Original Article

Prospective cohort study for postnatal CMV infection in preterm infants

Running title: Postnatal CMV infection in preterms

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【Abstract】

Aim: Cytomegalovirus (CMV) is a virus that can cause congenital and postnatal infections. Postnatal CMV is mainly transmitted via breast milk and blood transfusions. Frozen-thawed breast milk is used to prevent postnatal CMV infection. A prospective cohort study was conducted to determine the infection rate, risk, and clinical findings of postnatal CMV infection.

Methods: This prospective cohort study included infants born at 32 weeks or earlier than the gestational age. Participants were prospectively screened for infection in the urine by performing urine CMV DNA tests twice, i.e., once within the first 3 weeks of life and again after 35 weeks postmenstrual age. Postnatal CMV infection was defined as a case of CMV negative tests within 3 weeks of birth and CMV positive tests after 35 weeks postmenstrual age. CMV-negative blood products were used for transfusions in all cases.

Results: A total of 139 patients were subjected to two urine CMV DNA tests. The prevalence of postnatal CMV infection was 5.0%. One patient died of sepsis-like syndrome. The risk factors of postnatal CMV infection were younger gestational age and older age of the mother. The characteristic clinical findings of postnatal CMV infection were pneumonia.

Conclusions: Frozen-thawed breast milk feeding is not fully effective in preventing postnatal CMV infection. The prevention of postnatal CMV infection is important to further improve the survival rate of preterm infants. Development of guidelines on breast milk feeding for the prevention of postnatal CMV infection is necessary in Japan.

【Key words】

Breast milk; Cytomegalovirus; Cytomegalovirus-related sepsis-like syndrome; Premature infant; Prospective Studies

Introduction

Cytomegalovirus (CMV) can cause congenital and postnatal infections in the perinatal period. Postnatal cytomegalovirus (pCMV) is mainly transmitted via breast milk, blood transfusions, and cervical or vaginal secretions[1]. More than 80% of CMV-seropositive women will excrete the virus in their milk [2], and their DNA can be detected in breast milk from the 10th day postpartum with a peak between weeks 4 to 8. The use of frozen-thawed breast milk is recommended to prevent pCMV infection because freezing CMV at −20 °C for at least 3 days reduces its virulence by 85%–99%, but this method was proven ineffective[3]. Transfusion of CMV-negative blood products have also been recommended, as pCMV infection can also occur with blood transfusions [4–6]. CMV is present in the cervical or vaginal secretions of mothers with a history of infection and can be transmitted to the baby during vaginal delivery [1].

Although pCMV infection is generally a subclinical infection in term infants, preterm infants can develop severe symptoms such as sepsis-like syndrome (SLS), pneumonia, necrotizing enterocolitis (NEC), hepatitis, enteritis, biliary stasis, hepatomegaly, elevated liver enzymes, thrombocytopenia, and neutropenia [2,7–10].

Thus, pCMV infection is an important issue in the management of preterm infants. There are significant uncertainties in the management of premature infants with pCMV infection, which is in part due to our limited understanding of the natural history of this condition. Determining the incidence and clinical manifestations of pCMV infection is essential when screening tests and breastfeeding management are needed. Since the rate of CMV infection in pregnant women varies among countries, investigations are necessary for each country [11].

This prospective cohort study aimed to determine the rates, risk factors, and clinical presentation of pCMV infection in preterm infants born at ≤ 32 weeks of gestational age (GA), who are at high risk for pCMV infection[2]. Since there are few reports on pCMV infection in Japan, this study is important for understanding the epidemiology of pCMV infections in preterm infants in Japan.

Methods

Design and study participants

This prospective cohort study included infants born ≤32 weeks of gestation who were admitted to the neonatal intensive care unit (NICU) of Nagano Children's Hospital in Japan between July 2017 and March 2021. Nagano Children's Hospital has a level IV NICU at the perinatal medical center. The study was approved by the Medical Ethics Committee of Nagano Children's Hospital (Approval No. 29-11), and the parents provided written consent.

