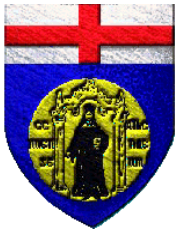

**Università degli Studi di Genova**

Dottorato di Ricerca in Scienze Pediatriche
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# Minor brain abnormalities in infants with congenital Cytomegalovirus infection

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*Alla mia famiglia*

## Background

Congenital Cytomegalovirus (cCMV) infection is the most common intrauterine infection and the leading cause of non-genetic sensori-neural hearing loss (SNHL) in children (1). Estimated prevalence is 0.6 to 0.7% live births in industrialized countries (2-4). In most neonates, the disease is asymptomatic. Symptomatic infection occurs in 10% of neonates, carrying a high risk for long-term sequelae mainly due to the involvement of the central nervous system (CNS) (5). Symptomatic cCMV infection is defined by the presence of physical, laboratory, or instrumental signs of either systemic or CNS involvement.

The spectrum of cerebral findings that can be detected in symptomatic neonates with cCMV infection is wide, ranging from extensive abnormalities (extensive calcifications, cortical malformations, microcephaly, severe ventriculomegaly, cerebellar hypoplasia) to minor signs of CNS involvement (e.g. isolated white matter abnormalities and germinolytic cysts) (6, 7). Of note, diagnostic accuracy of the different neuroimaging techniques (MRI, CT, ultrasound) in diagnosing each type of brain abnormality associated to cCMV varies widely, and a comprehensive neuroimaging evaluation has been advocated (7). SNHL may be identified in the neonatal period as well as develop later in childhood, even in previously asymptomatic subjects (8).

Despite the pathogenesis of cCMV-related sequelae has not been fully understood, persistent viral replication in the inner ear and CNS has been hypothesized. Studies investigating the correlation between blood or urine viral load in infancy and long-term hearing and neurological outcomes have shown conflicting results (9-13). Complete viral replication is possible in astrocytes, in endothelial cells of microvasculature and in neuronal precursor cells from the

subventricular zone of the cerebrum and the EGL of the cerebellum (14, 15). Viral DNA can arrest the cell cycle of these cells. Although not replicating in macrophages, these cells may play a role in viral dissemination. CMV hinders proliferation and neuronal differentiation in subventricular matrix. The virus migrates to the cortex with infected cells because infected cells may still migrate, although aberrantly; active neuroblast proliferation and migration between 5 and 25 weeks of gestation thus constitute a vulnerability window. Within the inner ear, CMV invades stria vascularis and endolymph spaces; the virus is not found in hair cells and ganglionic neurons (although they may disappear by atrophy); the infection eventually affects potassium homeostasis in endolymph spaces thus reducing auditory sensory cell activation: these phenomena lead to gradual impairment of auditory function in a slow and fluctuating process that may take up to three years In patients with cCMV infection, the virus may persist in the inner ear into adolescence. (16).

Among the available antiviral agents that are active against CMV, ganciclovir and its oral prodrug valganciclovir have been the best studied in neonates and infants. Ganciclovir is an analogue of nucleoside guanosine and was the first antiviral agent approved for the treatment of CMV infection (17). Ganciclovir is highly active against CMV, as shown in both “in vitro” and “in vivo” studies (18, 19). Viral and cellular enzymes of the host convert ganciclovir to ganciclovir triphosphate, which inhibits the synthesis of viral DNA (17, 19). This inhibition is enhanced by the persistence of significantly higher levels of ganciclovir triphosphate in CMV-infected compared to uninfected cells (20).

Due to the limited absorption of orally administered ganciclovir, the drug is given intravenously. The most common adverse event among infants treated with ganciclovir is bone marrow suppression (neutropenia, thrombocytopenia), which

is reversible after withdrawal of the drug (21, 22). The potential for permanent reproductive toxicity, mutagenicity and teratogenicity has been shown in animal studies (17, 18). Furthermore, recent studies have confirmed ganciclovir mutagenicity in humans (23, 24).

Valganciclovir is a prodrug developed to increase the bioavailability of orally administered ganciclovir. Valganciclovir oral bioavailability is around 60%, 10-fold higher than ganciclovir (25). Mechanism of action, adverse events and toxicity are essentially the same as those for ganciclovir (17).

For infants younger than 1 month with symptomatic cCMV infection, 6 months of valganciclovir therapy has been shown to be more beneficial for long-term hearing and neurodevelopmental outcomes than 6 weeks of treatment (26). Because of the potential toxicities associated with antiviral therapy, several Authors suggest that only symptomatic infants with CNS involvement who are more than 32 weeks gestation and less than 1 month of age should be considered for treatment with oral valganciclovir (1, 27, 28). For infants who do undergo treatment, regular monitoring for myelosuppression (neutropenia, thrombocytopenia, anemia) is recommended throughout the 6-month course. Antiviral treatment for infants with cCMV infection and signs of CNS involvement has been recommended by expert panels (27, 28).

White matter abnormalities (WMAs) and subependymal pseudocysts (SEPCs, also referred to as germinolysis, germinolytic cysts, or ependymal septations) are regarded to as minor neuroimaging abnormalities (when compared to severe ventriculomegaly, extensive parenchymal calcifications and cortical malformations) associated with cCMV infection (6). Subependymal germinal matrix injury can present with pseudocyst formation in the caudothalamic notch, loss of glial cell production, and disruption of cortical cytoarchitecture. These

pseudocysts were described in extenso by Larroche in 1972, in association with cCMV and rubella (29). Typical ultrasound findings consistent with germinolysis include hyperechoic matrix evolving into single or multiloculated (honeycomb-like) SEPCs. Similar postnatal changes are seen in preterm infants without postnatal CMV infection, so the cell reaction in matrix is not specific of cCMV infection. Moreover, extensive germinolysis can be found in newborns with metabolic and genetic disorders (30, 31).

SEPCs can be caudothalamic, parafrontal, temporal, and occipital (32). A lower sensitivity of MRI in detection of cCMV-related SEPCs compared to cranial ultrasound has been reported (7).

cCMV-related WMAs have been described as areas of increased signal intensity (SI) of the white matter (WM) on T2-weighted MRI and apparent diffusion coefficient (ADC) maps, which can be multifocal or diffuse. ISEPCs and WMAs have been described both in association with other brain lesions and as an isolated finding in infants with cCMV (33-36).

While major brain abnormalities correlate with poor outcomes in infants with cCMV, there are scant data about hearing and neurodevelopmental outcomes of patients with isolated SEPCs and WMAs (6).

Both WMAs and SEPCs can be found among infants with cCMV infection undergoing a comprehensive neuroimaging evaluation including neonatal MRI (6, 36) and have been recently included among the indications to antiviral treatment by an expert panel (27). However, previous randomized controlled trials have not specifically investigated the efficacy of antiviral treatment in infants with WMAs and/or SEPCs (37, 38). Antiviral agents may not be effective for the whole spectrum of brain abnormalities associated with cCMV infection, ranging from severe lesions to minor signs of CNS involvement. The potential for long-term

toxicity of ganciclovir and valganciclovir may raise concerns about their use in every infant with any sign of CNS involvement by cCMV infection irrespectively of the type of brain abnormality found through neuroimaging. On the other hand, treatment of even minor brain abnormalities might have beneficial effects on long-term hearing and neurodevelopment. With improved diagnosis resulting from prenatal and neonatal cCMV screening, and the increased use of fetal and postnatal MRI, counseling on treatment and outcome in patients with cCMV-related IWMAs and ISEPCs may be challenging.

## Objectives

1. To assess the prevalence of minor signs of CNS involvement detected by MRI (isolated subependymal pseudocysts and isolated white matter abnormalities) among term infants with cCMV infection.
2. To assess the impact of isolated minor signs of CNS involvement detected by MRI (isolated subependymal pseudocysts and isolated white matter abnormalities) on long-term hearing and neurodevelopmental outcomes in a multicenter cohort of term infants with cCMV infection.
3. To compare long-term hearing and neurodevelopmental outcomes between symptomatic and asymptomatic term infants with cCMV infection and isolated minor signs of CNS involvement.
4. To compare long-term hearing and neurodevelopmental outcomes between treated and untreated term infants with cCMV infection and isolated minor signs of CNS involvement.

## Methods

*Patient population*

This is a retrospective cohort study of infants with cCMV infection born before February 2021 from three European tertiary children's hospitals, with MRI performed within the first 3 months after birth available for analysis. Study centers were the following: IRCCS Istituto Giannina Gaslini, Genoa, Italy; Sant Joan de Deu University Hospital, Barcelona, Spain; Quironsalud Madrid University Hospital, Madrid, Spain. Preterm infants as well as patients with major malformations (except for cCMV-related brain malformations), genetic or chromosomal syndromes, or perinatal comorbidities with a potential impact on neurodevelopment were excluded from the study.

cCMV infection was defined as identification of viral DNA in urine or blood during the first 2 weeks after birth.

Due to the retrospective design of the study, antiviral treatment was proposed to infants' parents according to the best evidence-based standard practice, which changed over the study period following the publication of the results from two randomized controlled trials (26, 37): between 2003 and 2015, a 6-week course of ganciclovir or valganciclovir was proposed for symptomatic patients with signs of CNS involvement, starting within the first month after birth. From 2015, a 6-week course of ganciclovir or valganciclovir followed by 4.5 additional months of valganciclovir was considered for every symptomatic patient with cCMV infection and proposed to parents according to local protocols.

