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Original article

Prediction of poor neurological development in patients with symptomatic congenital cytomegalovirus diseases after oral valganciclovir treatment

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Abstract

Objective: This study aimed to evaluate the neurodevelopmental outcomes of infants with symptomatic congenital cytomegalovirus (SCCMV) disease after antiviral treatment and investigate the symptoms at birth associated with a developmental quotient (DQ) < 70.

Methods: In this prospective study conducted from 2009 to 2018, infants with SCCMV disease who received oral valganciclovir (VGCV; 32 mg/kg/day) for 6 weeks (November 2009 to June 2015) or 6 months (July 2015 to March 2018) were evaluated for their neurodevelopmental outcomes at around 18 months of corrected age. Sequelae were categorized as follows: no impairment with a DQ ≥ 80 and no hearing dysfunction; mild sequelae including unilateral hearing dysfunction or a DQ of 70–79; and severe sequelae with a DQ < 70, bilateral hearing dysfunction requiring hearing aids, blindness or epilepsy requiring anti-epileptic drugs. DQ was assessed using the Kyoto Scale of Psychological Development. Symptoms at birth associated with a DQ < 70 were determined using univariate and receiver operating characteristic curve analyses.

Results: Of the 24 treated infants, 21 reached > 18 months of corrected age. Six (29%) were no impairment, 4 (19%) had mild sequelae, and 11 (52%) developed severe sequelae. The symptoms at birth associated with a DQ < 70 were microcephaly and/or small for gestational age.

Conclusion: In our cohort of infants with SCCMV disease after VGCV treatment, the incidence of severe sequelae at 18 months of corrected age was around 50%. When microcephaly and/or small for gestational age are seen at birth, a low DQ may appear even after oral VGCV treatment.

Keywords: Antiviral treatment; Developmental quotient; Microcephaly; Sequelae; Small for gestational age

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1. Introduction

Congenital cytomegalovirus (CMV) infection is a major disease linked with neurological sequelae that occur during childhood. Approximately 70–90% of infants who show any symptoms of congenital CMV infection at birth (symptomatic congenital CMV [SCCMV] disease) have neurological sequelae later in life, such as hearing dysfunction, neuromuscular disorder (epilepsy, hemiparesis, and spasm), psychomotor retardation, ocular abnormality (optic atrophy and chorioretinitis), delayed language development, and intellectual disability [1–3]. The neurological sequelae of SCCMV disease occur at a high rate; therefore, many clinical studies have examined the neonatal predictive factors related to such sequelae [4–7]. For example, Kylat et al. reported that abnormalities in brainstem auditory-evoked potential (BAEP) and brain imaging on ultrasonography or computed tomography (CT) at birth, but not microcephaly and thrombocytopenia, were frequently seen in children with sequelae [4]. Noyola et al. evaluated the relationship between symptoms at birth and prognosis and found that microcephaly and abnormalities on a brain CT were the most specific predictive factors of psychomotor disorders [5]. Ito et al. reported that small for gestational age (SGA), microcephaly, brain imaging abnormalities, periventricular calcification, and disseminated intravascular coagulation were related to serious sequelae and death [6]. Lanzieri et al. reported that microcephaly or abnormalities on brain CT at birth was significantly related to hearing dysfunction, intellectual disability, or visual disturbance [7]. Although the evaluated ages and methods differ among clinical studies, microcephaly and brain abnormal imaging at birth are likely neonatal factors associated with the onset of neurological sequelae in untreated children with SCCMV disease.

Because newborns with SCCMV disease involving central nerve abnormalities often go on to develop neurological sequelae, antiviral treatment from the neonatal period is performed using intravenous ganciclovir (GCV) or oral valganciclovir (VGCV) to alleviate hearing dysfunction and neurodevelopmental delay later in life [8–13]. To date, however, few studies have assessed neonatal symptoms associated with neurological sequelae, such as a low developmental quotient (DQ), in children with SCCMV after antiviral treatment.