Participants were prospectively screened for infection in the urine by performing urine CMV DNA tests (Genelys, Shino-Test Corporation, Tokyo, Japan) twice, i.e., once within the first 3 weeks of life and again after 35 weeks postmenstrual age (PMA). In our NICU, breastfeeding is started at 35 weeks PMA and restriction on the use of fresh breast milk is discontinued. The CMV tests were performed at 35 weeks PMA before using fresh breast milk. In cases of CMV-SLS was suspected, a second test could be performed before 35 weeks PMA. If the second test was performed before 35 weeks PMA, protocol was to perform the third examination after 35 weeks PMA. These tests were conducted according to the package insert.

Congenital CMV infection was defined as positive CMV tests within 3 weeks of birth, pCMV infection was defined as negative CMV tests within 3 weeks of birth and positive CMV tests after 35 weeks PMA, and non-CMV infection as negative CMV tests at both time points. The exclusion criteria were death before 35 weeks PMA and no available urine sample at 35 weeks PMA. The presence of severe congenital disease was not included in the exclusion criteria, but there were no infants with severe congenital disease.

All mothers were encouraged to provide breast milk to their children. The infants were fed fresh breast milk until 1 week of age, frozen-thawed breast milk from 1 week of age to 35 weeks PMA, and fresh or frozen-thawed breast milk after 35 weeks PMA. No case deviated from this policy during the study period. Transfusion-associated CMV infection was prevented by the exclusive use of CMV-seronegative blood products. CMV-seronegative blood was used in all patients in whom transfusions were performed.

Data collection

Data were collected from patients' medical records until death or discharge from the NICUs. Patients were divided into the pCMV group and non-CMV group, and those with congenital CMV

infection were excluded.

The following demographic and clinical data were collected: GA, birth weight, sex, maternal age, number of deliveries and parity, fetal number, small for GA (SGA), Apgar scores at 1 and 5 min, congenital disease, mode of delivery, antenatal steroids, histological chorioamnionitis (CAM), the C-reactive protein (CRP) level upon admission, breastfeeding status, and number of transfusions. All infants were observed for signs of CMV infection, such as SLS, pneumonia, NEC, and hepatomegaly. Laboratory findings included elevated transaminases (aspartate transaminase [AST] > 140 U/L and alanine transaminase [ALT] > 50 U/L), elevated direct bilirubin (>1.0 mg/dL), decreased neutrophil count (<1500/μL), decreased platelet count (<150000/μL), and elevated CRP (>1.0mg/dL). Blood counts and biochemistry (transaminases, CRP levels, bilirubin levels) were determined weekly as part of the standard patient care until discharge. During the first and second urine CMV tests, we evaluated whether the maximum value of each item exceeded these data.

Definitions

GA was defined as the best estimate based on early prenatal ultrasound examination, last menstrual period, and physical examination of infants at birth. SGA was defined as a birth weight <10th percentile of the standard birth weight for GA published by the Japan Pediatric Society [12]. Antenatal corticosteroid use was defined as the administration of at least one dose of corticosteroid to the mother any time before delivery to accelerate fetal lung maturity. The histologic criterion used for CAM was the presence of accumulated leukocytes extending through the fetal membranes using Blanc's classification [13]. Breast milk was defined as breastfeeding at least once. Sepsis was considered when clinical signs and symptoms of infection were present, increased CRP level, and positive blood or cerebrospinal fluid culture. Pneumonia was diagnosed by sudden worsening of respiratory status, X-ray evidence of pneumonia, and CRP level elevations. NEC was defined as the presence of clinical symptoms of abdominal infection with pneumatosis intestinalis on an abdominal radiograph, according to the Bell criteria [14].

Statistical analyses

All statistical analyses were performed with EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface for R (The R Foundation for

Statistical Computing, Vienna, Austria). More precisely, it is a modified version of R commander designed to add statistical functions frequently used in biostatistics.

Maternal and infant demographic characteristics, perinatal risk factors, and incidence of mortality and morbidity were compared between pCMV infection groups and non-CMV infection groups using Fisher's exact test for categorical variables and the t-test for continuous variables. The Mann–Whitney U test was used when continuous data were not normally distributed. Multivariate logistic regression analysis was used to examine the impact of significant factors based on the univariate analysis of the two groups regarding pCMV infection, with two adjusted models. The first model included GA, whereas the second included maternal age. A p value <0.05 was considered significant.