*Neuroimaging studies*

MRI studies were performed on a 1.5 or 3 Tesla system. Sagittal, axial, and coronal T1- and T2-weighted images, as well as DWI, ADC maps, fluid-attenuated inversion recovery (FLAIR), gradient echo, 3D-T2 DRIVE, and susceptibility-weighted imaging (SWI) were reviewed where available. Both CUS scans and MRI scans were independently reviewed by two experts who were blinded to neonatal clinical data (other than the diagnosis of cCMV, the gestational age at birth, and the postnatal age at which the infant was scanned) and outcomes. Any discrepancy between the two readers was resolved by consensus agreement.

The presence of the following abnormalities associated with cCMV infection was recorded:

- Cortical malformation (polymicrogyria, pachygyria, or simplified gyral pattern);
- Cerebellar hypoplasia;
- Ventriculomegaly (at least one lateral ventricle width ≥7.5 mm on a coronal section at the level of the atria) (39);
- Intracranial calcifications (periventricular or subcortical calcifications).

SEPCs were defined as cystic lesions located along the inferior wall or adjacent to the lateral wall of the frontal horn or adjacent to the temporal or occipital horn of one or both the lateral ventricles. Occipital horn septations were classified as SEPCs as well. According to their location, SEPCs were classified as follows:

a) caudothalamic SEPCs: along the inferior wall of the frontal horn of the lateral ventricle, close to the caudo-thalamic notch (figure 1);

b) frontal SEPCs: adjacent to the lateral wall of the frontal horn of the lateral ventricle (figure 2);

c) temporal SEPCs: adjacent to the temporal horn of the lateral ventricle (figure 3);

d) occipital SEPCs: pseudocysts adjacent to the occipital horn of the lateral ventricle, or occipital horn septations (figure 4).

Patients were considered to have isolated SEPCs (ISEPCs) when these were the only abnormal finding detected by MRI.

