

# Congenital cytomegalovirus infection: current strategies and future perspectives

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**Abstract.** – **INTRODUCTION,** Cytomegalo-virus is the most common cause of congenital infections in humans and it produces considerable morbidity in newborns.

**AIMS,** The present study reviews current concepts on epidemiology, clinical manifestations, diagnosis, treatment, future strategies and prognosis of children with congenital cytomegalovirus infection.

**RESULTS,** Congenital cytomegalovirus infection can be symptomatic or not at birth, but about 10-20% of them all will exhibit neurological damage when followed up. Sensorineural hearing loss is the most frequent long-term consequence and is not manifest invariably at birth or in the neonatal period but in many cases becomes clinically apparent in later childhood. There are growing evidences that newborns with symptomatic congenital cytomegalovirus infection would benefit from treatment with either ganciclovir or valganciclovir, the most widely studied drugs in this setting. It is not yet clear if children with asymptomatic or pauci-symptomatic infection at birth would benefit from treatment.

**DISCUSSION,** Studies evaluating treatment and long-term follow-up of infants with both symptomatic and asymptomatic infection are necessary, in order to definitely evaluate the short and long-term effectiveness and safety of both ganciclovir and valganciclovir and to identify risk factors associated to the development of long-term sequelae. In this way it will be possible to select those children that might benefit for treatment.

*Key Words:*

Cytomegalovirus, Congenital cytomegalovirus infection, Diagnosis, Malformations, Complications, Ganciclovir, Valganciclovir.

## Introduction

Cytomegalovirus (CMV) is a double-stranded DNA,  $\beta$  human herpesvirus. The viral genome is divided into a unique long (UL) region, and a unique short (US) region, which are necessary for the synthesis of the *UL54* protein (DNA polymerase), the major target of antiviral drugs used to treat CMV infections, and the *UL97* protein (phosphotransferase protein), required for the phosphorylation of ganciclovir (GCV), a necessary step to form its active metabolite *in vivo*<sup>1</sup>.

The outer envelope of the virus, which is derived from the host cell nuclear membrane, contains multiple virally encoded glycoproteins<sup>2</sup>.

Glycoprotein B (gB) and glycoproteinH (gH) seem to be the major determinants of protective humoral immunity. Antibodies against these proteins are capable of neutralizing the virus, and these glycoproteins are under study for the development of CMV subunit vaccines<sup>1</sup>.

CMV is the most common cause of congenital infections in humans<sup>3</sup> and it produces considerable morbidity in newborns.

Congenital CMV (CCMV) infection is estimated to occur in 0.5 to 2% of all deliveries in the developed world<sup>4</sup>.

However, some Authors have recently suggested that the prevalence of CCMV infection in the developed world seems to be slightly lower, ranging between 0.6% and 0.7%<sup>5,6</sup>.

These data appear to be more precise than the range of 0.2-2.5% often reported in literature, in agreement with a study held in Lombardia (Italy) reporting a value of 0.47%<sup>7</sup>.

The seroprevalence of CMV varies significantly according to the analyzed population. In populations of higher socioeconomic status, about 40% of adolescents are CMV-seropositive, with overall annual seroconversion rates reported to be approximately 1% per year<sup>8</sup>.

CMV is transmitted by close contact between individuals, through contamination from urine, saliva, semen, cervical secretions and breast milk, while droplet contamination is thought to be less important<sup>9</sup>.

Children in daycare facilities represent an important reservoir of CMV. Transmission of virus from a day care attendee to a seronegative susceptible woman may, if she is pregnant, result in a primary maternal infection which in turn leads to CCMV infection of the fetus. Therefore, CMV-

seronegative women working in daycare centers are at increased risk of acquiring CMV infection, while there is no evidence that healthcare providers have an increased risk of acquiring CMV infection, compared with the general population<sup>1,10,11</sup>.

Primary CMV infection is reported in 1-4% of seronegative women during pregnancy and the risk of transmission to the fetus is estimated to be about 30 to 40%<sup>12</sup>.

Reactivation of CMV infection during pregnancy is reported in 10-30% of seropositive women and, in this circumstance, the risk of transmission of the virus is about 1-3%<sup>3,13,14</sup>.

CCMV infection most commonly occurs via intrauterine transmission, but since the virus is shed in body fluids, transmission can also be acquired during delivery or through breast milk.

Only infants born to mother who had a primary infection during pregnancy have symptomatic disease at birth when compared with those born to mother who had a recurrent infection. Also, they are at substantially higher risk for the development of long-term and severe sequelae<sup>15-17</sup>, even though a few studies have identified severe symptomatic disease in newborns born to women with preconceptional immunity<sup>13,18</sup>.

Moreover, women who are seropositive for CMV may become re-infected with a new strain during pregnancy, and this re-infection can lead to symptomatic disease in the neonate<sup>19</sup>.

The risk of severe consequences is much greater when CMV infection is acquired in the first half of pregnancy<sup>5,20</sup>.

In the first months of pregnancy, in fact, CMV has a teratogenic potential in the fetus, as CMV infections may result in migrational disturbances in the brain<sup>21-23</sup>.

Neocortical neurons migrate from their site of production in the periventricular germinative zone towards the cortical plate between the 12<sup>th</sup> and 24<sup>th</sup> week of gestation<sup>24</sup>.

During this period, CMV may disturb the normal development of the brain and produce malformations. Later in pregnancy, when the gross morphology of the brain is completed and myelination is occurring, white matter lesions without cerebral cortical malformations can develop<sup>25</sup>.

CMV infections acquired during delivery or via breast milk have no effect on future neurodevelopmental outcome in full term infants, but in premature infants and low birth-weight newborns have been demonstrated to cause symptomatic illness, including hepatitis, neutropenia, thrombocytopenia<sup>9,26,27</sup>, and “sepsis-like” symptoms<sup>28</sup>.

About 10-20% of children with CCMV, asymptomatic or symptomatic in the neonatal period, will exhibit neurological damage when followed up<sup>13,29,30</sup>.

### **Clinical Manifestations of CCMV Infection**

The majority of infants born with CCMV infection are asymptomatic at birth (asymptomatic CCMV infection is defined as the presence of CMV in any secretions within the first 3 weeks of life, but with normal clinical, laboratory and imaging evaluations)<sup>13</sup>, and only about 7 to 10% have clinically evident disease at birth<sup>31</sup>.