Results

A total of 151 preterm infants were born at ≤32 weeks of gestation during the study period. Figure 1 shows a flow diagram. Of these, 12 (7.9%) were excluded because of nonconsent (n = 6), death before 35 weeks PMA (n = 5) and transfer before the second test (n = 1). Death before 35 weeks PMA were caused by severe neonatal asphyxia (n = 2), disseminated intravascular coagulation (n = 1), and bacterial sepsis (n = 2). Finally, 139 patients underwent twice urine CMV DNA tests. In all cases, the first test was performed within the first 2 weeks of age, and the second test was performed between 35 and 37 weeks PMA. For the case of CMV-SLS, the time of onset coincided with the second urine CMV DNA test.

Of these patients, 3 (2.2%) were diagnosed with congenital CMV infection due to positive CMV tests within 3 weeks of birth, 7 (5.0%) were diagnosed with pCMV infection due to negative CMV tests within 3 weeks of birth and positive CMV tests after 35 weeks PMA, and 129 (92.8%) were negative for both CMV tests.

Table 1 presents the background of the seven patients with pCMV infection and 129 without CMV infection. Frozen-thawed breast milk were fed to 98.4% (127/129) patients in the non-CMV group and 100% (7/7) in the pCMV group. The duration of use of frozen-thawed breast milk was significantly longer in the pCMV group than in the non-CMV group (p = 0.03). GA was significantly shorter in the pCMV group than in the non-CMV group (p = 0.04). The highest infection rate was observed in infants aged 22–25 weeks, with a 10.5% (4/38) infection rate. Maternal age was

significantly older in the pCMV group (p = 0.001). The infection rate was 0 (0/63) in mothers aged ≤30 years, 7.5% (5/67) in those aged 31–40 years, and 33.3% (2/6) in those aged >41 years. Thus, the older the mother, the higher the infection rate possible.

Table 2 summarizes the clinical and laboratory findings of the pCMV group. One patient died of CMV-SLS. Five patients had pneumonia. No bacteria were detected or phagocytosed on the Gram staining of tracheal secretion. The four patients without CMV-SLS had a mildly elevated CRP level of 1–2 mg/dL. Blood test findings included elevated direct bilirubin (>1.0 mg/dL) in four patients, decreased neutrophil count (<1500/μL) in six, and decreased platelet count (<150,000/μL) in two. One patient had elevated AST or ALT levels. None of the patients received ganciclovir or valganciclovir as treatment for CMV infection.

Table 3 compares the frequency of clinical and laboratory findings between the non-CMV group and the pCMV group. Regarding clinical findings, the rates of pneumonia were higher in the pCMV group than in the non-CMV group (odds ratio [OR], 14.5; 95% CI, 2.62–80.1). Regarding laboratory findings, the rates of neutropenia (OR, 8.88; 95% CI, 1.04–76.0) and CRP levels (OR, 5.70; 95% CI, 1.16–28.0) were higher in the pCMV group than in the non-CMV group. Maternal age, pneumonia, and elevated CRP levels were significant on multivariate logistic regression analysis.

Discussion

This single-center, prospective cohort study examined the incidence of pCMV infection and its clinical presentation. The rate of pCMV infection in infants born at ≤ 32 weeks of GA was 5.0%. The diagnosis of pCMV infection was based on a negative CMV test on urine samples within the first 3 weeks of life and a positive CMV test after 35 weeks of PMA. This is an accurate way to diagnose pCMV infection rather than only testing suspected cases based on clinical symptoms or blood tests. Approximately 92% of the infants born during the study period were included in the study, allowing us to accurately determine the infection rate. Since CMV seronegative blood products were used, the possibility of transfusion-associated CMV infection is very low. However, the possibility of birth canal as a route of infection remains. In the pCMV infected group, there was one case of vaginal delivery. Since urinary CMV-DNA testing within 3 weeks of birth was negative, the possibility of birth canal infection is also considered low. Rather, the route of infection is likely

