of maternal primary or non-primary infection. Second, we have a larger number of study participants and a longer observation period compared with previous studies [14,23]. Furthermore, some studies are focused only on symptomatic cCMV patients [23]. We separately analyzed outcomes of symptomatic and asymptomatic cases.

A possible limit of this study is our high rate of symptomatic patients (about 56%) and the high rate of clinical pathological findings in non-primary infection group. However, this rate is in agreement with previous reports [26], and it might have more than one explanation. First, it can be due to the type of referral center. Our Perinatal Infection Unit serves a wide area in Southern Italy though mostly symptomatic infants will be referred. Second, in our Country there is no extensive active search of CMV in newborns in absence of clinical problems at birth. The lack of a capillary screening for cCMV may allow to a number of asymptomatic infants to be undetected. This happens mainly in the presence of preconceptional maternal CMV seroimmunity (maternal non-primary CMV infection). Maternal CMV infection is usually diagnosed by routine screening in CMV seronegative women. In case of preconceptional CMV seroimunity or CMV-specific IgG antibodies at the first trimester of pregnancy, no other maternal CMV test is usually performed. The majority of patients with cCMV disease due to a non-primary infection were referred to our center because of abnormal signs/symptoms at birth, while less cases were identified because of newborn CMV screening. This may represent a population bias because the study includes infants with more severe symptomatic infection. However, we should consider that the rates of symptomatic patients did not significantly differ between primary and non-primary infection groups, allowing a reliable interpretation of outcomes results. Finally, it is to note that a standard definition of symptomatic cCMV infection is lacking [27]. Another limit of this study is that was not possible to differentiate secondary infection due to reactivation from that due to reinfection with a new CMV strain. However, this data lacks in the majority of available studies.

In conclusion, although preconceptional seroimmunity provides protection against intrauterine transmission of CMV, once fetal infection occurs the risk to develop symptoms and sequelae is similar to primary infection. Our data point up that CMV prevention strategies should target both mothers not immune to CMV than those with a preconceptional immunity, and raise the need of universal newborn screen as a major tool for a prompt diagnosis of cCMV infection.

Contributors and authorship

AG participated to the design of the study, analyzed data, interpreted results and drafted the article; PDC and TF contributed to the revision of draft and interpretation of data; ADM, DDM, LB, MRA and LC were involved in the acquisition and analysis of data; CB performed and revised neurodevelopmental outcomes; FR conceived the study and revised the article critically for important intellectual content.

Funding

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

Ethical approval

The study was approved to the Ethical Committee of the University Federico II of Naples (Italy) (protocol number 274/16).

Conflict of interest

No conflict of interest for all Authors.

References

[1] W. Britt, Cytomegalovirus, in: Jack S. Remington, J.O. Klein, C.B. Wilson, C.J. Baker (Eds.), Infectious Diseases of the Fetus and Newborn Infant, seventh edition, W.B. Saunders, Philadelphia, 2011, pp. 706–755.

[2] M.M. Mussi-Pinhata, A.Y. Yamamoto, R.M. Moura Brito, I.M. de Lima, P.F. de Carvalho e Oliveira, S. Boppana, W.J. Britt, Birth prevalence and natural history of congenital cytomegalovirus infection in a highly seroimmune population, Clin. Infect. Dis. 49 (2009) 522–528, http://dx.doi.org/10.1086/600882.

[3] K. Ahlfors, S. Harris, S. Ivarsson, L. Svanberg, Secondary maternal cytomegalovirus infection causing symptomatic congenital infection, N. Engl. J. Med. 305 (1981) 284.

[4] C. Wang, X. Zhang, S. Bialek, M.J. Cannon, Attribution of congenital cytomegalovirus infection to primary versus non-primary maternal infection, Clin. Infect. Dis. 52 (2011) e11–e13, http://dx.doi.org/10.1093/cid/ciq085.

[5] K.B. Fowler, S. Stagno, R.F. Pass, Maternal immunity and prevention of congenital cytomegalovirus infection, JAMA 289 (2003) 1008–1011.

[6] W. Britt, Controversies in the natural history of congenital human cytomegalovirus infection: the paradox of infection and disease in offspring of women with immunity prior to pregnancy, Med. Microbiol. Immunol. 204 (2015) 263–271, http://dx.doi.org/10.1007/s00430-015-0399-9.

[7] A. Kenneson, M.J. Cannon, Review and meta-analysis of the epidemiology of congenital cytomegalovirus (CMV) infection, Rev. Med. Virol. 17 (2007) 253–276.

[8] M.A. Gaytant, G.I. Rours, E.A. Steegers, J.M. Galama, B.A. Semmekrot, Congenital cytomegalovirus infection after recurrent infection: case reports and review of the literature, Eur. J. Pediatr. 162 (2003) 248–253.

[9] S.B. Boppana, L.B. Rivera, K.B. Fowler, M. Mach, W.J. Britt, Intrauterine transmission of cytomegalovirus to infants of women with preconceptional immunity, N. Engl. J. Med. 344 (2001) 1366–1371.

[10] S. Stagno, R.F. Pass, M.E. Dworsky, R.E. Henderson, E.G. Moore, P.D. Walton, C.A. Alford, Congenital cytomegalovirus infection: the relative importance of primary and recurrent maternal infection, N. Engl. J. Med. 306 (1982) 945–949.