Jaundice (62%), petechiae (58%), and hepatosplenomegaly (50%) are the most frequently noted symptoms and constitute the classical triad on CCMV infection<sup>32</sup>.

Other clinical manifestations include sensorineural hearing loss (SNHL, present in about 30% of symptomatic infants at birth)<sup>33</sup>, oligohydramnios, polyhydramnios, prematurity, intrauterine growth retardation, non-immune hydrops, fetal ascites, hypotonia, poor feeding, lethargy, thermal instability, cerebral ventriculomegaly, microcephaly, intracranial calcifications (central nervous system (CNS) involvement is present in approximately two-thirds of infants with symptomatic CCMV infection)<sup>34</sup>, “blueberry muffin” spots, and chorioretinitis<sup>35,36</sup> and, less frequently, hepatitis, pneumonia, osteitis, and intracranial hemorrhage<sup>37</sup>.

Moreover, infants with symptomatic CCMV infection may be at increased risk for the presence of congenital malformations such as inguinal hernia in males, high-arched palate, hydrocephalus, clasp thumb deformity, and clubfoot<sup>38,39</sup>.

Therefore, children with CCMV infection need to be evaluated carefully for the research of such malformations.

True mortality rates are difficult to obtain and have been reported to be as high as 30% for symptomatic infants<sup>40</sup> but other Authors have suggested a more likely average of about 5-10%<sup>41</sup>. Death is usually due to non-CNS manifestations of the infection, such as hepatic dysfunction or bleeding<sup>42</sup>.

### **Diagnosis**

The diagnosis of CCMV infection in a neonate is based on demonstration of the virus by isolation from urine, by identification of CMV-DNA by polymerase chain reaction (PCR) in urine, blood, saliva and cerebrospinal fluid (CSF) sampled before 3 weeks of age or by detection of antigen or

CMV-IgM in blood. A rapid diagnosis may be obtained by detection of CMV antigen in blood but the sensitivity is low. IgG antibodies in neonates are mostly maternally transferred antibodies, while the demonstration of IgM antibodies in the newborn is indicative of congenital infection, because maternal IgM antibodies can't cross the placenta. However, only 70% of neonates with CCMV infection have IgM antibodies at birth<sup>43</sup>.

Concerning the mother, seroconversion of CMV-IgG between two serum samples obtained in 2-3 weeks distance provides the most reliable diagnosis of primary infection. The presence of CMV-IgM suggests a recent or ongoing infection, but they have a low specificity. However, further confirmation of a diagnosis of primary CMV in pregnancy is always required. The CMV-IgG avidity test, a measure of the binding capacity of CMV-IgG antibodies, is a useful tool for confirmation and for dating the time of a primary CMV infection<sup>44-47</sup>.

Low avidity IgG indicates antibody-production induced by acute or recent primary CMV infection, whereas high avidity IgG indicates no current or recent primary infection<sup>45,48-52</sup>.

If a high avidity is found in the first 12-16 weeks of gestation, a recent infection can be ruled out<sup>30</sup>.

Table I shows diagnostic methods for diagnosing maternal, fetal and neonatal CMV infection.

### Outcome of CCMV Infection

About 10-20% of all children with CCMV infection, symptomatic or not in the neonatal peri-

od, will exhibit neurological damage when followed up<sup>13,29,30</sup>.

SNHL, mental retardation, seizures, psychomotor and speech delays, learning disabilities, chorioretinitis, optic nerve atrophy, and defects in dentition are the most common long-term consequences<sup>53</sup>.

SNHL is the most frequent long-term consequence and is not manifest invariably at birth or in the neonatal period but in many cases may fluctuate and be progressive in nature<sup>54-56</sup>, becoming clinically apparent in later childhood (during the first 6 years of life)<sup>56</sup>.

The prevalence of SNHL caused by CCMV infection (symptomatic and asymptomatic) at birth is 5.2% and late-onset hearing loss at 6 years is 15.4%<sup>40,57-59</sup>.

Generally, children with symptomatic neonatal infection have hearing loss at an earlier age and with greater severity than infants with asymptomatic infection<sup>30,60</sup>.

An estimated 40-58% of infants with symptomatic CCMV infection suffer from severe neurologic sequelae<sup>40,61</sup>, and mortality rates range from 5%<sup>41</sup> to 30% of them<sup>40,62</sup>.

It is now recognized that also asymptomatic CCMV infection is associated with increased risk of SNHL<sup>41,42</sup>. In particular, different studies report that 6 to 25% of asymptomatic children will develop late-onset sequelae, overall neurological ones, the most important of them being SNHL, making CCMV infection as the probable leading non-genetic cause of SNHL in childhood<sup>13,54,56-58,63-69</sup>.

**Table I.** Diagnostic methods available for the diagnosis of maternal, fetal and neonatal CMV infection. Adapted from Ref. 5.

Type of patient	Diagnostic method	Comments
Maternal infection	IgG seroconversion (appearance of virus-specific IgG in the serum of a pregnant woman who was previously seronegative) Presence of anti-CMV IgM and IgG antibodies Anti-CMV IgG avidity test	Two consecutive maternal blood samples need to be collected 2-3 weeks apart. IgM can be detected in: reactivations or reinfections; until more than one year after CMV primary infection; interference due to rheumatoid factor of the IgM class or cellular antigen; false positive during other viral infections (B19 Virus, Epstein Barr Virus, etc.). Low avidity means recent maternal infection, but threshold differs between virological methods.
Fetal infection	Amniocentesis to assess the presence of CMV by PCR	Perform the test after the 21 <sup>st</sup> week of gestation and after 5-6 weeks from the estimated onset of infection. Indications are: woman with compatible clinical signs of primary CMV infection; compatible ultrasound abnormalities; serologic suspicion of a recent maternal infection.
Neonatal infection	Culture or CMV-DNA testing by PCR in urine, blood, throat and CSF.	If infection is confirmed, classify as symptomatic or asymptomatic and follow-up at 1, 3, 6 and 12 months and annually until school age in order to detect sequelae with delayed onset.