The aims of this study were to prospectively evaluate the neurodevelopmental outcomes at around 18 months of corrected age in patients with SCCMV disease who were treated with oral VGCV and identify the neonatal symptoms associated with a DQ < 70 in a Japanese cohort after antiviral treatment with VGCV.

2. Patients and methods

2.1. Study design

This single-center prospective cohort study was conducted from 2009 to 2018 at Kobe University Hospital to evaluate the neurodevelopmental outcomes at around 18 months of corrected age in children with SCCMV disease after antiviral treatment with oral VGCV and investigate the symptoms at birth associated with a DQ < 70. Oral VGCV treatment for congenital CMV disease is currently an off-label treatment; therefore, prior to starting the VGCV treatment, the risks and benefits were thoroughly explained to the patients' parents. This clinical study obtained written informed consent and approval from the ethics committee of Kobe University Graduate School of Medicine (no. 1214).

2.2. Diagnosis of congenital CMV infection

Congenital CMV infection was diagnosed by the detection of CMV DNA in urine samples collected within 3 weeks of age by real-time quantitative polymerase chain reaction (PCR) as we previously reported [14].

2.3. Definition of SCCMV disease

SCCMV disease was defined as a case with at least one of the following findings: microcephaly (head circumference at birth, less than the 10th percentile) [15]; SGA (standard deviation [SD] score of birth weight and/or birth height, <−2.0); hepatitis (serum aspartate aminotransferase level, ≥100 U/L); thrombocytopenia (<100,000/μL); abnormalities on brain imaging by ultrasonography, CT, and magnetic resonance imaging (cortical dysplasia, white-matter abnormality, ventricular dilation, and calcification); ocular complications such as chorioretinitis; and abnormal BAEP (no detection of V-wave at 30 dB at ≥37 weeks of corrected gestational age and at 40 dB at 34–36 weeks of corrected gestational age) [10]. Findings of brain imaging abnormalities were decided according to the interpretations of 2 pediatric radiologists who were blinded to the patients' clinical courses. SD scores for birth weight, height, and head circumference were calculated using nordiFIT® (Novo Nordisk Pharma, Tokyo, Japan). This can calculate SD scores for birth weight, height, and head circumference by gestational age at birth according to sex-specific standards based on the Japanese population [16].

2.4. Oral VGCV treatment protocol

The treatment was oral VGCV 32 mg/kg/day for 6 weeks in November 2009 to June 2015. From July

2015, oral VGCV 32 mg/kg/day was administered for 6 months based on the evidence for a better prognosis than that for 6 weeks [11]. Oral VGCV was administered during hospitalization for the 6-week treatment. The 6-month treatment was continued on an outpatient basis depending on each patient's condition after the first 6 weeks. Infants hospitalized for 6 weeks were examined by a neonatal pediatrician every day, and blood tests were performed at least once a week to evaluate the treatment efficacy and adverse events. The VGCV treatment was discontinued temporarily when the neutrophil count was $<500/mm^3$ and resumed when it recovered [8,10,11]. When the discontinuation period was prolonged, a decrease in VGCV dose or the administration of granulocyte-colony stimulating factor was decided by the attending pediatrician.

2.5. Measurement methods for CMV load in blood and urine

According to our methods described previously [14], we extracted DNA from peripheral blood and urine using QIAamp DNA Mini kits (Qiagen Corp., Tokyo, Japan). CMV DNA copy numbers were measured using real-time quantitative PCR. Blood CMV DNA was expressed as copy number/10^6 leukocytes, while urine CMV DNA was expressed as copy number/mL.

2.6. Neurodevelopmental outcomes

At around 18 months of corrected age, neurodevelopmental outcomes (DQ, hearing dysfunction, blindness and epilepsy status) were evaluated. No impairment was defined as a DQ $\geq$ 80 and no hearing dysfunction; mild sequelae was defined as unilateral hearing dysfunction or a DQ of 70–79; and severe sequelae was defined as a DQ $<$ 70, bilateral hearing dysfunction requiring hearing aids, blindness or epilepsy requiring antiepileptic drugs [10]. DQ was assessed using the Kyoto Scale of Psychological Development, the most commonly used method for determining developmental outcomes in Japan [17]. Overall DQ was calculated as follows:

Overall DQ = (developmental age obtained from the Kyoto Scale of Psychological Development/corrected chronological age) × 100

We recorded DQ in 3 specific areas, namely postural-motor, cognitive-adaptive, and language-social, and an overall DQ.