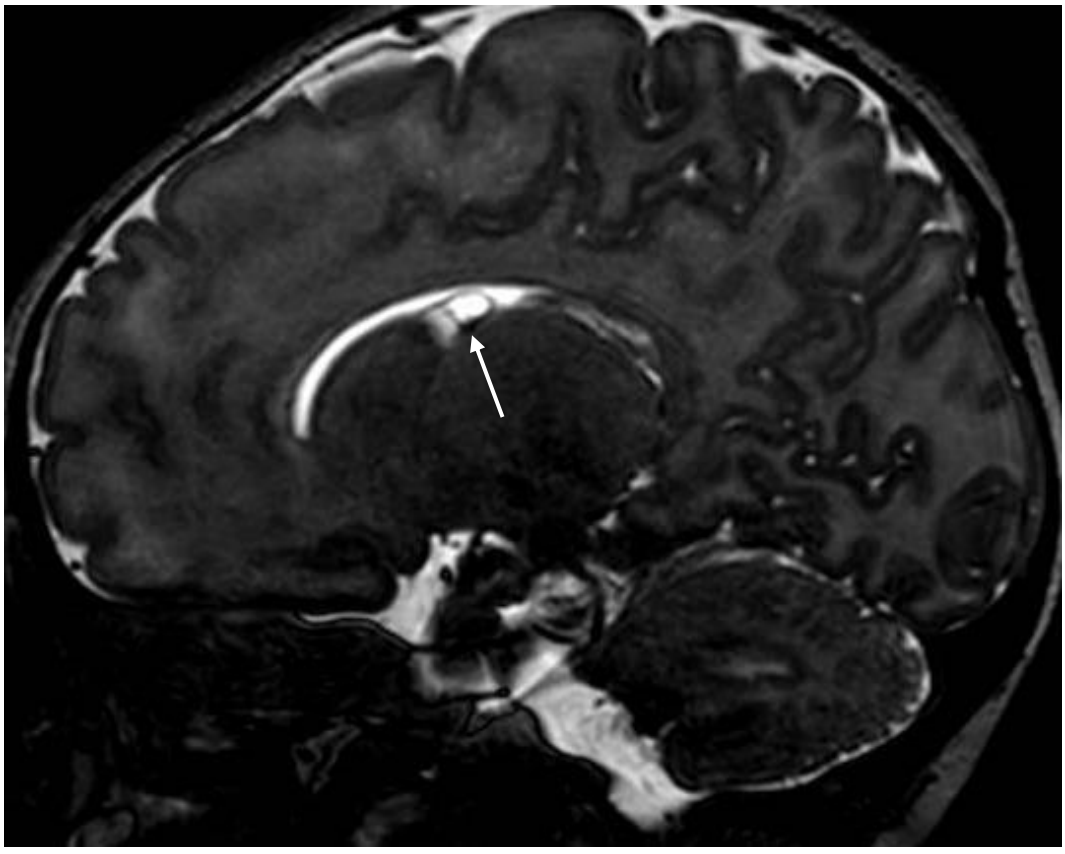


**Figure 1.** Caudo-thalamic SEPC (arrow).

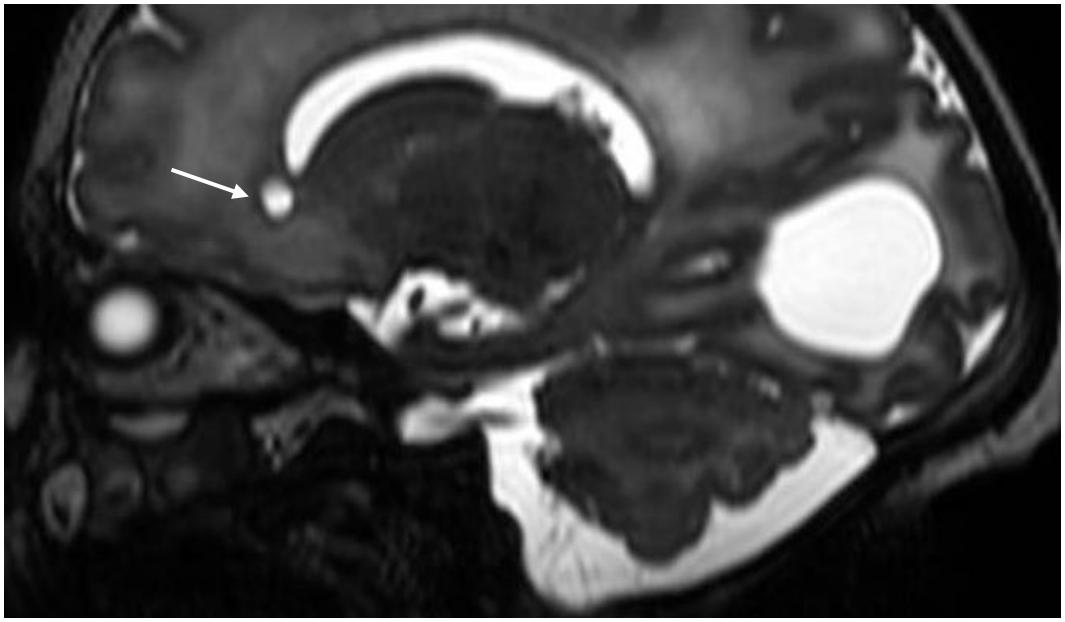


**Figure 2.** Frontal SEPC (arrow).

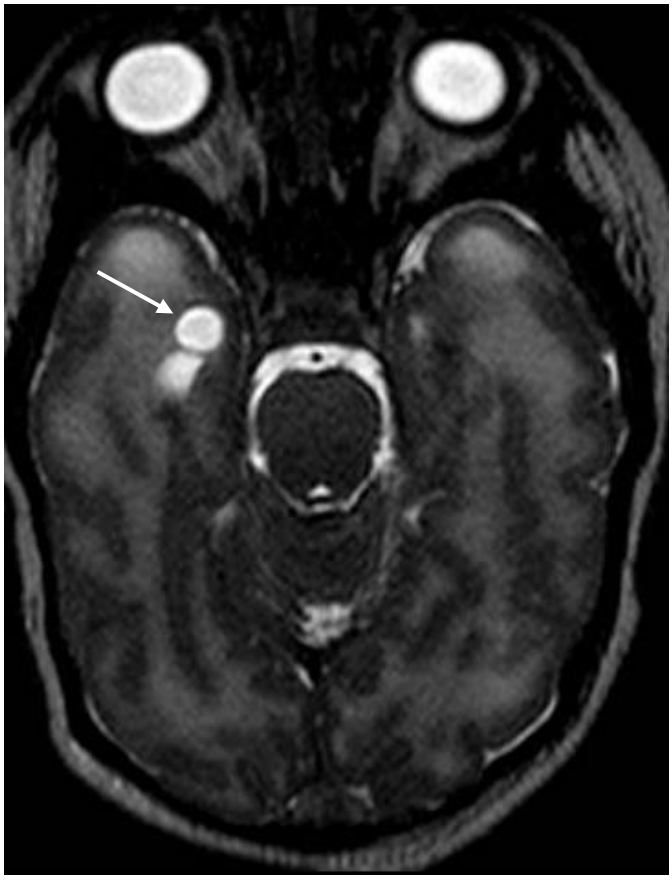


**Figure 3.** Temporal ISEPC (arrow).

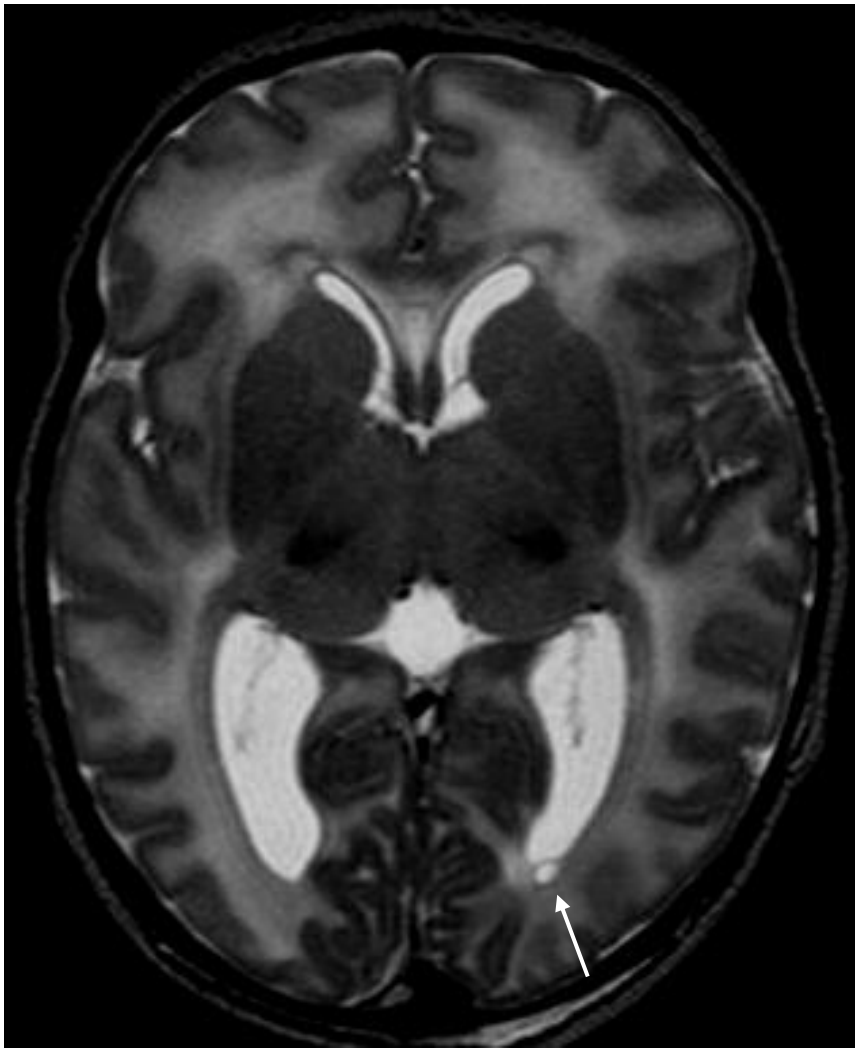


**Figure 4.** Occipital SEPC.

WMAs were established in the presence of abnormally high SI on T2- and low SI on T1-weighted MRI. WMAs were classified as multifocal (non-confluent frontal, parieto-occipital, and/or temporal) or diffuse (confluent) (34) (figure 5). Visual assessment of DWI and ADC maps was combined with that of T1- and T2-weighted MRI in order to support WMA identification and to further characterize the pattern of WMAs. Patients were considered to have isolated WMAs (IWMAs) when these were the only abnormal finding detected by MRI, or were associated solely with SEPCs.

According to a previous trial, symptomatic disease was defined as one or more of the following: thrombocytopenia (platelet count <100x$10^3$/µL); petechiae; hepatomegaly; splenomegaly; birth weight adjusted for gestational age <-2 SD; hepatitis (alanine aminotransferase >100 U/L) or cholestasis (direct bilirubin >3 mg/dL); microcephaly (head circumference adjusted for gestational age <-2 SD); intracranial calcifications; abnormal indices or CMV-DNA detection in cerebrospinal fluid; chorioretinitis; sensorineural hearing loss (SNHL), defined as hearing threshold >20 dB by auditory brainstem responses (26).

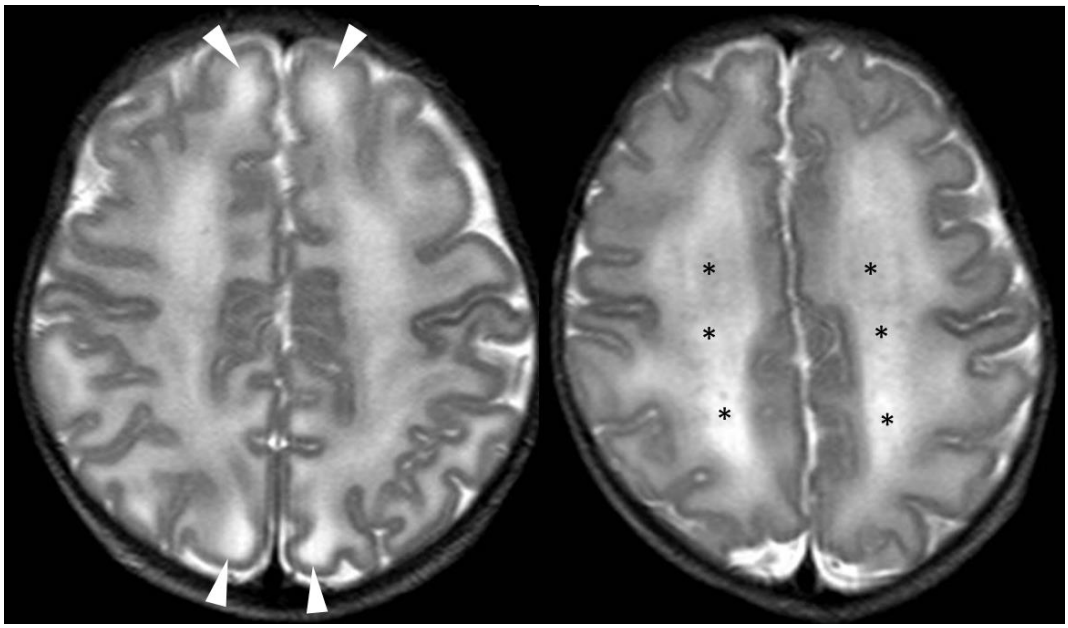


**Figure 5.** Multifocal WMAs (arrowheads) and diffuse WMAs (asterisks).

*Neurodevelopmental outcome*

Neurodevelopmental data were only recorded for children with follow-up ≥24 months. Follow-up visits included: clinical interview; screening of atypical neurodevelopment; and physical examination, including structured neurological examination. Regular hearing evaluations consisted of auditory brainstem responses or audiometry, depending on the child's age. The presence of cerebral palsy (40), visual impairment (visual acuity <20/40 or poor fixation behaviour

caused by cortical, optic nerve, and/or retinal abnormalities), and epilepsy were also recorded.

Between 24 and 42 months of age, children with atypical neurodevelopment as identified by screening during follow-up visits underwent standardized neurodevelopmental assessment using the Griffiths Mental Development Scales (GMDS) (41), the Bayley Scales of Infant Development II (BSID-II) (42), or the Bayley Scales of Infant and Toddler Development third edition (Bayley-III) (43). From 43 months, cognitive functioning in children with concerns was measured with the use of the Kaufman Assessment Battery for Children (44), the Wechsler Preschool and Primary Scale of Intelligence (45), or the Wechsler Intelligence Scale for Children (46). Children from one centre (IRCCS Istituto Giannina Gaslini, Genoa, Italy) were systematically assessed with one of these standardized assessment tools according to age at last follow-up visit, irrespectively of concerns emerging from the screening visit.

Moderate or severe disability was defined as one of the following: cerebral palsy; GMDS developmental quotient (DQ), BSID-II Mental Developmental Index (MDI) or Psychomotor Developmental Index (PDI), Bayley-III composite cognitive or motor score, or overall IQ <-2 SD or not testable because of severe impairment; epilepsy requiring antiseizure medication; severe SNHL (>70 dB threshold and/or requiring cochlear implants); or visual impairment.

Non-severe SNHL or a score between -1 and -2 SD on the GMDS DQ, BSID-II MDI or PDI, Bayley-III composite cognitive or motor scales, or overall IQ were classified as mild disabilities.

Children with typical neurodevelopment or no concerns arising from screening follow-up visits, as well as children with GMDS DQ, BSID-II MDI or PDI,

Bayley-III composite cognitive or motor scales, or overall IQ >-1 SD, were classified as having normal neurodevelopment or normal cognitive functioning.

*Statistical analysis*

Descriptive statistics were generated for the whole cohort and data were expressed as mean and standard deviation (SD) for continuous variables. Moreover, median value and range were calculated and reported, as were absolute or relative frequencies for categorical variables.

Non-parametric analysis (Mann–Whitney U-test) for continuous variables and Chi square or Fisher's exact test for categorical variables were used to measure differences between the groups. A *P* value less than 0.05 was considered statistically significant. and all values were based on two-tailed tests. Statistical analysis was performed using SPSS for Windows (SPSS Inc. Chicago. Illinois USA).

## Results

A total of 106 patients were identified, born between June 2009 and January 2021. Fifteen infants were excluded due to preterm birth, while two infants were excluded due to comorbidities (1 with hypoxic-ischemic encephalopathy, 1 with a non-cCMV related malformation syndrome involving the CNS). Of the 89 patients included, 47 (52.8%) were girls and 42 (47.2%) were boys. Median gestational age at birth was 39 weeks (range 37-41 weeks). Timing of maternal infection was unknown in most cases (49/89, 55.1%).

Clinical presentation at birth was categorized as symptomatic in 42 (47.2%) infants, asymptomatic in 47 (52.8%). The most common features at birth leading to the definition of symptomatic disease were SNHL (20/42 symptomatic infants, 47.6%), thrombocytopenia (14/42 infants, 33.3%), microcephaly (13/42 infants, 31.0%), and intrauterine growth restriction (12/42 infants, 28.6%).

A total of 44 (49.4%) infants were treated with ganciclovir or valganciclovir. Treatment rate was significantly higher among infants with symptomatic disease compared to asymptomatic patients at birth (table 1). Neonatal and neurodevelopmental characteristics of study patients according to disease presentation at birth (symptomatic or asymptomatic) are shown in Tables 1 and 2. Overall, a significantly higher rate of adverse outcome was observed among symptomatic patients compared to patients with asymptomatic cCMV infection at birth.