In a longitudinal investigation of CMV-associated deafness in a cohort of 307 newborns with asymptomatic CCMV infection, 22 (7.2%) had SNHL. Among children with hearing loss, further deterioration of hearing occurred in 50%. Delayed-onset SNHL was observed in 18.2% of the children, with the median age of detection being 27 months<sup>70</sup>.

The same Authors demonstrated on 388 congenitally-infected neonates that a single audiological screening in neonatal period identified less than 50% of patients suffering from hearing deficit compared with repetitive screening until the age of 6 years (3.9% vs 8.3%)<sup>68</sup>. The implications of these observations are important for hearing-screening programs for newborns because the universal screening of hearing in neonates is estimated to detect less than half of all SNHL caused by CCMV infection<sup>68</sup>.

Therefore, infants with documented CCMV infection, but normal hearing at the time of the newborn screening, should be monitored throughout childhood for evidence of progression to SNHL.

Little attention has been focused on the influence of CCMV infection on children's physical growth and intellectual development. By following-up asymptotically infected infants from 2003 to 2007, Shan et al<sup>62</sup> investigated changes in audiology, nervous behavior, intellectual development, and behavioral development in order to find out the impact of asymptomatic CCMV infection. 52 asymptomatic newborns were enrolled in the infection group. At one year of age, seven ears of 5 cases showed mild abnormal auditory thresholds in V waves with an abnormal rate of 14%, while no abnormalities were found in 21 cases in the control group, with a statistically significant difference between the 2 groups. Five ears in 4 cases in the infection group showed prolonged intervals in I-V waves, whereas 3 ears in 2 cases in the control group showed this abnormality (no statistically significant difference). No significant differences in mental development index (MDI) and psychomotive development index (PDI) were found. No abnormalities were found on cranial B-ultrasonographies and cranial computed tomography scans. This study indicated that asymptomatic CCMV infection had an impact on infant hearing.

Fowler et al<sup>54</sup>, through comparison with a control group which consisted of siblings or randomly selected children, reported that SNHL was only found in the asymptomatic infection group.

Numazaki and Fujikawa<sup>66</sup> found that, among 17 cases of asymptomatic CCMV infection, 2 children developed late-onset SNHL, including 1

case of moderately binaural hearing loss and 1 case of unilateral hearing loss.

Whether infants with asymptomatic infection are at increased risk of mental retardation is controversial.

According to some studies, CCMV infection did not have a significant influence on total Intelligence Quotient of infants<sup>62,71,72</sup>.

In contrast, Hanshaw et al<sup>73</sup> compared 44 children with asymptomatic CCMV infection with controls and found school failure and deafness to be associated with asymptomatic CCMV infection<sup>39,73</sup>.

Some factors associated with the development of long-term sequelae have been found.

An analysis of the data of 180 children with CCMV infection showed that the presence of petechiae and intrauterine growth retardation were independently associated with the development of hearing loss<sup>74,75</sup>.

Microcephaly, after adjustment for weight deficit, had a 100% specificity for the prediction of mental retardation and/or major motor deficits<sup>61,74,76</sup>, but not with an increased risk for the development of SNHL<sup>69,74</sup>.

Some investigations<sup>61,69,74,76-78</sup> have shown that development of neonates with CNS involvement at birth is impaired, as > 90% of surviving infants developed significant CNS sequelae, perceptual defects or both within the first 2 years of life.

Normal neuro-imaging at birth in symptomatic CCMV infection predicts a good long-term neurologic outcome<sup>61,69,74,76-78</sup>.

On the opposite, intracranial lesions on neuroimaging are associated with severe intellectual impairment in > 80% of cases<sup>61,77</sup>.

Some studies have shown that the amount of CMV copies in blood correlates with neurological outcome irrespective of whether children are considered symptomatic or asymptomatic at birth<sup>59,75,79-81</sup>.

Four studies have demonstrated that a high viral load in early infancy expressed by a high amount of virus in the urine (450,000 PFU/mL) is highly predictive of audiologic impairment<sup>59,74,77,80</sup>.

Greater than 70% of symptomatic (with or without CNS involvement) infants with viruria of > 5 · 10<sup>4</sup> PFU/mL will have poor neurodevelopmental outcome when compared with only 4% with viruria of < 3.5 · 10<sup>3</sup> pfu/mL<sup>77,82</sup>.

### ***Pathogenesis of Hearing Loss in CCMV infection***

The viral and/or host inflammatory mechanisms involved in the pathogenesis of CCMV-related auditory dysfunction are still unclear<sup>84</sup>.



CMV-induced hearing loss is believed to be caused by virus-induced labyrinthitis<sup>84</sup>. In fact, the progressive nature of SNHL suggests that there may be a chronic infection in the CNS or endolabyrinth that continues to be active throughout early childhood<sup>83</sup>. Supporting this hypothesis, inner ear histology from congenitally infected infants shows damage to structures such as the vestibular endolymphatic system and the vestibular organs (sacculle and utricle), along with collapse of the saccular membrane<sup>85</sup>.

It has been postulated that CMV enters the endolymph via the stria vascularis<sup>86</sup>; this hypothesis is confirmed by viral DNA detection in the perilymph by quantitative PCR analysis<sup>87,88</sup>.

While the exact mechanism of injury is unknown<sup>89</sup>, evidence shows that both direct cytopathic viral and localized inflammatory response play roles in pathogenesis<sup>54,84</sup>.

Several studies suggest that the primitive damage could be established either by direct chromosomal injury or by modulation of developmental gene expression<sup>83</sup>.

Supporting the first hypothesis, two loci present near the chromosomal breakpoint are remarkable: *DFNA7* and *USH2A*<sup>84</sup>. The *DFNA7* gene has been linked to the inheritance of an autosomal dominant, nonsyndromic, progressive form of hearing loss<sup>90</sup>. Perturbation of the *DFNA7* gene caused by CMV-induced breakage could conceivably be linked to the development of the progressive SNHL. The *USH2A* gene, which is physically closest to the most prevalent CMV-induced break<sup>91</sup>, encodes a protein important in the pathogenesis of *Usher's syndrome type II*, an autosomal recessive disorder responsible for both SNHL and blindness<sup>91,92</sup>.