2.7. Physical data at follow-up

Height and body weight were collected at follow-up. Height was measured with a digital height meter, body weight was measured with a digital scale, and head circumference was measured by a ruler tape.

2.8. Study methods and statistical analyses

To investigate the incidence of sequelae, enrolled children with SCCMV disease after oral VGCV treatment were categorized into the aforementioned categories of no impairment, mild sequelae, and severe sequelae. The patients at around 18 months of corrected age were divided into groups of DQ $<$ 70 and DQ $\geq$ 70. Body weight, height, and head circumference at around 18 months of corrected age were compared. SD scores for body weight, height, and head circumference at follow-up were calculated according to age- and sex-specific standards based on the Japanese population [18]. The neonatal symptoms associated with a DQ $<$ 70 were examined in a univariate analysis with the Wilcoxon signed-rank or Fisher exact test. Neonatal symptoms with p values $<$ 0.10 were noted, and the neonatal symptoms associated with a DQ $<$ 70 were determined using sensitivity, specificity, and the Youden index on receiver operating characteristic (ROC) curve analysis. The Youden index is the point farthest from the boundary delineating the area under the curve (0.500 on the ROC curve) and represents the (sensitivity + specificity − 1) value [19].

3. Results

3.1. Enrolled infants with SCCMV disease

Of 72 infants diagnosed with congenital CMV infection during the study period, 27 were symptomatic. Two patients who were treated with intravenous GCV, one who was followed up with observation but no treatment, and 3 who were younger than 12 months of age were excluded. The 21 infants treated with VGCV whose neurodevelopmental outcomes were evaluated at around

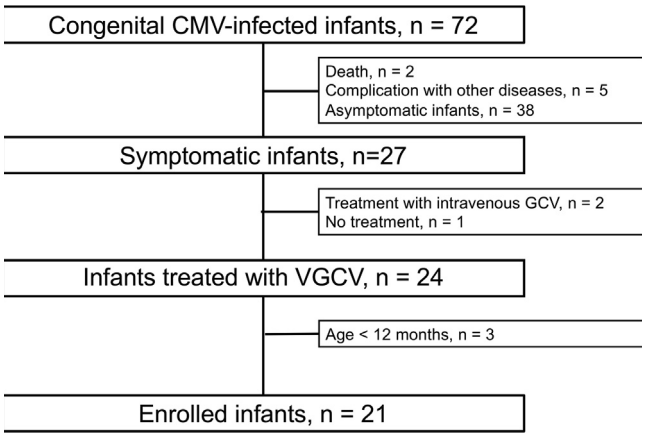

Fig. 1. Flowchart of the subject selection and enrolment. CMV, cytomegalovirus; GCV, ganciclovir; VGCV, valganciclovir.

Table 1
Clinical characteristics at birth.

	N = 21
Gestational age at birth (weeks)	36 (31–40)
Male	5 (24)
Birth weight (g)	2192 (940–3312)
Birth height (cm)	44.5 (35.0–51.0)
Head circumference at birth (cm)	31.5 (25.5–34.5)
Symptoms	
Abnormality on brain image	19 (90)
Abnormal brainstem auditory evoked potential	17 (81)
Thrombocytopenia	11 (52)
Small for gestational age	9 (43)
Microcephaly	8 (38)
Hepatitis	8 (38)
Ocular complication	6 (28)

Data are shown as median (range) or number (percentage).

18 months of corrected age were selected as this study subjects (Fig. 1).