to be via breastfeeding. Although frozen-thawed breast milk is used in our hospital, it is not sufficient to prevent breast milk CMV infection. Although comparisons are difficult because the status of CMV infection of the mothers is unknown, we can use previous literature as a basis. In a systematic review by Lanzieri et al., the rate of pCMV infection in infants born to CMV-seropositive mothers was 13% (range, 7%–24%) in the frozen-thawed breast milk group [15]. In another systematic review, the pCMV infection rate among breastfed infants with CMV-seropositive mothers was 11% with frozen-thawed breast milk [3]. In a previous report from Japan, CMV DNA was detected in the breast milk of 21/25 mothers (84%) with birth weights of < 1000 g and/or 28 weeks of GA [16]. Only 1 (4.3%) infant had infection. Another report from Japan states that pCMV infection was confirmed in 3/30 (10%) of infants born < 34 weeks of GA or weighing < 2000 g at birth [17]. Furthermore, in 2018, it was reported that 4/65 (6.2%) of very low birth weight infants fed frozen-thawed breast milk were infected with pCMV [6] . The route of transmission was identified by CMV DNA as breast milk. The slight differences in study participants may have affected the results.

The risk factors of pCMV infection were lower GA and older maternal age. The older the mother, the higher the infection rate possible. The duration of use of frozen-thawed breast milk was significantly longer in the pCMV group than in the non-CMV group. The duration of frozen–thawed breast milk was associated with shorter GA because we used it for 35 weeks PMA in this study. Risk factors for pCMV infection are extremely low birth weight, low GA, and low infantile IgG titers [7,18–21]. In this study, low GA was similar to previous reports. No cases of pCMV infection were recorded after 30 weeks of GA. Maternal old age was also a risk for pCMV infection, but there have been no reports on this. Since we were unable to assess the status of maternal CMV infections in this study, it is difficult to conclude what this might suggest. An examination of viral seroprevalence estimated from cord blood samples showed a yearly decrease in prevalence from 76% in 2001–2002 to 67% in 2013 [22]. The prevalence of CMV is lower among younger mothers, making pCMV infection less likely to occur. Future large-scale surveys involving the determination of maternal infection status and the presence of CMV in breast milk are required.

In this study, 1 (0.7%) patient died of CMV-SLS caused by pCMV infection. In a Japanese nationwide survey of CMV-SLS cases conducted for 3 years (2017–2020), 12 cases were reported [23].

This report indicates that most CMV-SLS cases were those of extremely premature infants (< 28 weeks) fed with frozen-thawed breast milk. Notably, 5 (71.4%) patients had pneumonia. it was unclear whether pCMV infection was the cause of the pneumonia because real-time polymerase chain reaction was not performed. The mildly elevated CRP level and no bacteria and phagocytosis on tracheal secretion Gram staining suggest the possibility of viral infection.

On laboratory findings, neutropenia and elevated CRP levels were characteristic findings. Neuberger et al. reported that neutropenia, mild thrombocytopenia, and mildly elevated CRP (1–2 mg/dL) were significantly more frequent in the pCMV group than in the GA-matched control group [24]. In a systematic review by Kurath et al., the most common laboratory findings were thrombocytopenia, decreased neutrophil count, and elevated transaminases, in that order [25].

The prevention of pCMV infection is important to further improve the survival rate of preterm infants in Japan. However, no guidelines for the prevention of pCMV infection have been established in Japan. Furthermore, serological screening for mothers has not been recommended, and there is no consensus on handling breast milk. Frozen-thawed breast milk feeding is commonly used, but as mentioned earlier, it is not fully effective in preventing the infection. Meanwhile, France and Germany both recommend pasteurization to inactivate CMV for preterm infants born to CMV-positive mothers [26,27]. In addition to pasteurization, ultraviolet-C and microwave radiation at a high-power setting are promising and safe methods for CMV inactivation [28,29]. Large prospective cohort studies and development of guidelines for the prevention of pCMV infection are needed in Japan.