Schraff et al<sup>89</sup> findings on Guinea Pigs (GP) model support instead the CMV-modulated inflammatory response hypothesis, stating that recombinant GPCMV, with the MIP 1- $\alpha$  homolog gene deleted, has an attenuated ability to recruit leukocytes to the site of viral replication and, therefore, to produce deafness and inflammatory labyrinthitis.

This evidence suggests that this gene plays an important role in GPCMV-related hearing loss following direct cochlear challenge with virus<sup>89</sup>.

These findings are significant since therapies and vaccines can be directed toward further research in preventing the propagation of CMV-related inflammatory mediators and decreasing auditory injury<sup>89</sup>.

### **Treatment: "the GCV Experience"**

Ganciclovir (GCV) was the first compound licensed specifically for treatment of CMV infection. GCV is a synthetic acyclic nucleoside analogue, structurally similar to guanine. GCV requires phosphorylation to achieve antiviral activity. The enzyme responsible for its phosphorylation is the product of the CMV *UL97* gene, a protein that functions as a protein kinase and phosphotransferase<sup>94,95</sup>, which generate the triphosphate form. GCV triphosphate competitively inhibits DNA synthesis catalyzed by inhibition of the CMV polymerase (encoded by the *UL54* gene).

Clearance of GCV occurs by renal mechanisms, and reduction in dose is recommended in the setting of renal failure. GCV penetrates well into the CNS<sup>96</sup>, an observation that may be important for treatment in the setting of CMV-induced neurodevelopmental injury. Resistance to GCV is an important clinical problem that may emerge during therapy<sup>97</sup>.

To date, resistance has not been reported in newborns being treated for CCMV infection, although it has been described in an immunocompromised infant being treated for CMV acquired in the perinatal period<sup>98</sup>.

Although oral GCV obviates the need for an indwelling venous catheter in many immunocompromised adult patients, the lack of a suspension formulation, as well as its relatively poor oral bioavailability, renders this drug a less attractive option for pediatric patients, and no information is available about the use of this agent in newborns and young children<sup>99,100</sup>.

The suggestion that antiviral therapy might be of value in newborns with CCMV infection was first raised in a number of reports in the late 1960s and early 1970s<sup>101-104</sup>. Nevertheless, the first use of GCV therapy for CCMV infection dates to the late 1980s<sup>105</sup>. In subsequent reports<sup>106-115</sup>, GCV therapy had been shown to be generally safe and well-tolerated when used in newborns, and it has appeared to be useful in ameliorating the severity of a number of focal, end-organ CMV disease syndromes. Although GCV appeared to be of value in the short-term management of CMV infection in infants in some settings, it was less clear whether the use of GCV provided any long-term benefit for congenitally or perinatally acquired CMV infection.

Afterwards, multicenter studies conducted by the National Institute of Allergy and Infectious Diseases Collaborative Antiviral Study Group

(CASG) have shed light on the potential long-term benefits of antiviral therapy<sup>116-120</sup>. These trials have focused on the impact of antiviral therapy on symptomatic CCMV infection with CNS involvement. The first studies conducted by the CASG were performed to determine the safety and pharmacokinetics of GCV in newborns and young infants<sup>116,117</sup>. A subsequent phase II CASG study evaluated the toxicity, virologic response, and clinical outcomes in newborns with symptomatic CCMV infection receiving 6 weeks of parenteral GCV therapy<sup>118</sup>. These are the first well-done studies that showed the efficacy of GCV therapy for CCMV infection (hearing improvement or stabilization of hearing was noted in 16 percent of 30 infants when they were 6 months of age or older) and GCV-related side-effects (thrombocytopenia and neutropenia). These observations supported further studies on efficacy and safety of GCV therapy for CCMV infection.

A pilot study in 1994 compared two different regimens of GCV treatment<sup>112</sup> started within the first 2 weeks of life in two small groups (6 vs 6) of neonates with symptomatic CCMV infection. Group 1 was treated with GCV 5 mg/kg twice daily for two weeks. Group 2 was treated with GCV 7.5 mg/kg twice daily for 2 weeks, followed by 10 mg/kg three times a week for 3 months. In group 1 viral shedding disappeared in 3/6 infants, whereas in group 2 all six infants showed cessation of viruria. In all babies viral shedding reappeared after treatment was discontinued. Two infants in group 1 and four in group 2 had normal neurologic outcomes at 18 months of age. In one of them microcephaly had disappeared. Two babies with initial chorioretinitis had normal eye examination at 18 months. Patients in the low-dose short-duration regimen showed no side-effects. Side effects in the higher-dose longer-duration regimen were transient neutropenia (2/6 patients), transient elevation of liver enzymes (2/6 patients) and difficulties for venous access (1/6 patients). Long-term complications related to the use of GCV were not mentioned. Three patients (two in group 1 and one in group 2) had hearing loss on follow-up.

A larger phase II study compared two 6-week regimens of GCV (8 mg/kg/die versus 12 mg/kg/die, in 14 and 28 infants, respectively) for toxicity, virologic response, and clinical and neurologic outcome in newborns with CCMV infection with CNS involvement<sup>118</sup>. The 12 mg/kg/die group showed a more pronounced antiviral effect in urine that was associated with a normal neuro-

logic examination at 18 months of age ( $p = 0.036$ ). However, in all children viral shedding in urine reappeared following discontinuation of antiviral therapy. Audiologic data, available for 30 out of 42 infants, did not differ by treatment group. 11 out of 13 infants with normal baseline hearing developed deafness. Of 14 infants with initial chorioretinitis (12 in the 12 mg/kg/die group), 8 had normal eye examinations at 6 months. Of the infants with baseline normal eye examinations, 3 developed retinal scarring. 8 of 33 children (24%) evaluated at  $\geq 2$  years of age had normal neurologic development, which did not differ by GCV dosage<sup>69,121</sup>.