Follow-up data were available for 83 patients. Median age at the latest follow-up assessment was 4.3 years (range 24 months to 13.4 years). Seventy-one (85.5%) were ≥36 months at follow-up, and 20 (24.1%) were ≥6 years.

| | **Presentation at birth** | | |
|---|---|---|---|
| | **Asymptomatic** (N = 47) | **Symptomatic** (N = 42) | **p** |
| **Neonatal characteristics** | | | |
| Sex, n (%) | | | 0.39 |
| Male | 24 (51.0) | 18 (42.8) | |
| Female | 23 (48.9) | 24 (57.1) | |
| Gestational age, weeks, mean ± SD | 38.8 ± 1.1 | 38.6 ± 1.2 | 0.55 |
| Birth weight, grams, mean ±SD | 3231 ± 476 | 2745 ± 513 | 0.0001 |
| Antiviral treatment, n (%) | 17 (36.1) | 27 (64.2) | 0.008 |

**Table 1.** Neonatal characteristics of the study population according to cCMV presentation at birth.

| | **Presentation at birth** | | |
|---|---|---|---|
| | Asymptomatic | Symptomatic | p |
| **Outcome data** | | | |
| Outcome available, n/total (%) | 43/47 (91.5) | 40/42 (95.2) | 0.48 |
| Age at follow-up, months, mean ± SD | 65.6 ± 33.5 | 50.1 ± 21.1 | 0.06 |
| Moderate or severe disability, n/total (%) | 0 (0.0) | 15 (37.5) | 0.0001 |
| Cerebral palsy, n/total (%) | 0 (0.0) | 9 (22.5) | 0.001 |
| Moderate or severe cognitive or neurodevelopmental deficit, n/total (%) | 0 (0.0) | 8 (20.0) | 0.002 |
| Epilepsy requiring antiseizure medication, n/total (%) | 0 (0.0) | 5 (12.5) | 0.02 |
| Severe SNHL, n/total (%) | 0 (0.0) | 12 (0.3) | 0.0001 |
| Visual impairment, n/total (%) | 0 (0.0) | 2 (5.0) | 0.21 |
| Death, n (%) | 0 (0.0) | 0 (0.0) | - |
| Mild disability, n/total (%) | 2 (4.6) | 7 (17.5) | 0.06 |
| Non-severe SNHL without other moderate or severe disabilities, n/total (%) | 0 (0.0) | 5 (12.5) | 0.07 |
| Mild cognitive or neurodevelopmental deficit, n/total (%) | 2 (4.6) | 2 (5.0) | 1 |
| Any disability, n/total (%) | 2 (4.6) | 22 (55.0) | 0.0001 |

**Table 2.** Neurodevelopmental characteristics of the study population according to cCMV presentation at birth.

Brain MRI was performed at a median postnatal age of 11 days (range 0-90 days). In 72 (80.8 %) of them the examination was performed within the first month after birth. Neuroimaging characteristics according to disease presentation at birth are summarized in Table 3.

| **MRI findings** | **Presentation at birth** | | |
|---|---|---|---|
| | **Asymptomatic** (N = 47) | **Symptomatic** (N = 42) | **p** |
| Normal MRI, n/total (%) | 17/47 (36.2) | 14/42 (33.3) | 1 |
| Subependymal pseudocysts (SEPCs) | 25/47 (53.2) | 27/42 (64.3) | 0.40 |
| Caudothalamic SEPCs | 24/25 (96.0) | 26/27 (96.3) | 1 |
| Frontal SEPCs | 1/25 (4.0) | 4/27 (14.8) | 0.18 |
| Temporal SEPCs | 3/25 (12.0) | 9/27 (33.3) | 0.07 |
| Occipital SEPCs | 1/25 (4.0) | 6/27 (22.2) | 0.05 |
| Isolated subependymal pseudocysts (ISEPCs) | 13/47 (27.1) | 6/42 (14.3) | 0.20 |
| Caudothalamic ISEPCs | 12/13 (92.3) | 6/6 (100) | 1 |
| Frontal ISEPCs | 0/13 (0.0) | 0/6 (0.0) | - |
| Temporal ISEPCs | 1/13 (8.3) | 0/6 (0.0) | 0.60 |
| Occipital ISEPCs | 0/13 (0.0) | 0/6 (0.0) | - |
| White matter abnormalities (WMAs) | 15/47 (31.9) | 21/42 (50.0) | 0.08 |
| Isolated white matter abnormalities (IWMAs) | 13/47 (27.6) | 7/42 (16.7) | 0.21 |
| Multifocal IWMAs | 8/13 (61.5) | 4/7 (57.1) | 1 |
| Diffuse IWMAs | 5/13 (38.5) | 3/7 (42.8) | 1 |
| Ventriculomegaly | 3/47 (6.4) | 14/42 (33.3) | <0.001 |
| Periventricular or subcortical calcifications | 1/47 (2.1) | 9/42 (21.4) | 0.005 |
| Cortical malformation | 1/47 (2.1) | 9/42 (22.0) | 0.005 |
| Cerebellar hypoplasia | 0/47 (0.0) | 5/42 (12.2) | 0.02 |

**Table 3.** Neuroimaging characteristics according to cCMV presentation at birth.

In 31 (34.8%) infants, no abnormalities were identified on MRI scans (normal MRI group). SEPCs were identified in 52 (58.4%) infants, 50 of which (96.2%) were classified as caudo-thalamic SEPCs. In 33 (37.1%) cases, SEPCs were concomitant with other lesions, while in 19 (21.3%) they were an isolated finding (ISEPCs). WMAs were found in 36 (40.4%) infants. WMAs were associated with

MRI abnormalities other than SEPCs in 16 (18.0%) infants, while in 20 (22.5%) infants they were isolated or associated solely with SEPCs (IWMAs). Overall, ISEPCs and IWMAs were identified in 39 (43.8%) infants and were more frequent among asymptomatic infants (26/47, 55.3%) compared to patients with symptomatic disease at birth (13/42, 31.0%) ($p = 0.03$). Median age at MRI was 10 days in the group of infants with normal MRI (range 2-90 days), as well as in the ISEPC group (range 4-64 days) and in the IWMA group (range 0-27 days).

Table 4 shows antiviral treatment rates, hearing outcome, and neurodevelopmental outcome in the normal MRI and ISEPC groups: patients with ISEPCs and patients with normal MRI had comparable rates of antiviral treatment as well as of disability. None of the patients with ISEPCs had any severe disability at the latest follow-up assessment. The comparison of outcomes between symptomatic and asymptomatic patients with ISEPCs is shown in table 5. Treated and untreated patients with ISEPCs had comparable rates of disability (table 6).

| | **Normal MRI** (N = 31) | **ISEPCs** (N = 19) | **P** |
|---|---|---|---|
| Antiviral treatment, n (%) | 13 (41.9) | 8 (42.1) | 1 |
| **Outcome data** | | | |
| Age at follow-up, months, mean ± SD | 57.2 ± 26.3 | 52.3 ± 21.6 | 0.60 |
| Moderate or severe disability, n/total (%) | 2/28 (7.1) | 0/17 (0.0) | 0.52 |
| Cerebral palsy, n/total (%) | 0/28 (0.0) | 0/17 (0.0) | - |
| Moderate or severe cognitive or neurodevelopmental deficit, n/total (%) | 0/28 (0.0) | 0/17 (0.0) | - |
| Epilepsy requiring antiseizure medication, n/total (%) | 0/28 (0.0) | 0/17 (0.0) | - |
| Severe SNHL, n/total (%) | 2/28 (7.1) | 0/19 (0.0) | 0.51 |
| Visual impairment, n/total (%) | 0/28 (0.0) | 0/17 (0.0) | - |
| Death, n (%) | 0/28 (0.0) | 0/19 (0.0) | - |
| Mild disability, n/total (%) | 1/28 (3.6) | 2/17 (11.8) | 0.55 |
| Non-severe SNHL without other moderate/severe disabilities, n/total (%) | 1/28 (3.6) | 2/19 (10.5) | 0.56 |
| Mild cognitive or neurodevelopmental deficit, n/total (%) | 0/28 (0.0) | 0/17 (0.0) | - |
| Any disability, n/total (%) | 3/28 (10.7) | 2/17 (11.8) | 1 |

**Table 4.** Antiviral treatment rates, hearing outcome, and neurodevelopmental outcome in the normal MRI and ISEPC groups.

| | **Asymptom. ISEPCs** (N = 13) | **Symptomatic ISEPCs** (N = 6) | **P** |
|---|---|---|---|
| Antiviral treatment, n (%) | 5 (38.5) | 3 (50.0) | 1 |
| **Outcome data** | | | |
| Moderate or severe disability, n/total (%) | 0/12 (0.0) | 0/5 (0.0) | - |
| Cerebral palsy, n/total (%) | 0/12 (0.0) | 0/5 (0.0) | - |
| Moderate or severe cognitive or neurodevelopmental deficit, n/total (%) | 0/12 (0.0) | 0/5 (0.0) | - |
| Epilepsy requiring antiseizure medication, n/total (%) | 0/12 (0.0) | 0/5 (0.0) | - |
| Severe SNHL, n/total (%) | 0/13 (0.0) | 0/6 (0.0) | - |
| Visual impairment, n/total (%) | 0/12 (0.0) | 0/5 (0.0) | - |
| Death, n (%) | 0/13 (0.0) | 0/6 (0.0) | - |
| Mild disability, n/total (%) | 0/12 (0.0) | 2/5 (40.0) | 0.07 |
| Non-severe SNHL without other moderate/severe disabilities, n/total (%) | 0/13 (0.0) | 2/6 (33.3) | 0.11 |
| Mild cognitive or neurodevelopmental deficit, n/total (%) | 0/12 (0.0) | 0/5 (0.0) | - |
| Any disability, n/total (%) | 0/12 (0.0) | 2/5 (40.0) | 0.07 |