3.2. Clinical characteristics at birth

Clinical characteristics at birth for the 21 enrolled infants are shown in Table 1. The median gestational age at birth was 36 weeks, while the median birth weight was 2192 g. All infants had symptoms (100%). The most common symptom was brain imaging abnormalities (90%), followed by abnormal BAEP (81%) and thrombocytopenia (52%; Tables 1 and 2). Regarding the brain imaging abnormalities, white-matter abnormalities were found in 18 (86%), ventricular dilation in 16 (76%), calcification in 9 (43%), and cortical dysplasia in 6 (29%) patients. Of the 21 enrolled infants, 16 (76%) were treated with oral VGCV for 6 weeks and 5 (24%) were treated for 6 months. The VGCV treatment was started at a median 13 days of age (range, 4–77 days). Side effects were seen in 9 (43%) patients, the most common being neutropenia (n = 7; 33%).

3.3. Physique and neurodevelopmental outcomes at follow-up

At a median 18 months of corrected age, no impairment was observed in 6 (29%); mild sequelae were seen in 4 (19%); and severe sequelae were seen in 11 (52%) patients (Table 2). No patients with blindness were observed. When DQ was the focus as an indicator of neurodevelopmental outcome, the enrolled children were divided into the DQ < 70 (n = 9; 43%) and DQ ≥ 70 (n = 12; 57%) groups (Tables 2 and 3). The DQ values and physical data were compared between the 2 groups. All DQ values were significantly lower in the DQ < 70 group than in the DQ ≥ 70 group ($p < 0.01$). No significant intergroup difference was found in body weight, height, or head circumference SD scores (Table 3).

3.4. Neonatal symptoms associated with a DQ < 70

The univariate analyses revealed that SD scores of height and head circumference at birth were significantly lower in the DQ < 70 group than in the DQ ≥ 70 group ($p < 0.05$; Table 4). Among the symptoms, microcephaly, SGA, and thrombocytopenia had high prevalence rates in the DQ < 70 group ($p < 0.10$; Table 4). The sensitivity, specificity, and Youden Index that predicted a DQ < 70 based on these 3 factors are shown in Table 5. Microcephaly and/or SGA showed the highest Youden Index values (0.63; Table 5).

4. Discussion

The current study investigated the neurodevelopmental outcome of patients with SCCMV disease who were treated with oral VGCV and the neonatal symptoms associated with a DQ < 70. This Japanese cohort included patients with severe sequelae; however, mild sequelae or no impairment was observed in approximately 50% of the patients with SCCMV disease after oral VGCV treatment. Furthermore, oral VGCV treatment revealed that the neonatal symptoms associated with a DQ < 70 at 18 months of corrected age were microcephaly and/or SGA.

Sequelae developed in approximately 70–90% of infants with untreated SCCMV disease [1,2]. Therefore, since the year 2000, treatment with antiviral drugs from the neonatal period has been attempted to reduce the sequelae. Kimberlin et al. administered GCV 12 mg/kg/-day intravenously for 6 weeks to 24 infants with SCCMV disease involving central nervous system abnormalities. One year later, hearing improved in some patients (4/24; 17%) and the number of patients with worsened hearing level decreased significantly (treatment group: 21%; untreated group: 68%) [8]. Oliver et al. reported that the psychomotor delay scores evaluated using the Denver II test at 6 and 12 months of age decreased significantly in the treated group (GCV 12 mg/kg/day intravenously for 6 weeks) versus those in the untreated group [9]. Recently, the number of reports on treatment with oral VGCV has been increasing compared with those on intravenous GCV treatment [10–13,20]. However, most of the reports focused on the effect on hearing impairment, while those regarding neurodevelopmental outcomes are limited. Providing recent evidence, Kimberlin et al. reported that neurodevelopmental outcomes, particularly language development evaluated using the Bayley III scale, improved significantly at 12 and 24 months of age by VGCV treatment for 6 months compared with that for 6 weeks [11]. Even in our cohort, approximately 50% of children had severe sequelae that impaired performance in daily living and approximately 40% of children had a DQ < 70. However, some infants with SCCMV disease involving cen-

Table 2
Neonatal symptoms and neurodevelopmental outcomes.