[11] K.B. Fowler, S. Stagno, R.F. Pass, W.J. Britt, T.J. Boll, C.A. Alford, The outcome of congenital cytomegalovirus infection in relation to maternal antibody status, N. Engl. J. Med. 326 (1992) 663–667.

[12] A. Ornoy, O. Diav-Citrin, Fetal effects of primary and secondary cytomegalovirus infection in pregnancy, Reprod. Toxicol. 21 (2006) 399–409.

[13] S.A. Ross, K.B. Fowler, G. Ashrith, S. Stagno, W.J. Britt, R.F. Pass, S.B. Boppana, Hearing loss in children with congenital cytomegalovirus infection born to mothers with preexisting immunity, J. Pediatr. 148 (2006) 332–336.

[14] S.B. Boppana, K.B. Fowler, W.J. Britt, S. Stagno, R.F. Pass, Symptomatic congenital cytomegalovirus infection in infants born to mothers with preexisting immunity to cytomegalovirus, Pediatrics 104 (1999) 55–60.

[15] C.L. Townsend, M. Forsgren, K. Ahlfors, S.A. Ivarsson, P.A. Tookey, C.S. Peckham, Long-term outcomes of congenital cytomegalovirus infection in Sweden and the United Kingdom, Clin. Infect. Dis. 56 (2013) 1232–1239.

[16] S.B. Boppana, K.B. Fowler, Y. Vaid, G. Hedlund, S. Stagno, W.J. Britt, R.F. Pass, Neuroradiographic findings in the newborn period and long term outcome in children with symptomatic congenital cytomegalovirus infection, Pediatrics 99 (1997) 404–414.

[17] A. Giannattasio, P. Di Costanzo, P. Milite, D. De Martino, E. Capone, A. Romano, C. Bravaccio, L. Capasso, F. Raimondi, Is lenticulostriated vasculopathy an unfavorable prognostic finding in infants with congenital cytomegalovirus infection? J. Clin. Virol. 91 (2017) 31–35, http://dx.doi.org/10.1016/j.jcv.2017.04.006.

[18] Società Italiana di Neonatologia (SIN), Percorsi diagnostico-assistenziali in Ostetricia-Ginecologia e Neonatologia: Citomegalovirus (2012). Available at: https://www.neonatologia.it/upload/725_CITOMEGALOVIRUS_Aprile_2012.pdf.

[19] S. Bernard, S. Wiener-Vacher, T. Van Den Abbeele, N. Teissier, Vestibular disorders in children with congenital cytomegalovirus infection, Pediatrics 136 (2015) e887–e895, http://dx.doi.org/10.1542/peds.2015-0908.

[20] F.A. Colugnati, S.A. Staras, S.C. Dollard, M.J. Cannon, Incidence of cytomegalovirus infection among the general population and pregnant women in the United States, BMC Infect. Dis. 7 (2007) 71.

[21] J.J. de Vries, E.W. van Zwet, F.W. Dekker, A.C. Kroes, P.H. Verkerk, A.C. Vossen, The apparent paradox of maternal seropositivity as a risk factor for congenital cytomegalovirus infection: a population-based prediction model, Rev. Med. Virol. 23 (2013) 241–249, http://dx.doi.org/10.1002/rmv.1744 Epub 2013 Apr 5.

[22] E. Hadar, L. Salzer, E. Dorfman, J. Amir, J. Pardo, Antenatal risk factors for symptomatic congenital CMV disease following primary maternal CMV infection, J. Perinat. Med. 44 (2016) 339–344, http://dx.doi.org/10.1515/jpm-2014-0322.

[23] E. Hadar, E. Dorfman, R. Bardin, R. Gabbay-Benziv, J. Amir, J. Pardo, Symptomatic congenital cytomegalovirus disease following non-primary maternal infection: a retrospective cohort study, BMC Infect. Dis. 17 (2017) 31, http://dx.doi.org/10.1186/s12879-016-2161-3.

[24] M. Barbi, S. Binda, S. Caroppo, U. Ambrosetti, C. Corbetta, P. Sergi, A wider role for congenital cytomegalovirus infection in sensorineural hearing loss, Pediatr. Infect. Dis. J. 22 (2003) 39–42.

[25] A.J. Dahle, K.B. Fowler, J.D. Wright, S.B. Boppana, W.J. Britt, R.F. Pass, Longitudinal investigation of hearing disorders in children with congenital cytomegalovirus, J. Am. Acad. Audiol. 11 (5) (2000) 283–290.

[26] N. Oosterom, J. Nijman, J. Gunkel, T.F. Wolfs, F. Groenendaal, M.A. Verboon-Maciolek, L.S. de Vries, Neuro-imaging findings in infants with congenital cytomegalovirus infection: relation to trimester of infection, Neonatology 107 (2015) 289–296, http://dx.doi.org/10.1159/000375439.

[27] S.B. Boppana, S.A. Ross, K.B. Fowler, Congenital cytomegalovirus infection: clinical outcome, Clin. Infect. Dis. 57 (2013) S178–S181, http://dx.doi.org/10.1093/cid/cit629.