**Table 5.** Antiviral treatment rates, hearing outcome, and neurodevelopmental outcome of patients with ISEPCs according to disease presentation at birth.

| | **Untreated ISEPCs** (N = 11) | **Treated ISEPCs** (N = 8) | **P** |
|---|---|---|---|
| Symptomatic disease, n (%) | 3/11 (27.3) | 3/8 (37.5) | 1 |
| **Outcome data** | | | |
| Age at follow-up, months, mean ± SD | 49.9 ± 22.9 | 56.4 ± 22.9 | 0.55 |
| Moderate or severe disability, n/total (%) | 0/9 (0.0) | 0/8 (0.0) | - |
| Cerebral palsy, n/total (%) | 0/9 (0.0) | 0/8 (0.0) | - |
| Moderate or severe cognitive or neurodevelopmental deficit, n/total (%) | 0/9 (0.0) | 0/8 (0.0) | - |
| Epilepsy requiring antiseizure medication, n/total (%) | 0/9 (0.0) | 0/8 (0.0) | - |
| Severe SNHL, n/total (%) | 0/11 (0.0) | 0/8 (0.0) | - |
| Visual impairment, n/total (%) | 0/9 (0.0) | 0/8 (0.0) | - |
| Death, n (%) | 0/11 (0.0) | 0/8 (0.0) | - |
| Mild disability, n/total (%) | 1/9 (11.1) | 1/8 (12.5) | 1 |
| Non-severe SNHL without other moderate or severe disabilities, n/total (%) | 1/11 (9.1) | 1/8 (12.5) | 1 |
| Mild cognitive or neurodevelopmental deficit, n/total (%) | 0/9 (0.0) | 0/8 (0.0) | - |
| Any disability, n/total (%) | 1/9 (11.1) | 1/8 (12.5) | 1 |

**Table 6.** Hearing and neurodevelopmental outcomes of patients with ISEPCs according to antiviral treatment.

Table 7 shows antiviral treatment rates, hearing outcome, and neurodevelopmental outcome in the normal MRI and IWMA groups: patients with IWMAs had higher rates of disability compared to patients with normal MRI, although not statistically significant, whilst rates of antiviral treatment were comparable between the two groups. Among patients with IWMAs, 1/20 (5.0%) had a moderate/severe disability (severe SNHL) at the latest follow-up visit. Within the IWMA group, a trend towards a higher rate of disability was observed among patients with symptomatic cCMV infection at birth compared to those with asymptomatic disease, although not statistically significant (table 8). Treated and untreated patients with IWMAs had comparable rates of disability (table 9). Hearing and neurodevelopmental outcomes of treated and untreated patients with

multifocal IWMAs and diffuse IWMAs are summarized in tables 10 and 11, respectively.

| | **Normal MRI** (N = 31) | **IWMAs** (N = 20) | **p** |
|---|---|---|---|
| Antiviral treatment, n (%) | 13 (41.9) | 9 (45.0) | 1 |
| **Outcome data** | | | |
| Age at follow-up, months, mean ± SD | 57.2 ± 26.3 | 53.2 ± 24.0 | 0.59 |
| Moderate or severe disability, n/total (%) | 2/28 (7.1) | 1/20 (5.0) | 1 |
| Cerebral palsy, n/total (%) | 0/28 (0.0) | 0/20 (0.0) | - |
| Moderate or severe cognitive or neurodevelopmental deficit, n/total (%) | 0/28 (0.0) | 0/20 (0.0) | - |
| Epilepsy requiring antiseizure medication, n/total (%) | 0/28 (0.0) | 0/20 (0.0) | - |
| Severe SNHL, n/total (%) | 2/28 (7.1) | 1/20 (5.0) | 1 |
| Visual impairment, n/total (%) | 0/28 (0.0) | 0/20 (0.0) | - |
| Death, n (%) | 0/28 (0.0) | 0/20 (0.0) | - |
| Mild disability, n/total (%) | 1/28 (3.6) | 5/20 (25.0) | 0.07 |
| Non-severe SNHL without other moderate/severe disabilities, n/total (%) | 1/28 (3.6) | 2/20 (10.0) | 0.56 |
| Mild cognitive or neurodevelopmental deficit, n/total (%) | 0/28 (0.0) | 3/20 (15.0) | 0.07 |
| Any disability, n/total (%) | 3/28 (10.7) | 6/20 (30.0) | 0.14 |

**Table 7.** Antiviral treatment rates, hearing outcome, and neurodevelopmental outcome in the normal MRI and IWMA groups.

| | **Asymptom. IWMAs** (N = 13) | **Symptomatic IWMAs** (N = 7) | **p** |
|---|---|---|---|
| Antiviral treatment, n (%) | 4 (30.1) | 5 (71.4) | 0.20 |
| **Outcome data** | | | |
| Moderate or severe disability, n/total (%) | 0/13 (0.0) | 1/7 (14.3) | 0.30 |
| Cerebral palsy, n/total (%) | 0/13 (0.0) | 0/7 (0.0) | - |
| Moderate or severe cognitive or neurodevelopmental deficit, n/total (%) | 0/13 (0.0) | 0/7 (0.0) | - |
| Epilepsy requiring antiseizure medication, n/total (%) | 0/13 (0.0) | 0/7 (0.0) | - |
| Severe SNHL, n/total (%) | 0/13 (0.0) | 1/7 (14.3) | 0.30 |
| Visual impairment, n/total (%) | 0/13 (0.0) | 0/7 (0.0) | - |
| Death, n (%) | 0/13 (0.0) | 0/7 (0.0) | - |
| Mild disability, n/total (%) | 2/13 (15.4) | 3/7 (42.9) | 0.29 |
| Non-severe SNHL without other moderate/severe disabilities, n/total (%) | 0/13 (0.0) | 2/7 (28.6) | 0.11 |
| Mild cognitive or neurodevelopmental deficit, n/total (%) | 2/13 (15.4) | 1/7 (14.3) | 1 |
| Any disability, n/total (%) | 2/13 (15.4) | 4/7 (57.1) | 0.15 |

**Table 8.** Antiviral treatment rates, hearing outcome, and neurodevelopmental outcome of patients with IWMAs according to disease presentation at birth.

| | **Untreated IWMAs** (N = 11) | **Treated IWMAs** (N = 9) | **p** |
|---|---|---|---|
| Symptomatic disease, n (%) | 2/11 (18.2) | 5/9 (44.4) | 0.20 |
| **Outcome data** | | | |
| Age at follow-up, months, mean ± SD | 58.6 ± 23.1 | 46.4 ± 23.0 | 0.40 |
| Moderate or severe disability, n/total (%) | 0/11 (0.0) | 1/9 (11.1) | 0.45 |
| Cerebral palsy, n/total (%) | 0/11 (0.0) | 0/9 (0.0) | - |
| Moderate or severe cognitive or neurodevelopmental deficit, n/total (%) | 0/11 (0.0) | 0/9 (0.0) | - |
| Epilepsy requiring antiseizure medication, n/total (%) | 0/11 (0.0) | 0/9 (0.0) | - |
| Severe SNHL, n/total (%) | 0/11 (0.0) | 1/9 (11.1) | 0.45 |
| Visual impairment, n/total (%) | 0/11 (0.0) | 0/9 (0.0) | - |
| Death, n (%) | 0/11 (0.0) | 0/9 (0.0) | - |
| Mild disability, n/total (%) | 3/11 (27.3) | 2/9 (22.2) | 1 |
| Non-severe SNHL without other moderate or severe disabilities, n/total (%) | 1/11 (9.1) | 1/9 (11.1) | 1 |
| Mild cognitive or neurodevelopmental deficit, n/total (%) | 2/11 (18.2) | 1/9 (11.1) | 1 |
| Any disability, n/total (%) | 3/11 (27.3) | 3/9 (33.3) | 1 |

**Table 9.** Hearing and neurodevelopmental outcomes of patients with IWMAs according to antiviral treatment.

| | **Untreated multifocal IWMAs** (N = 7) | **Treated multifocal IWMAs** (N = 5) | **p** |
|---|---|---|---|
| Symptomatic disease, n (%) | 2/7 (28.6) | 2/5 (40.0) | 1 |
| **Outcome data** | | | |
| Moderate or severe disability, n/total (%) | 0/7 (0.0) | 0/5 (0.0) | - |
| Cerebral palsy, n/total (%) | 0/7 (0.0) | 0/5 (0.0) | - |
| Moderate or severe cognitive or neurodevelopmental deficit, n/total (%) | 0/7 (0.0) | 0/5 (0.0) | - |
| Epilepsy requiring antiseizure medication, n/total (%) | 0/7 (0.0) | 0/5 (0.0) | - |
| Severe SNHL, n/total (%) | 0/7 (0.0) | 0/5 (0.0) | - |
| Visual impairment, n/total (%) | 0/7 (0.0) | 0/5 (0.0) | - |
| Death, n (%) | 0/7 (0.0) | 0/5 (0.0) | - |
| Mild disability, n/total (%) | 2/7 (28.6) | 0/5 (0.0) | 0.47 |
| Non-severe SNHL without other moderate or severe disabilities, n/total (%) | 1/7 (14.3) | 0/5 (0.0) | 1 |
| Mild cognitive or neurodevelopmental deficit, n/total (%) | 1/7 (14.3) | 0/5 (0.0) | 1 |
| Any disability, n/total (%) | 2/7 (28.6) | 0/5 (0.0) | 0.47 |

**Table 10.** Hearing and neurodevelopmental outcomes of patients with multifocal IWMAs according to antiviral treatment.