In 2003, results of a phase III randomized, double blind, not placebo-controlled (because of the ethical concerns regarding the prolonged intravenous administration of a placebo through a central venous catheter) CASG trial, evaluating a 6 weeks therapy with intravenous (IV) GCV in neonates with CCMV-related CNS infection have been reported<sup>119</sup>. The primary endpoint was improved brainstem-evoked response (BSER) between baseline and 6-month follow-up or, for those infants with normal hearing at enrollment, maintenance of normal hearing between baseline and 6-month follow-up. Of the 100 patients enrolled in the study, only 42 patients completed the 6-month follow-up. 21 (84%) out of 25 GCV recipients either had improved hearing or maintained normal hearing between baseline and 6 months. In contrast, only 10 (59%) out of 17 control patients had improved or stable hearing ( $p = 0.06$ ). Moreover, none of 25 GCV recipients had worsening in hearing between baseline and 6-month follow-up, compared with 7 (41%) out of 17 control patients ( $p < 0.01$ ). Eventually, among 43 patients who had a BSER at both baseline and at 1 year or beyond, 5 (21%) out of 24 GCV recipients had worsening of hearing versus 13 (68%) of 19 control patients ( $p < 0.01$ )<sup>1</sup>. During therapy the viral excretion in urine decreases, but returns to near pretreatment levels after cessation of therapy. Nevertheless, the large proportion of unevaluable infants (58 out of 100 subjects enrolled) raises concerns about follow-up bias<sup>12</sup>.

Additional analyses of this randomized controlled CASG trial suggested that GCV may also improve neurodevelopmental outcome. The numbers of milestones not met ("delays") were determined for each subject. At 6-month follow-up, the average number of delays was 4.46 and 7.51, respectively, for GCV recipients and "no treatment" subjects ( $p = 0.02$ ). At 12 months, the average

number of delays was 10.06 and 17.14, respectively ( $p = 0.007$ ). In a multivariate regression model, the effect of GCV therapy remained statistically significant at 12 months ( $p = 0.007$ )<sup>120</sup>.

Kimberlin et al<sup>42</sup> are currently recruiting patients in a study to document pubertal development and cancer history of subjects enrolled in CASG studies. The study has now been concluded but results are not yet available. (<http://www.clinicaltrials.gov/ct/show/NCT00031421>. Accessed 1 December 2010).

A number of reports on treatment and outcome of CCMV-related chorioretinitis have been reviewed by Shoji et al<sup>23</sup>. A total of 36 newborns treated with IV GCV for a duration of therapy ranging from 2 to 7 weeks have been reported, with improvement or resolution of chorioretinitis in 18 cases, no changes in 12 cases, worsening in 6 cases.

The same Authors recently described a case of chorioretinitis which required a 6-month course of antiviral therapy (9 weeks of GCV, followed by Val-GCV) to control the ocular lesion<sup>123</sup>.

Single reports of improvement in infants with CCMV-related cholestasis<sup>124</sup> and chorioretinitis<sup>125</sup> after IV GCV have also been published<sup>30</sup>.

#### **Treatment: "the Val-GCV Experience"**

Val-GCV is a mono-valyl ester pro-drug of GCV with high oral bioavailability.

First studies on the use of Val-GCV were conducted by Italian researchers, which reported that in 5 newborns taking different Val-GCV dosages (2 patients on 10 mg/kg/12 h and 3 patients on 15 mg/kg/12 h) the 15 mg/kg twice daily dose increased GCV plasma concentrations<sup>126</sup>.

Two case reports provided pharmacokinetic data on oral treatment with Val-GCV<sup>127,128</sup>.

One infant with encephalitis due to perinatal HIV-CMV co-infection was treated for 1 year. Val-GCV inhibited CMV replication without side effects<sup>127</sup>. In another case, a continuous adaptation dose of 280 to 850 mg/m<sup>2</sup> was needed during 5.5 months of treatment of a symptomatic infant to achieve plasma levels that made CMV-DNA undetectable in the urine<sup>128</sup>.

Schulzke et al<sup>129</sup> administered Val-GCV to a newborn up to 56 mg/kg/day<sup>129</sup> while Jansen et al<sup>128</sup> gave a dose ranging from 280 to 850 mg/m<sup>2</sup> twice daily to a 1-month old child measuring 0.25 m<sup>2</sup>.

Five studies<sup>121,130-134</sup> have shown that Val-GCV has 10 times greater oral bioavailability than oral GCV (53.6% vs. 4.8%). Once absorbed, it is rapidly metabolized in the bowel by cleavage of the valine ester into GCV.

Galli et al<sup>133</sup> treated four newborns with Val-GCV 15 mg/kg every 24h, but achieving low plasma GCV concentration; they therefore increased to 15 mg/kg every 12h, achieving a significant increase in concentrations.

Kimberlin et al<sup>134</sup> confirmed these results, showing that a 16 mg/kg dose of oral Val-GCV solution administered twice daily provided GCV concentration comparable with that of 6 mg/kg/dose of IV GCV.

Lombardi et al<sup>135</sup> treated 13 newborns suffering from symptomatic CCMV with oral Val-GCV 15 mg/kg every 12 h. At baseline and on days 6, 15, 30 and at the end of therapy, viral assessment was performed in blood and urine. Pharmacokinetic analyses demonstrated that mean C<sub>trough</sub> concentration was 0.51±0.3 mg/mL and mean C<sub>2h</sub> (presumed C<sub>max</sub>) was 3.81±1.37 mg/mL. GCV plasma concentration was stable and within suggested therapeutic range.

One newborn discontinued therapy because of thrombocytopenia, another finished with a neutrophils count of 1,000/μl. At the end of therapy 6 out of 12 and 8 out of 12 newborns were negative for CMV in urine and plasma. At the end of the 6<sup>th</sup> week of therapy, 8 newborns had negative CMV-DNA value in the blood and the 4 newborns that were still positive showed a 90% reduction relative to pre-therapy CMV-DNA values. Clinically, the 4 patients reporting hepatic disease and the 3 patients with thrombocytopenia recovered after 6 weeks of therapy. 8 newborns suffered from SNHL; at the 6-month follow-up no patient had worsened, 2 had improved, and no deterioration was reported in the 3 newborns with chorioretinitis scarring<sup>122</sup>.