Three patients (2.2%) had a congenital CMV infection. This is higher than the 0.31% reported in the Japanese large-scale screening conducted by Koyano et al [30]. The presence of twin cases had a significant influence on the percentage. Furthermore, preterm delivery is said to be more common in cases of congenital CMV infections [31]. Thus, the inclusion of preterm infants born at ≤ 32 weeks of GA may have also affected the congenital CMV infection rate.

The strength of this study is the relatively large number of cases compared with the pCMV infection rate studies conducted in Japan[6,16,17]. It also suggests the possibility of detailed clinical manifestations, although a direct relationship could not be demonstrated.

This study has some limitations. First, statistical studies are limited because of the insufficient

number of cases in this single-center cohort study. Second, because we have not evaluated the presence or absence of maternal CMV infection, the infection rate and other factors cannot be adequately compared with those in previous studies. Third, the causal relationship between findings and pCMV infection is not certain because real-time PCR assays were not performed. Finally, this study could not prove that CMV-DNA was present in breast milk fed to infants infected with pCMV. Therefore, it could not be ruled out that the pCMV infection was due to breast milk. There remains a small possibility of trans-birth canal infection or transfusion-related infection.

This prospective cohort study investigated the incidence, risk factors, and findings of pCMV infection. The pCMV infection rate in this study was 5.0%, which approximated the results of other related studies in Japan[6,16,17]. Frozen-thawed breast milk feeding is not fully effective in the prevention of pCMV infection. Risk factors of pCMV infection were younger GA and older age of the mother. Cases of pCMV infection cases characterized by pneumonia, elevated CRP levels, and decreased neutrophil count. The prevention of pCMV infection is important to further improve the survival rate of preterm infants. Guidelines of breast milk feeding for the prevention of pCMV infection are necessary in Japan.

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Disclosure

Authors declare no Conflict of Interests for this article.

Author contributions

R.O. made substantial contributions to the conception and design of the study and analysis and interpretation of the data. A.K performed the urine CMV DNA tests and contributed to revising the article critically for important intellectual content. T.H., M.T., Y.I., and T.N. contributed to revising

the article critically for important intellectual content. All authors approved the final version to be published.

Data availability statement

The data that supports the findings of this study are available in the supplementary material of this article.

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Tables

Table 1. Characteristics of the mothers and their infants

	Non-CMV group	**Postnatal CMV group**	
	n = 129	n = 7	*p* value
Gestational age, median, week (IQR)	28.3 (25.6–30.1)	25.5 (24.5–27.1)	0.04*
22–25 weeks, n	34	4	
26–29 weeks, n	49	3	
30–32 weeks, n	46	0	
Birth weight, median, g (IQR)	993 (653–1300)	802 (721–1003)	0.28
Male sex, n (%)	72 (55.8)	3 (42.9)	0.27
Maternal age, median, years (IQR)	31 (27–35)	37 (35–40)	0.001*
≤30 years, n	63	0	
31–40 years, n	62	5	
≥41 years, n	4	2	
Multipara, n (%)	60 (46.5)	3 (42.9)	1.00
Singleton, n (%)	84 (65.1)	7 (100)	0.30
SGA, n (%)	39 (30.2)	0	0.19
Apgar score 1 min, median (IQR)	4 (2–7)	4 (2–5)	0.29
Apgar score 5 min, median (IQR)	7 (5–9)	6 (4–7)	0.22
Cesarean section, n (%)	94 (72.9)	6 (85.7)	0.70
Antenatal steroids, n (%)	108 (83.7)	6 (85.7)	1.00
Histologic CAM, n (%)	61/120 (50.8)	5/6 (83.3)	0.21
CRP, median, mg/dL (IQR)	0.01 (0–0.01)	0.02 (0–0.04)	0.10
Frozen-thawed breast milk, n (%)	127 (98.4)	7 (100)	1.00
Duration of frozen-thawed breast milk, median, days (IQR)	46 (33–64)	65 (55-72)	0.03*
Number of transfusions, median, n (IQR)	0 (0–2)	2 (1–3)	0.29

The second test, median, weeks of PMA (IQR)	35.1 (35.0–35.3)	35.2 (35.1–35.2)	0.59

*Statistically significant.