| | **Untreated diffuse IWMAs** (N = 4) | **Treated diffuse IWMAs** (N = 4) | **p** |
|---|---|---|---|
| Symptomatic disease, n (%) | 0/4 (0.0) | 3/4 (75.0) | 0.20 |
| **Outcome data** | | | |
| Moderate or severe disability, n/total (%) | 0/4 (0.0) | 1/4 (25.0) | 1 |
| Cerebral palsy, n/total (%) | 0/4 (0.0) | 0/4 (0.0) | - |
| Moderate or severe cognitive or neurodevelopmental deficit, n/total (%) | 0/4 (0.0) | 0/4 (0.0) | - |
| Epilepsy requiring antiseizure medication, n/total (%) | 0/4 (0.0) | 0/4 (0.0) | - |
| Severe SNHL, n/total (%) | 0/4 (0.0) | 1/4 (25.0) | 1 |
| Visual impairment, n/total (%) | 0/4 (0.0) | 0/4 (0.0) | - |
| Death, n (%) | 0/4 (0.0) | 0/4 (0.0) | - |
| Mild disability, n/total (%) | 1/4 (25.0) | 2/4 (50.0) | 1 |
| Non-severe SNHL without other moderate or severe disabilities, n/total (%) | 0/4 (0.0) | 1/4 (25.0) | 1 |
| Mild cognitive or neurodevelopmental deficit, n/total (%) | 1/4 (25.0) | 1/4 (25.0) | 1 |
| Any disability, n/total (%) | 1/4 (25.0) | 3/4 (75.0) | 0.49 |

**Table 11.** Hearing and neurodevelopmental outcomes of patients with diffuse IWMAs according to antiviral treatment.

A trend towards a higher rate of disability was observed among patients with diffuse IWMAs compared to patients with multifocal IWMAs, although not statistically significant (table 12).

Overall disability rate among symptomatic infants with minor MRI abnormalities (either ISEPCs or IWMAs) was significantly higher compared to asymptomatic infants with the same neuroimaging findings (table 13, figures 6a-6b and 7a-7b). Within the whole population of children with minor MRI abnormalities (either ISEPCs or IWMAs), 7/37 (18.9%) had a mild disability, while 1/37 (2.7%) had a moderate/severe disability (table 13, figures 8a-8b).

There were no differences in outcome of patients with minor MRI abnormalities (either ISEPCs or IWMAs) in relation to antiviral treatment (table 14).

Table 15 provides a description of neonatal characteristics and neuroimaging findings of the 11 patients with no or minor MRI abnormalities (either normal MRI or ISEPCs or IWMAs) and any disability at last follow-up assessment.

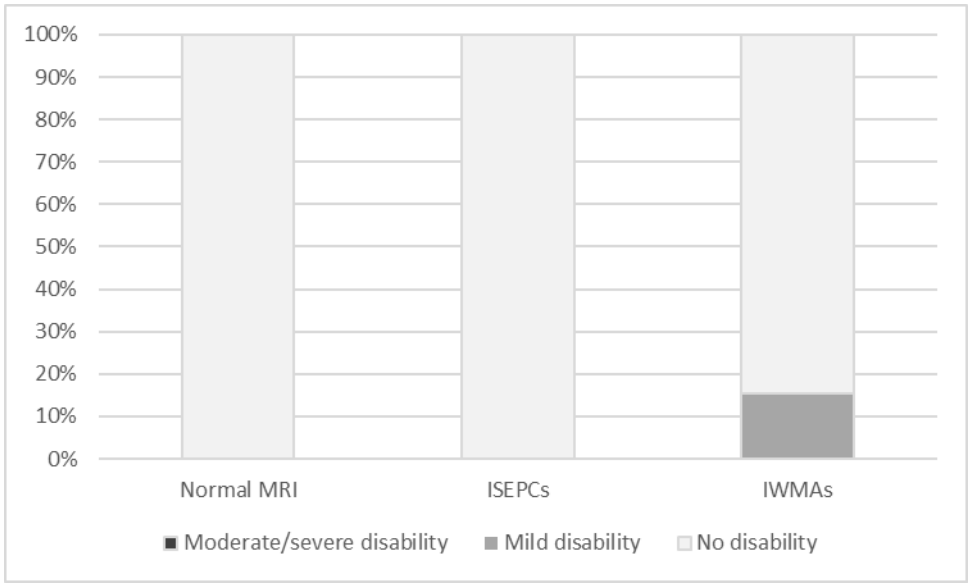
Within the “Gaslini” subgroup, treated and untreated patients with SEPCs and IWMAs had comparable mean DQs/IQs, as shown in table 16. Comparable DQs/IQs were also observed between 9 patients with multifocal IWMAS (101.9 ± 9.4, range 80-110, at a mean age of 48 months) and 8 patients with diffuse IWMAs (96.3 ± 13.9, range 71-110, at a mean age of 61 months) (p=0.28).

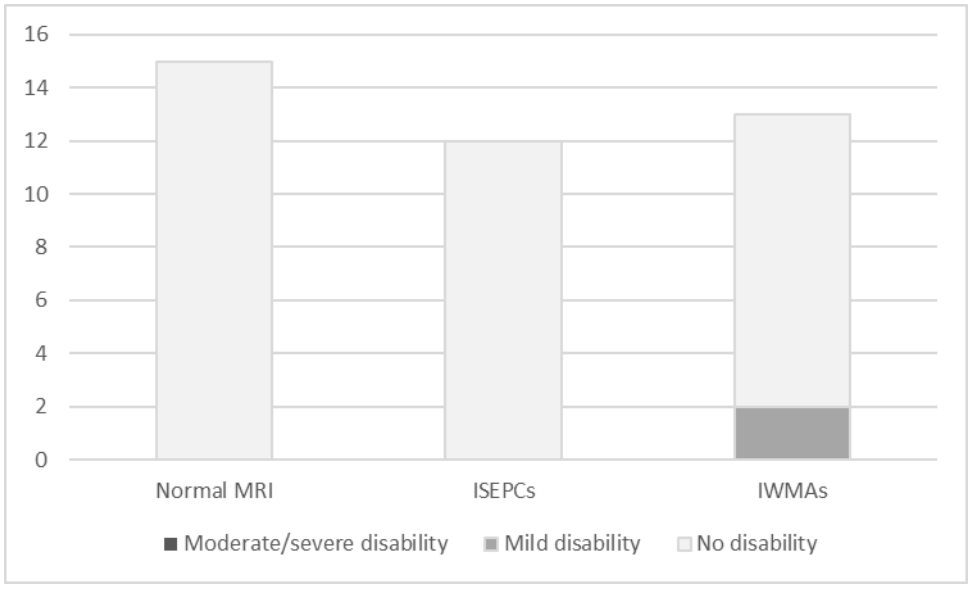
| | **Multifocal IWMAs** (N = 12) | **Diffuse IWMAs** (N = 8) | **p** |
|---|---|---|---|
| Antiviral treatment, n (%) | 5/12 (41.7) | 4/8 (50.0) | 1 |
| **Outcome data** | | | |
| Moderate or severe disability, n/total (%) | 0/12 (0.0) | 1/8 (11.1) | 0.40 |
| Cerebral palsy, n/total (%) | 0/12 (0.0) | 0/8 (0.0) | - |
| Moderate or severe cognitive or neurodevelopmental deficit, n/total (%) | 0/12 (0.0) | 0/8 (0.0) | - |
| Epilepsy requiring antiseizure medication, n/total (%) | 0/12 (0.0) | 0/8 (0.0) | - |
| Severe SNHL, n/total (%) | 0/12 (0.0) | 1/8 (12.5) | 0.40 |
| Visual impairment, n/total (%) | 0/12 (0.0) | 0/8 (0.0) | - |
| Death, n (%) | 0/12 (0.0) | 0/8 (0.0) | - |
| Mild disability, n/total (%) | 2/12 (16.7) | 3/8 (37.5) | 0.35 |
| Non-severe SNHL without other moderate or severe disabilities, n/total (%) | 1/12 (8.3) | 1/8 (12.5) | 1 |
| Mild cognitive or neurodevelopmental deficit, n/total (%) | 1/12 (8.3) | 2/8 (25.0) | 0.54 |
| Any disability, n/total (%) | 2/12 (16.7) | 4/8 (50.0) | 0.11 |

**Table 12.** Comparison of hearing and neurodevelopmental outcomes between patients with multifocal IWMAs and patients with diffuse IWMAs.