Patient no.	Neonatal symptoms							Neurological outcome				Sequelae
	SGA	Microcephaly	Thrombocytopenia	Hepatitis	Ocular complication	Abnormality on brain image	Abnormal BAEP	DQ < 70	70 ≤ DQ < 80	Hearing dysfunction	Epilepsy	
1		●			●	●	●	●		Bilateral	●	Severe Sequelae, 11 (52%)
2	●	●	●	●	●	●	●	●		Bilateral	●	
3	●	●	●	●		●		●		Bilateral	●	
4	●		●			●	●	●		Unilateral		
5	●	●	●			●	●	●				
6	●	●	●	●		●	●	●		Bilateral		
7	●					●	●	●		Bilateral		
8		●	●	●		●	●	●				
9			●			●	●	●		Bilateral		
10	●	●		●		●	●		●	Bilateral		
11			●		●	●	●			Bilateral		
12			●			●	●		●			Mild sequelae, 4 (19%)
13			●	●		●	●			Unilateral		
14						●				Unilateral		
15						●	●			Unilateral		
16							●					No impairment, 6 (29%)
17	●			●		●	●					
18	●	●	●	●	●	●	●					
19					●							
20						●	●					
21					●	●						

BAEP, brainstem auditory-evoked potential; DQ, developmental quotient; SGA, small for gestational age.

Table 3
Developmental quotients and physical data at follow-up.

	DQ < 70 n = 9	DQ ≥ 70 n = 12	P value
Corrected age at evaluation, months	18 (14–19)	18 (17–26)	0.35
DQ			
Overall	42 (10–69)*	90 (78–99)	<0.01
Postural-motor	31 (10–77)*	70 (56–108)	<0.01
Cognitive-adaptive	49 (10–69)*	93 (80–100)	<0.01
Language-social	44 (10–67)*	94 (67–113)	<0.01
Physique			
Body weight (g)	7950 (7030–9695)	9249 (7555–13760)	0.06
Body weight SD score	−1.8 (−3.0–−0.5)	−0.8 (−2.6–1.4)	0.08
Height (cm)	76.7 (73.2–81.1)	77.7 (72.9–87.9)	0.22
Height SD score	−1.2 (-2.5–0.3)	−0.6 (−2.4–0.3)	0.70
Head circumference (cm)	43.1 (39.5–46.6)	44.7 (42.2–49.2)	0.02
Head circumference SD score	−2.4 (−4.7–−0.1)	−1.3 (−2.9–1.1)	0.06

DQ, developmental quotient; SD, standard deviation.
* One case showed as DQ = 10 in all areas for severe motor and intellectual disability.

Table 4
Univariate analyses.

	DQ < 70, n = 9	DQ ≥ 70, n = 12	P value
Gestational age at birth (weeks)	36 (32–39)	37 (31–40)	0.97
Male	1 (11)	4 (33)	0.34
Apgar scores at 5 min ≤7	3 (33)	0 (0)	0.06
Birth weight (g)	2184 (940–2548)	2321 (1378–3312)	0.23
Birth weight SD score	−2.08 (−3.59–0.42)	−0.585 (−3.79–2.62)	0.12
Birth height (cm)	43.0 (35.0–46.0)	45.75 (38.6–51.0)	0.04
Birth height SD score	−2.24 (−3.21–−0.93)	−0.485 (−3.3–1.47)	0.02
Birth head circumference (cm)	29.5 (25.5–33)	31.8 (28.6–34.5)	0.04
Birth head circumference SD score	−2.07 (−3.63–0.3)	−0.1 (−2.72–1.07)	0.03
CMV load in blood before treatment (copies/10^6 leukocytes)	3.1×10^2 (2.1×10–9.3×10^3)	5.35×10^2 (2.2×10–6.9×10^4)	0.50
CMV load in urine before treatment (copies/mL)	5.4×10^7 (4.0×10^4–2.9×10^8)	9.6×10^6 ($1.9 \times 10^{4-}$–2.1×10^8)	0.16
Symptoms			
Microcephaly	6 (67)	2 (17)	0.03
Small for gestational age	6 (67)	3(25)	0.09
Thrombocytopenia	7 (78)	4 (33)	0.08
Hepatitis	4 (44)	4 (33)	0.67
Ocular complication	2 (22)	4 (33)	0.66
Abnormality on brain image	9 (1 0 0)	10 (83)	0.49
Abnormal brainstem auditory-evoked potential	8 (89)	9 (75)	0.60

Data are shown as median (range) or number (percentage).
CMV, cytomegalovirus; DQ, developmental quotient; SD, standard deviation.