IQR, interquartile range; SGA, small for gestational age; CAM, chorioamnioniti

Table 2. Clinical and laboratory findings of cases with postnatal CMV infection

Case	Gestational age, week	Birth weight, g	Sex	Second test, weeks of PMA	Clinical findings (weeks of PMA)	Laboratory findings, value (weeks of PMA)					Death before discharge
						AST and ALT (U/L)	Direct bilirubin (mg/dL)	Neutrophil (/μL)	Platelet (/μL)	CRP (mg/dL)	
1	22	449	M	35	−	−	−	823 (35)	−	−	−
2	24	662	F	35	Pneumonia (31)	−	1.2 (29)	487 (35)	107000 (32)	1.0 (31)	−
3	25	780	M	35	Pneumonia (28)	−	−	1433 (35)	−	1.4 (28)	−
4	25	802	M	35	Pneumonia (29)	−	−	1072 (35)	−	1.8 (29)	−
5	27	950	F	35	SLS, Pneumonia, NEC, Hepatomegaly (35)	AST208 ALT71 (35)	4.8 (35)	1176 (35)	51000 (35)	29.7 (35)	(39)
6	27	1056	F	35	Pneumonia (34)	−	1.5 (31)	690 (32)	−	1.3 (34)	−
7	29	1134	F	35	−	−	1.1 (28)	−	−	−	−

SLS, sepsis-like syndrome; NEC, necrotizing enterocolitis

In clinical findings and laboratory findings, numbers in parentheses indicated the weeks of PMA when it appeared.

In Laboratory findings, the numbers in the table indicate the worst and abnormal values for each test item.

Table 3. Clinical and laboratory findings of infants and perinatal information according to postnatal CMV infection

	Non CMV infection	**Postnatal CMV infection**	Odds ratio[a] (95% CI)	Adjusted odds ratio[b] (95% CI)	Adjusted odds ratio[c] (95% CI)
	n = 129	n = 7			
Perinatal information					
Gestational age <28wks, n (%)	56 (43.4)	6 (85.7)	7.82 (0.92–66.8)	–	6.44 (0.71–58.9)
Maternal age, median, years (IQR)	31 (27–35)	37 (35–40)	1.33 (1.09–1.62)*	1.32 (1.07–1.62)*	–
Multipara, n (%)	60 (46.5)	3 (42.9)	0.86 (0.19–4.01)	0.65 (0.13–3.13)	0.16 (0.02–1.15)
Transfusion, n (%)	62 (48.0)	5 (71.4)	2.70 (0.51–14.4)	0.53 (0.06–4.44)	2.27 (0.39–13.2)
Clinical findings					
Sepsis/sepsis like syndrome, n (%)	6 (4.7)	1 (14.3)	3.42 (0.35–33.1)	1.97 (0.19–19.9)	2.47 (0.19–32.8)
Pneumonia, n (%)	19 (14.7)	5 (71.4)	14.5 (2.62–80.1)*	9.57 (1.60–57.4)*	12.8 (1.99–82.3)*
NEC, n (%)	1 (0.8)	1 (14.3)	5.21 (0.50–54.0)	11.0 (0.60–204.0)	13.8 (0.30–631.0)
Hepatomegaly, n (%)	3 (2.3)	1 (14.3)	7.00 (0.63–77.7)	7.62 (0.57–103.0)	5.89 (0.30–116.0)
Death before discharge, n (%)	0	1 (14.3)	–	–	–
Laboratory findings					
AST >140 and ALT >50, n (%)	4 (3.1)	1 (14.3)	5.21 (0.50–54.0)	6.82 (0.53–87.2)	4.72 (0.28–79.0)

Direct bilirubin >1.0mg/dL, n (%)	22 (17.1)	3 (42.9)	3.65 (0.76–17.5)	4.51 (0.88–23.2)	2.43 (0.45–13.1)
Neutrophil <1500/μL, n (%)	52 (40.3)	6 (85.7)	8.88 (1.04–76.0)*	5.87 (0.65–52.8)	9.11 (0.97–85.4)
Platelet <15*10^4/μL, n (%)	20 (15.5)	1 (14.2)	0.91 (0.10–7.95)	0.56 (0.06–5.12)	1.04 (0.10–11.2)
CRP >1mg/dL	15 (11.6)	5 (71.4)	19.0 (3.38–107.0)*	12.9 (2.12–77.9)*	14.6 (2.29–93.4)*

*Statistically significant.