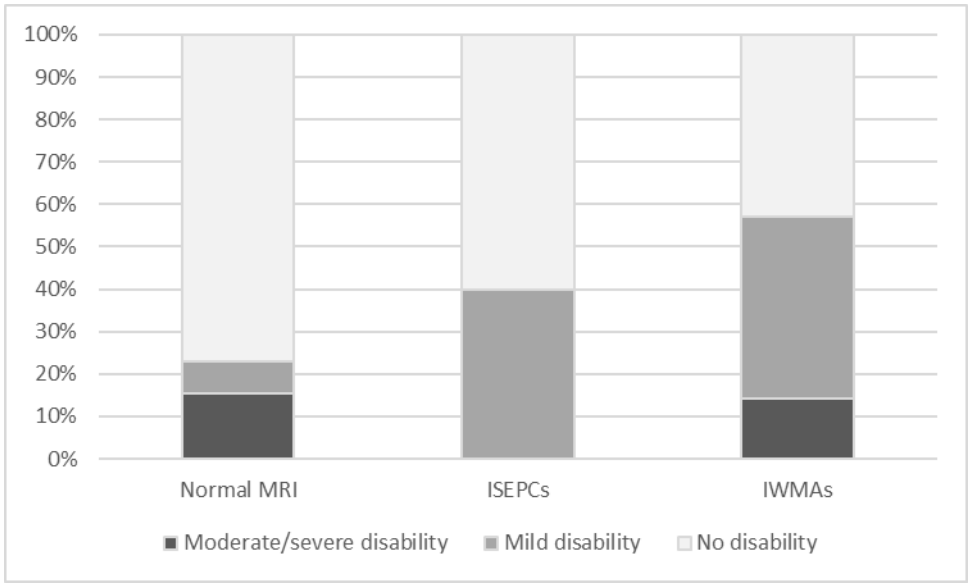
| | **Asymptom. ISEPCs or IWMAs** (N = 26) | **Symptomatic ISEPCs or IWMAs** (N = 13) | **p** |
|---|---|---|---|
| Antiviral treatment, n (%) | 9 (34.6) | 8 (61.5) | 0.21 |
| **Outcome data** | | | |
| Moderate or severe disability, n/total (%) | 0/25 (0.0) | 1/12 (8.3) | 0.20 |
| Cerebral palsy, n/total (%) | 0/25 (0.0) | 0/12 (0.0) | - |
| Moderate or severe cognitive or neurodevelopmental deficit, n/total (%) | 0/25 (0.0) | 0/12 (0.0) | - |
| Epilepsy requiring antiseizure medication, n/total (%) | 0/25 (0.0) | 0/12 (0.0) | - |
| Severe SNHL, n/total (%) | 0/26 (0.0) | 1/13 (7.7) | 0.20 |
| Visual impairment, n/total (%) | 0/25 (0.0) | 0/12 (0.0) | - |
| Death, n (%) | 0/26 (0.0) | 0/13 (0.0) | - |
| Mild disability, n/total (%) | 2/25 (8.0) | 5/12 (41.7) | 0.02 |
| Non-severe SNHL without other moderate/severe disabilities, n/total (%) | 0/26 (0.0) | 4/13 (30.8) | 0.009 |
| Mild cognitive or neurodevelopmental deficit, n/total (%) | 2/25 (8.0) | 1/12 (8.3) | 0.54 |
| Any disability, n/total (%) | 2/25 (8.0) | 6/12 (50.0) | 0.008 |

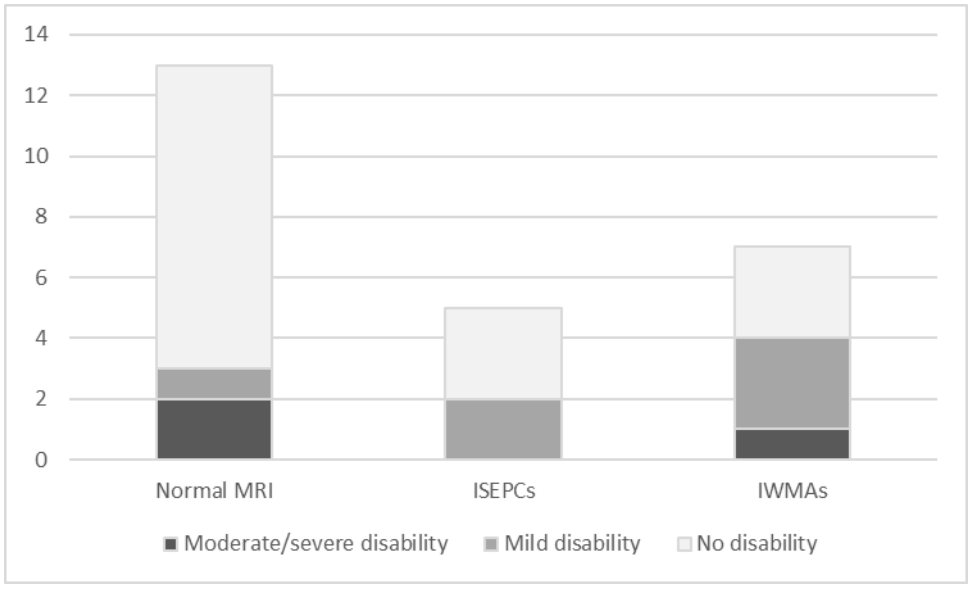
**Table 13.** Hearing and neurodevelopmental outcomes of patients with either ISEPCs or IWMAs according to disease presentation at birth.



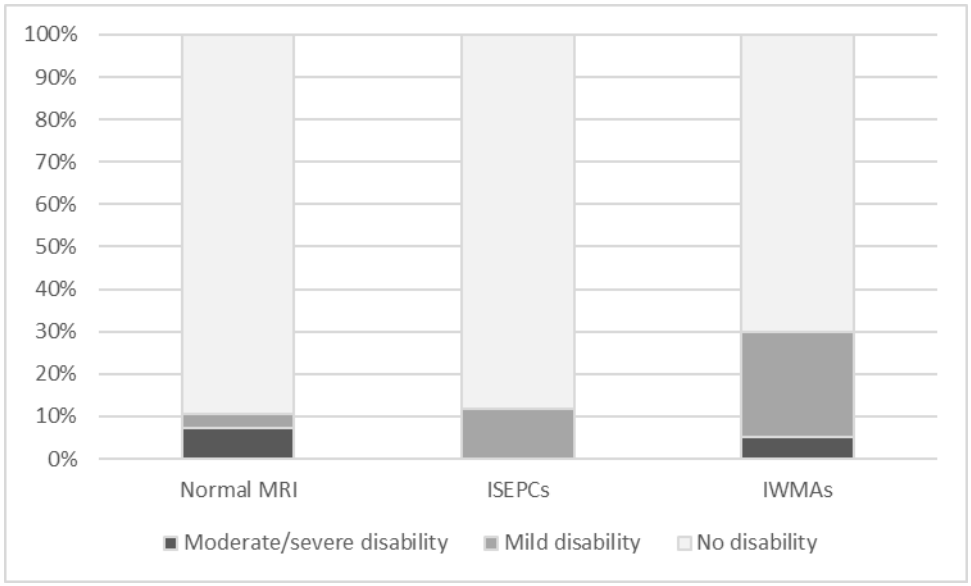



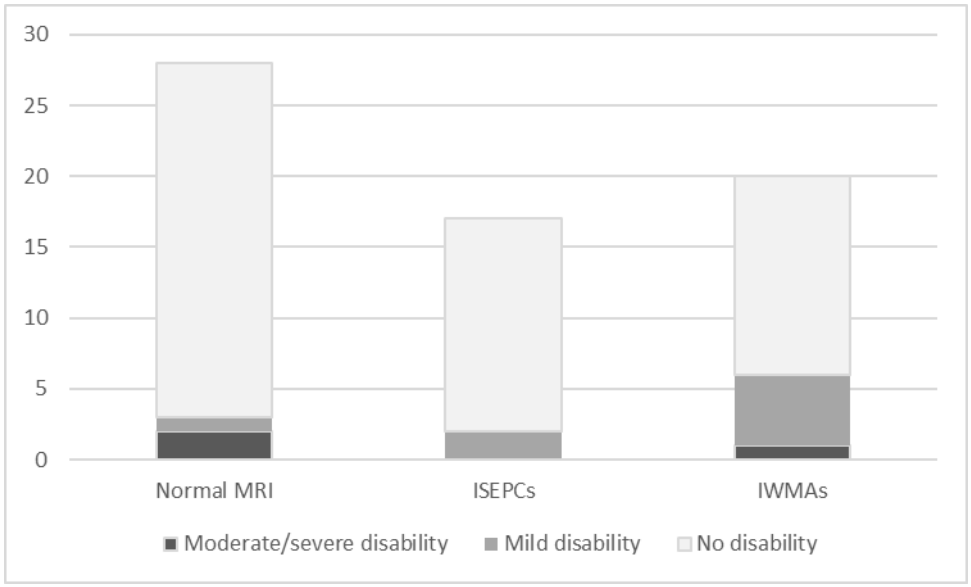

**Figures 6a and 6b.** Disability rates and absolute numbers of patients with disability according to MRI findings in the subgroups of asymptomatic patients with normal MRI, ISEPCs, and IWMAs.




**Figures 7a and 7b.** Disability rates and absolute numbers of patients with disability according to MRI findings in the subgroups of symptomatic patients with normal MRI, ISEPCs, and IWMAs.




**Figures 8a and 8b.** Disability rates and absolute numbers of patients with disability according to MRI findings in the subgroups of patients with normal MRI, ISEPCs, and IWMAs.

| | **Untreated**<br><br>**ISEPCS or IWMAs** (N = 22) | **Treated**<br><br>**ISEPCs or IWMAs** (N = 17) | **p** |
|---|---|---|---|
| Symptomatic disease, n (%) | 5/22 (22.7) | 8/17 (47.1) | 0.20 |
| **Outcome data** | | | |
| Moderate or severe disability, n/total (%) | 0/20 (0.0) | 1/17 (5.9) | 0.30 |
| Cerebral palsy, n/total (%) | 0/20 (0.0) | 0/17 (0.0) | - |
| Moderate or severe cognitive or neurodevelopmental deficit, n/total (%) | 0/20 (0.0) | 0/17 (0.0) | - |
| Epilepsy requiring antiseizure medication, n/total (%) | 0/20 (0.0) | 0/17 (0.0) | - |
| Severe SNHL, n/total (%) | 0/22 (0.0) | 1/17 (5.9) | 0.30 |
| Visual impairment, n/total (%) | 0/20 (0.0) | 0/17 (0.0) | - |
| Death, n (%) | 0/22 (0.0) | 0/17 (0.0) | - |
| Mild disability, n/total (%) | 4/20 (20.0) | 3/17 (17.6) | 0.81 |
| Non-severe SNHL without other moderate or severe disabilities, n/total (%) | 2/22 (9.1) | 2/17 (11.8) | 0.88 |
| Mild cognitive or neurodevelopmental deficit, n/total (%) | 2/20 (10.0) | 1/17 (5.9) | 0.88 |
| Any disability, n/total (%) | 4/20 (20.0) | 4/17 (23.5) | 0.89 |