Amir et al<sup>136</sup> treated 23 infants with symptomatic CCMV infection with IV GCV (5 mg/kg, was administered every 12h for 6 weeks) followed by oral Val-GCV until the age of 12 months (17-18 mg/kg every 12h for 6 weeks followed by one daily suppressive dose). At age ≥ 1 year, hearing was normal in 76% of affected ears compared to baseline (54%). In 25 normal ears at birth no deterioration was found at ≥1 year. These results were significantly better than reported in a historical control group of similar infants treated for 6 weeks GCV therapy ( $p = 0.001$ )<sup>119</sup>.

The 18% rate of psychomotor retardation at age ≥1 year was considerably lower than the 55% reported in the past<sup>137</sup>. Viral load monitoring demonstrated sustained virological response. The main side effect of treatment was transient neutropenia. BSER at ≥1 year was better in patients who received prolonged treatment.

Moreover, Hilgendorff et al<sup>138</sup> treated with oral Val-GCV a congenitally infected infant with SNHL first detected at 4 months of age, resulting in a beneficial effect on retaining normal hearing and avoiding deterioration<sup>6,135</sup>.

The CASG group is performing a study on short-term (6 weeks) against long-term (6 months) oral Val-GCV treatment speculating that the long-lasting therapy may decrease long-term sequelae keeping the viral replication controlled for a longer period (ClinicalTrials.gov NCT00466817, accessed 1 December, 2010)<sup>139</sup>.

Recently, a case of CCMV-associated chorioretinitis, which required a 6-month course of antiviral therapy to be controlled (IV GCV, 12 mg/kg/day every 12 hours, for a total of 9 weeks followed by oral Val-GCV, 32 mg/kg/day administered every 12 hours), has been described. In addition to chorioretinitis, the patient had hearing impairment at the beginning of initial GCV treatment, and significant recovery of hearing of the better ear was observed after the initial GCV therapy; hearing level remained the same 6 months after the cessation of Val-GCV therapy. Pharmacokinetic data confirmed that oral Val-GCV dose was sufficient to achieve GCV levels equivalent to those of the IV GCV administration. Neutropenia did not develop during the entire course of therapy<sup>123</sup>.

#### ***Treatment: Asymptomatic CCMV Infection***

Even though it seems that asymptomatic patients have an increased risk for neurodevelopmental sequelae in general<sup>79</sup> and SNHL in particular<sup>140</sup> there has been only one recent study evaluating the effect on hearing of GCV therapy for asymptomatic CCMV infection<sup>141</sup>.

12 asymptomatic CCMV infants were treated with intravenous GCV for a period of 21 days and a 4 to 10-year follow-up demonstrated no hearing loss in this group when compared with 11.1% hearing loss occurring in the asymptomatic untreated group<sup>82,141</sup>.

#### ***GCV and Val-GCV-related Side Effects***

GCV is associated with a number of drug toxicities. Myelosuppression (such a granulocytopenia, anemia, thrombocytopenia) often is a dose-limiting toxicity in immunocompromised patients and in newborns.

The major toxicity in patients receiving GCV is hematologic abnormalities, overall neutropenia (100). However, the incidence of neutropenia in CCMV infected infants varies significantly. For

example, in the clinical trial conducted by Kimberlin et al<sup>119</sup>, neutropenia developed in 3% of infants with CCMV infection who received GCV during the first 6 weeks of treatment.

Tanaka-Kitajima et al<sup>142</sup> reported that neutropenia did not develop in any of 6 Japanese cases of congenital CMV during GCV treatment.

Similarly, Nigro et al<sup>112</sup> found neutropenia only in 1 of 12 cases of congenital CMV treated with GCV.

In two studies<sup>118,125</sup> neutropenia was present in up to 63% children undergoing GCV therapy and up to 38% children undergoing Val-GCV. Nevertheless, neutropenia-related sepsis was rarely a problem. Other rare side effects are bone marrow suppression, raised liver enzymes, hypokalemia and renal impairment.

For GCV-induced neutropenia, it has been demonstrated that Granulocyte Colony Stimulating Factor could be used to increase the absolute neutrophil count, while continuing long-term GCV therapy. All these side effects are reversible after stopping the drug for 3-7 days or decreasing the dose of the drug according to company product information (<http://www.rocheuk.com> Accessed December 2010).

Animal experiments with high dose GCV showed that short-term exposure induces testicular damage, affects sperm viability and may have carcinogenic effects<sup>143</sup>, but long-term effects in humans are not established. Thus, further follow-up is necessary for such infants who required long-term GCV or Val-GCV therapy; an observational study has been made in order to evaluate long-term side effects related to GCV and Val-GCV therapy, but results are not yet available (<http://www.clinicaltrials.gov/ct/show/NCT00031421>. Accessed 1 December 2010).

However, GCV has been used in the treatment of CMV retinitis and prevention of CMV disease in pediatric transplant patients for many decades. Despite prolonged treatment in these patients, reports of malignancy associated with its use have not been reported<sup>144</sup>.

The US-FDA has listed GCV as a pregnancy Category C drug<sup>82</sup>.

#### ***Strategies Under Development: Hyperimmune Globulin Treatment, New Antiviral Drugs and Vaccines***

Experiments using animal models of CMV infection and observational studies in humans indicate that CMV hyperimmune globulin (HIG) administered to pregnant women with a primary



CMV infection should be effective for both treatment and prevention of fetal infection<sup>145</sup>.

Some studies evaluating IV HIG treatment in pregnant women with primary CMV infection have recently been claimed to protect the fetus<sup>146-148</sup>. However, further studies should be performed to evaluate this result<sup>5,30,149</sup>.

Several anti-CMV agents are under development.

Maribavir (GW1263W94) is a benzimidazole L-riboside<sup>150,151</sup> whose mechanism of activity is thought to be inhibition of nuclear egress of newly-formed viral capsids<sup>152</sup>.

Because one of maribavir's targets, *UL97*, is necessary for GCV antiviral activity, there is a concern that the two agents could be antagonistic if used together<sup>153</sup>.

Due to Maribavir's recent failure in a phase 3 trial of a hematopoietic stem cell transplant population, it is unlikely that it will be investigated as a potential drug for CCMV infection.

Other antiviral agents with activity towards CMV that are in development or in clinical trials include GW275175X, tomeglovir (BAY 38-4766), hexadecyloxypropyl-cidofovir (HDPCDV), and octadecyloxyethyl-cidofovir (ODE-CDV)<sup>42</sup>.