Table 5
Sensitivity, specificity, and Youden Index on the ROC curve analyses.

	Sensitivity	Specificity	Youden Index
Microcephaly	0.67	0.83	0.50
SGA	0.67	0.75	0.42
Thrombocytopenia	0.78	0.67	0.44
Microcephaly and/or SGA	**0.89**	**0.75**	**0.63**
Microcephaly and/or thrombocytopenia	0.89	0.58	0.47
SGA and/or thrombocytopenia	0.89	0.50	0.39
Microcephaly and/or SGA and/or thrombocytopenia	1.00	0.50	0.50

BW, birth weight; SD, standard deviation; SGA, small for gestational age; ROC, receiver operating characteristic
The bold has shown the symptoms with the highest Youden index.

tral nerve abnormalities might show improvement with oral VGCV treatment.

Microcephaly and/or SGA were identified as neonatal symptoms associated with a DQ < 70 in our cohort. The mechanism of mother-to-child CMV infection is a transplacental transmission of CMV; the CMV disseminates to various organs of the fetus. Invasion of the central nerves results in widespread infection of the various central nerve cells. A recent *in vitro* study has shown that expression of the human CMV immediate-early 2 protein affects the proliferation and self-renewal capacity of neural progenitor cells and induces the premature differentiation of neural stem cells [21]. This results in central nervous system abnormalities, decreased brain volume, and microcephaly [22–26]. In addition, CMV infection has been shown to cause avascular regions in the placenta, progression of fibrosis, and decreased blood flow that results in placental dysfunction and poor fetal growth due to fetal nutritional disorder [27–29]. Recently, Tsuge et al. have reported that CMV-infected placenta affects the fetal body weight gain and the increases in fetal abdominal circumference or femur length [30], suggesting that CMV-infected placenta is closely associated with fetal growth. Thus, children affected by fetal growth due to CMV infection may have poor neurodevelopmental outcomes even when antiviral treatment is given soon after birth. Our results that birth height, birth circumference, and their SD scores were significantly lower in the DQ < 70 group than in the DQ ≥ 70 group (Table 4), would support this theory.

This study has four limitations. First, because it was conducted at a single center over approximately 10 years, there was a limited number of patients. However, none of the patients withdrew from the follow-up and the study was performed on the basis of a uniform treatment and follow-up protocol, which is a strength of this study. Second, the neurodevelopmental evaluation age was low; therefore, the long-term prognosis could not be evaluated. However, an evaluation based on a longer period will be affected by factors other than CMV infection, such as the patients' environments. Therefore, the evaluation age in this study was set to around 18 months of corrected age. Third, because recent studies showing the efficacy of antiviral treatment for infants with SCCMV disease have been increasing [10–13,20], we could not set the non-treatment control group due to ethical issues. Finally, the 6-week and 6-month treatments were mixed in this study. We hope that the most effective treatment method is chosen for each patient. As mentioned, the 6-month treatment with VGCV was reportedly more effective than the 6-week treatment in 2015 [11]; for this reason, the treatment method had to be changed midway.

5. Conclusions

In our cohort of infants with SCCMV disease after VGCV treatment, the incidence of severe sequelae at around 18 months of corrected age was around 50%, while approximately 50% demonstrated mild sequelae and normal development. The presence of microcephaly and/or SGA at birth may carry the risk of a DQ < 70 even after VGCV treatment.

Acknowledgments

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Conflict of interest

The authors declare no financial or personal relationships that could pose a conflict of interest in this study.

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