**Table 14.** Hearing and neurodevelopmental outcomes of patients with either ISEPCs or IWMAs according to antiviral treatment.

| | First month of life | | | | | Last follow-up visit | | |
|---|---|---|---|---|---|---|---|---|
| Patient | Birth weight (g) | GA (weeks) | Disease symptoms | MRI abnormalities | Antiviral treatment | Age (months) | SNHL | Cognitive or neurodev. deficit |
| 1 | 2630 | 38 | CMV-DNA in CSF | No | Ganciclovir (6 weeks) | 100 | Severe | No |
| 2 | 2340 | 37 | SNHL | No | Valganciclovir (6 months) | 52 | Severe | No |
| 3 | 2165 | 37 | SNHL | No | Valganciclovir (6 months) | 36 | Non-severe | No |
| 4 | 3900 | 40 | SNHL | Caudo-thalamic ISEPCs | No | 40 | Non-severe | No |
| 5 | 3575 | 38 | SNHL | Caudo-thalamic ISEPCs | Ganciclovir (6 weeks) | 93 | Non-severe | No |
| 6 | 2930 | 39 | SNHL | Caudo-thalamic SEPCs Multifocal IWMAs | No | 100 | Non-severe | No |
| 7 | 2640 | 38 | Microcephaly | Temporal SEPCs Diffuse IWMAs | Valganciclovir (6 months) | 71 | Non-severe | No |
| 8 | 2960 | 39 | SNHL | Caudo-thalamic SEPCs Diffuse IWMAs | Valganciclovir (6 months) | 61 | Severe | No |
| 9 | 3690 | 39 | No | Caudo-thalamic SEPCs Diffuse IWMAs | No | 38 | No | Mild (GMDS 80) |
| 10 | 3680 | 40 | No | Caudo-thalamic SEPCs Temporal SEPCs Diffuse IWMAs | Valganciclovir (6 months) | 36 | No | Mild (GMDS 71) |
| 11 | 2890 | 39 | Microcephaly | Caudo-thalamic SEPCs Multifocal IWMAs | No | 36 | No | Mild (GMDS 80) |

Table 15. neonatal characteristics and neuroimaging findings of the 11 patients with no or minor MRI abnormalities and any disability.

| | **Untreated** | **Treated** | **p** |
|---|---|---|---|
| **ISEPCs** | | | |
| Patients, n/total (%) | 7/8 (87.5%) | 1/8 (12.5%) | - |
| Mean DQ/IQ ± SD (range) | 97.1 ± 6.7 (range 88-104) | 99 | - |
| Mean age at last follow-up ± SD, months | 49.1±18.9 | 56.4 ±25.5 | 0.45 |
| | | | |
| **IWMAs** | | | |
| Patients, n/total (%) | 10/17 (58.8%) | 7/17 (41.2%) | - |
| Mean DQ/IQ ± SD | 98.0 ± 10.6 (range 80-109) | 101.0 ± 13.9 (range 71-110) | 0.27 |
| Mean age at last follow-up ± SD, months | 58.6 ± 29.4 | 46.4 ± 14.1 | 0.41 |

**Table 16.** Developmental Quotients (DQs) or Intelligence Quotients (IQs) in patients with ISEPCs and IWMAs according to antiviral treatment.

## Discussion

The present study focuses on qualitative magnetic resonance imaging of isolated minor brain abnormalities (ISEPCs and IWMAs) in a large, multicentre cohort of infants with cCMV infection, both symptomatic and asymptomatic. This work provides further insights into the impact of minor brain abnormalities related to cCMV infection on long-term hearing and neurodevelopmental outcomes.

Consistent with other studies, minor brain abnormalities were common in our cohort (6, 36). Systematic and qualitative assessment of MRI findings allowed to identify patients with isolated minor brain abnormalities. In our cohort, overall prevalence of ISEPCs and IWMAs was high (43.8% of the study population), and 78.4% of patients with such minor MRI findings had a normal outcome. Of note, a higher proportion of ISEPCs and IWMAs was observed among asymptomatic compared to symptomatic infants (55.3% vs 31.0%). This might reflect the increasing use of MRI in routine clinical care for assessment of brain abnormalities in otherwise "healthy-appearing" and asymptomatic neonates. Furthermore, the routine MRI scanning protocol at one of the three participating Centres (Istituto Giannina Gaslini, Genoa, Italy) for assessment of brain abnormalities in cCMV infected infants included the 3D-T2 DRIVE sequence, which is highly sensitive in the detection of thin structures surrounded by static fluids such as cyst walls (47): this may have increased diagnostic accuracy of MRI for identification of ISEPCs in our cohort.

Subgroup analysis within the IWMA group showed a non-significant trend towards a higher rate of disability among patients with diffuse compared to multifocal IWMAs. However, due to the small size of these subgroups, this finding warrants further investigation in a larger cohort.

Interestingly, rates of antiviral treatment were high among both ISEPCs and IWMAs (42.1% and 45.0%, respectively). Considerable rates of antiviral treatment were also observed in the subgroups of patients with minor brain abnormalities and asymptomatic cCMV infection (38.5% of asymptomatic ISEPCs and 30.1% of asymptomatic IWMAs). The high prevalence of such abnormalities among cCMV infected infants may challenge the management of these "mildly affected" patients, often judged asymptomatic. Most asymptomatic patients with ISEPCs or IWMAs (92%) had normal hearing and neurodevelopmental outcomes at last follow-up assessment, whilst half of the 12 patients with symptomatic cCMV infection and such minor brain abnormalities were diagnosed with disability, including 1 with mild neurodevelopmental deficit, 4 with non-severe SNHL, and 1 with severe SNHL. In our study, we used the same criteria to define symptomatic cCMV disease according to a recent trial (26). Data from the same trial support treating neonates born with symptomatic cCMV with valganciclovir for 6 months (26). Antiviral treatment for cCMV infected infants with signs of CNS involvement has been recommended by expert panels (27, 28). Despite toxicity of valganciclovir and lack of evidence about these specific scenarios, WMAs and SEPCs have been recently included among the indications to antiviral treatment (28). However, no trial exists where allocation of treatment was based on severity of neonatal neuroimaging. To the best of our knowledge, large-scale data on long-term outcomes of cCMV infected infants with ISEPCs and IWMAs are not available. The results of the present study might contribute to identify patients at a higher risk for sequelae, or to clarify the role of treatment in the challenging scenarios of cCMV infected patients with minor brain abnormalities. In our cohort, treatment decisions were made on a case-by-case basis in asymptomatic infants with ISEPCs and IWMAs.

Overall, treated and untreated patients with ISEPCs or IWMAs had comparable rates of disability and comparable mean DQs or QIs within the Gaslini subgroup. Due to the observational nature of our study, we could not ascertain the effectiveness of treatment in infants with such minor MRI findings: however, our results together with the lack of specific evidence do not support the routine use of ganciclovir and/or valganciclovir in this group of infants, as the expected benefits likely do not outweigh the risks for long-term toxicity (17, 23, 24). Given the demonstrated mutagenicity of ganciclovir in humans, we believe that randomized controlled trials investigating new anti-CMV agents (e.g. terminase complex inhibitors) with patient stratification according to neuroimaging may open the road to the use of less toxic antiviral drugs in neonates with cCMV infection and provide better evidence about the effectiveness and safety of treatment for different expressions of the same disease (48).

Although our study design allowed the inclusion of a large number of infants from three European tertiary centres and the selection of a homogeneous group of infants with mild brain abnormalities related to cCMV, it has some limitations derived from its retrospective observational nature.

Routine prenatal serological cCMV screening was only performed in one institution, which explains the greater proportion of symptomatic cCMV infected infants compared to literature data. Unfortunately, we could not investigate the association between minor brain abnormalities related to cCMV infection and type and timing of maternal infection, as this information was lacking in most of our cases.

Subgroup analysis (asymptomatic vs symptomatic, treated vs untreated, etc.) should be interpreted with caution because of the limited sample size of each subgroup.

We did not include cranial ultrasound data because of the known limitations of retrospective assessment of ultrasound scans due to the intrinsic features of any ultrasound examination (real-time, operator-dependent): this may have led to underestimate the frequency of ISEPCs according to published data (7). However, disability rates among subjects with potentially missed ISEPCs (i.e. with normal MRI) and those with ISEPCs were comparable, and none of the asymptomatic infants with normal MRI or ISEPCs had any disability. Moreover, all three patients with normal MRI and disability were symptomatic and received antiviral treatment at birth, meaning that the inclusion of ultrasound data would not have modified the main results of the study.

Brain MRI protocols and equipment varied between centres and throughout the study period. Nevertheless, all images were reviewed specifically for this study in a standardized manner by two blinded independent experts. Quantitative analysis of ADC values in patients with IWMAs was not performed because multicentre and multi-device studies are almost always subject to measurement bias due to scarce reproducibility of ADC measurements across different MR systems (49).

In conclusion, ISEPCs and IWMAs are common MRI findings among cCMV infected infants. Asymptomatic patients with ISEPCs and IWMAs have normal outcome and a low rate of mild disability, respectively, whilst infants with symptomatic cCMV disease and such minor brain abnormalities carry a higher risk for adverse hearing or neurodevelopmental outcomes, regardless of antiviral treatment.

In the era of individualized therapies, there is urgent need for well-designed trials investigating the role of modern and non-mutagenic anti-CMV agents, which may provide novel therapeutic options to clinicians and families facing the dilemma of treatment for this kaleidoscopic disease.

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