However, the ideal method of prevention of CMV infection clearly is active immunization. The development of CMV vaccine to prevent CCMV is ranked as a top priority<sup>154</sup>. CMV vaccines in preclinical and clinical development have been reviewed by Schleiss and Heineman<sup>155</sup> (a comprehensive review of current vaccines is not the purpose of the current review).

## Discussion

Currently, CMV is the most common cause of congenital infections in humans and has a profound impact on individual and society's health.

Even though there is a growing number of studies on CCMV infection, its management is not yet well defined.

At this time, GCV and its orally-available pro-drug Val-GCV are the two most studied drugs that have been shown to be effective in the treatment of neonates with CCMV infection.

Based on reviewed data and overall on studies conducted by the National Institute of Allergy and Infectious Diseases Collaborative Antiviral Study Group (CASG studies)<sup>116-120</sup>,

a 6-week course of IV GCV therapy should be considered in the management of newborns with symptomatic CCMV disease involving the CNS.

Treatment should be initiated within the first month of life and patients should be monitored closely for toxicity, especially neutropenia.

These data cannot be extrapolated to neonates with other manifestations of CMV disease and to asymptomatic and symptomatic (other than CNS involvement) babies<sup>120</sup>, but there are growing data suggesting that GCV therapy might be considered for neonates with disseminated CCMV infection (defined as presence of asymmetric intrauterine growth retardation, epatosplenomegaly, hepatitis, anaemia and conjugated hyperbilirubinaemia) and high viral ( $> 5 \cdot 10^4$  PF/mL) or thrombocytopenia, because it may improve their long-term neurodevelopmental outcome<sup>56</sup>.

It is unclear whether prolonged or repeated treatment of CCMV infection more than 6 weeks is necessary as viral shedding in urine returns at a similar or lower viral load level after stopping treatment<sup>81</sup>.

The urine test is, in fact, the last parameter to turn negative because CMV replicates and concentrates in renal tubules and is usually eliminated via the kidneys, which makes a negative urine test difficult to achieve.

There are only few small studies to date that have administered GCV for prolonged periods without conclusive results<sup>156</sup>. Some Authors tried a prolonged therapy of symptomatic CCMV infection with IV GCV followed by oral Val-GCV, and it appeared safe and effective, as it led to a better auditory outcome than short-term treatment<sup>136</sup>.

The virus presence in blood is less frequent, but it could be more significant, as it indicates a systemic involvement<sup>82</sup>. Therefore, monitoring of viremia could be considered by a physician as a useful tool in deciding whether stopping or prolonging the treatment after a standard 6 weeks regimen.

Whether the orally bioavailable Val-GCV is as effective as GCV in improving SNHL in symptomatic newborns still need to be demonstrated.

However, evidence in neonates with Val-GCV is growing and pharmacokinetic data currently recommend a dose of 15 mg/kg twice a day (majority of studies have evaluated a regimen with doses ranging from 14 to 20 mg/kg twice daily) for 6 weeks, which provides plasma concentrations of GCV comparable to those achieved with administration of IV GCV<sup>5,127-129,132-134,145,157,158</sup>.

Uncontrolled case series advocated a more prolonged course of therapy for optimal outcome, partially with oral Val-GCV and apparently without higher risk of side effects<sup>111,112,127,159</sup>.

At this time, however, due to the lack of controlled trials, there is no evidence for longer

treatment. Oral treatment with Val-GCV should be limited to clinical trials, which should include pharmacokinetic data gathering and viral load determination in urine and blood as a marker of drug efficacy<sup>69</sup>.

If the effectiveness and safety of Val-GCV will be demonstrated by randomized controlled trials, therapy of CCMV infection will drastically change. In fact, the advantage of Val-GCV is the possibility of administering therapy at home, greatly simplifying the management of congenitally-infected newborns because indwelling intravascular access (necessary for GCV therapy) are no longer required with Val-GCV (*per os* administration) and children are no longer exposed to hospital-acquired infections. For the same reasons, neutropenia (related to both GCV and Val-GCV, even though it seems to occur less frequently and to be milder with Val-GCV) would be no longer as worrisome as usually thought. Home therapy also decreases the cost to the National Health Service<sup>1</sup>.

Regarding asymptomatic infection, which affects the highest proportion of infants with CCMV infection, problems related to infected children's growth and early use of antiviral drugs remains unclear<sup>1</sup>.

Even though it seems that asymptomatic patients have an increased risk for neurodevelopmental sequelae in general<sup>79</sup> and SNHL in particular<sup>80</sup>, at this time antiviral treatment in this population cannot be recommended. There is, in fact, not enough literature data on treatment of asymptomatic children; this should only be done within the setting of clinical trials, preferably randomized ones. Such trial should preferably include asymptomatic babies with high viral load in urine or blood, as they are at higher risk of hearing loss. It will be important to elucidate those viral or host factors that predispose to progression to SNHL in

early childhood, so that the ideal candidates for antiviral treatment may be identified.

The risks and side-effects related to antiviral therapy are thought to be greater than the risks of long-term consequences of asymptomatic infection, and its long-term benefit still unknown<sup>82</sup>.

A limitation of GCV and Val-GCV is the potential for toxicity, overall neutropenia, which can be particularly dangerous in symptomatic newborns because of prematurity, residence in intensive care units and, in the case of GCV, the risks of indwelling catheters for drug infusion, even though dangerous neutropenia have rarely been described, and it is easily resolved by diminishing drug doses or interrupting therapy for 3-7 days<sup>140</sup>.

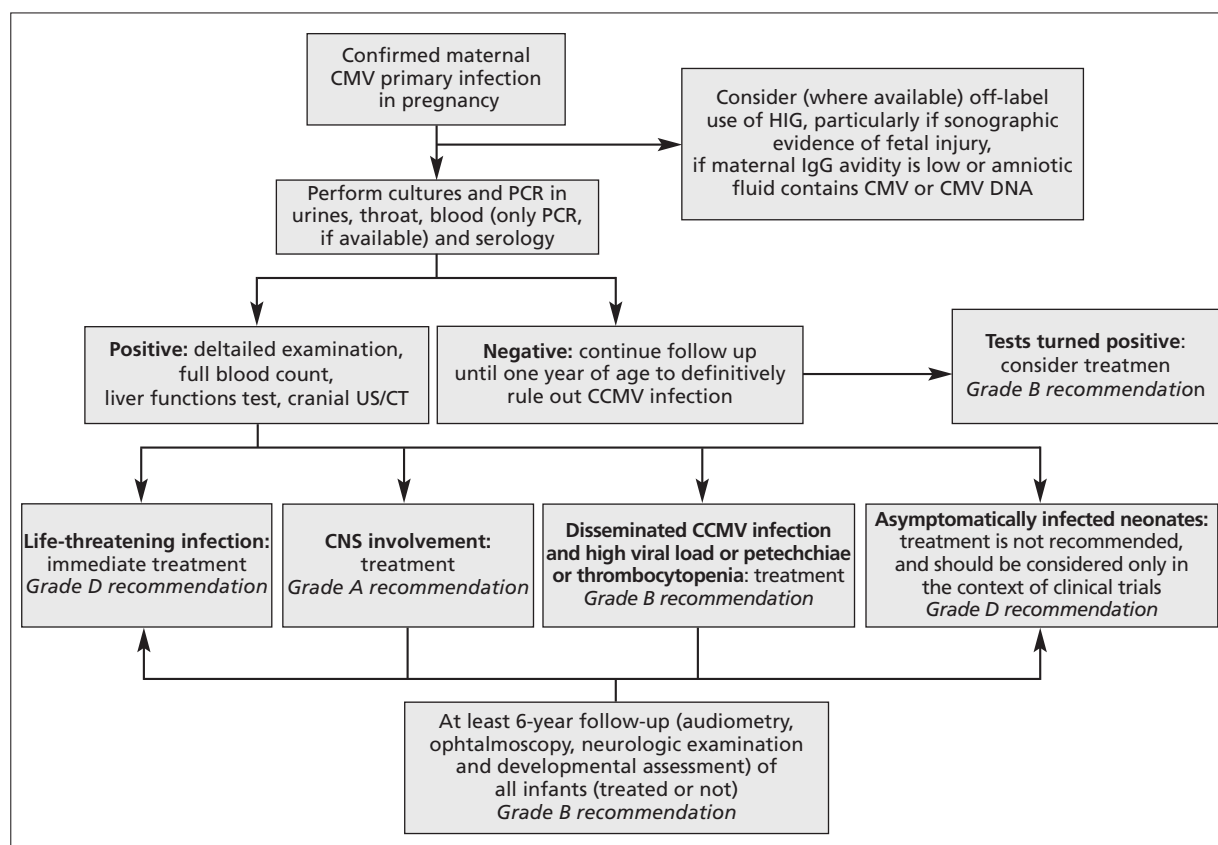
Another limitation of antiviral therapy is the persistence of viral replication within the cochlear perilymph and renal parenchyma. Though it had previously been shown that CMV can be isolated from the perilymph of affected infants<sup>85</sup>, more recent studies utilizing PCR have shown evidence of ongoing viral replication within the perilymph in children up to 4 years of age<sup>87,88</sup>. Since these patients had not been previously treated, it is not certain that this persistence of CMV within the perilymph also occurs in infants who receive GCV.

A report that there is no significant difference in the duration of urinary excretion of CMV between treated and untreated patients with congenital CMV infection<sup>81</sup> suggests that it is at least possible for persistence of viral replication to occur regardless of treatment. This may indicate that SNHL is an ongoing process over the first several years of life rather than a single hit early in infancy. In the face of an ongoing viral process such as this, a one-time, relatively short course of therapy might not be the optimal treatment duration<sup>1</sup>.

Therefore, studies evaluating standard (6 weeks) versus longer regimens are urgently needed. Table

**Table II.** Treatment of CCMV infection. Adapted from Gandhi et al, 2010; 99: 509-515<sup>82</sup>.

Drug	Regimen	Monitoring	Comments
Ganciclovir	6 mg/kg twice daily, intravenous, per 6 weeks.	Full blood count, liver function tests, creatinine, urea and electrolytes	Suspend treatment if absolute neutrophil count < 500 cells/ $\mu$ L or platelet count < 25 000 cells/ $\mu$ L
Valganciclovir	15 mg/kg twice daily, per os, per 6 weeks.	Full blood count, liver function tests, creatinine, urea and electrolytes	Suspend treatment if absolute neutrophil count < 500 cells/ $\mu$ L or platelet count < 25 000 cells/ $\mu$ L



**Figure 1.** Management of CCMV infection. Adapted from Ref. 82. Grades of recommendation: *Grade A*, Consistent level 1 studies. *Grade B*, Consistent level 2 or 3 studies or extrapolations from level 1 studies. *Grade C*, Level 4 studies or extrapolations from level 2 or 3 studies. *Grade D*, Level 5 evidence or troublingly inconsistent or inconclusive studies of any level. Levels of evidence: *Level 1*, Systematic review (SR) of randomized controlled trials (RCT), SR of prospective cohort studies, individual RCT, prospective cohort study with good follow-up). *Level 2*, SR of cohort studies, SR of either retrospective cohort studies or untreated control groups in RCT, individual cohort study, retrospective cohort study or poor follow-up, retrospective cohort study or follow-up of untreated control patients in an RCT). *Level 3*, SR of case-control studies, individual case-control study). *Level 4*, Case series. *Level 5*; Expert opinion without explicit critical appraisal.

II summarizes current recommendations on the management of CCMV infection.

Figure 1 shows an algorithm for the management of CCMV infection.

In conclusion, there are growing evidences that newborns with CCMV infection would benefit from treatment, and continuous researches are being made to understand the long-term role of antiviral therapies. Studies looking at identification of risk factors in asymptomatic newborns associated to the development of long-term sequelae are required, in order to identify those asymptotically-infected children who might benefit for treatment. Moreover, randomized controlled trials evaluating treatment and long-term follow-up of infants with both symptomatic and asymptomatic CCMV are necessary, in order to definitely evaluate the short and long-term effectiveness and safety of both GCV and Val-GCV<sup>82</sup>.

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