CI, confidence interval; NEC, necrotizing enterocolitis;

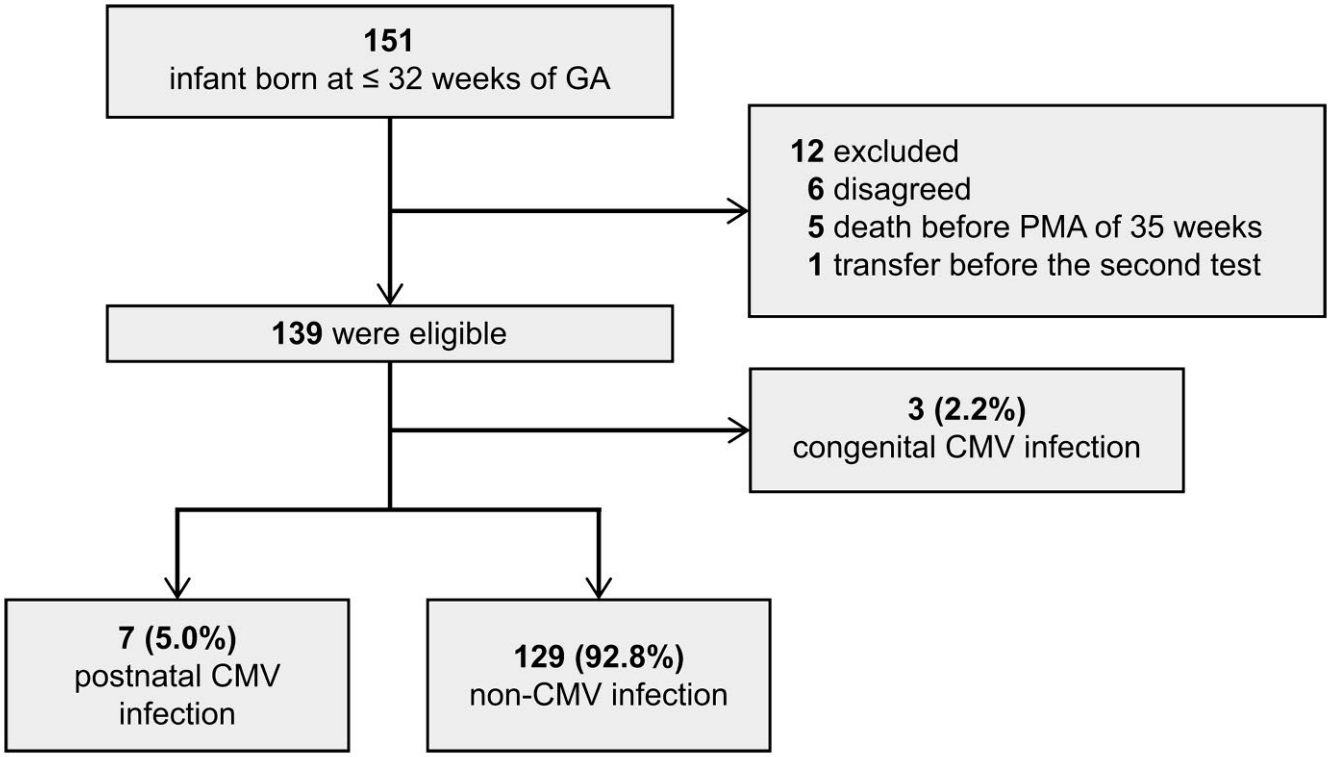
[a]Odds ratios were for used logistic regression analysis.

[b]Adjusted for gestational age.

[c]Adjusted for maternal age.

Figure legend

Figure 1. Flow diagram of the study



There were 151 preterm infants born at ≤32 weeks of gestation during the study period. Of these, 12 (7.9%) were excluded. A total of 139 patients underwent two urine CMV DNA tests. Three patients (2.2%) were diagnosed with congenital CMV infection. Seven cases (5.0%) were diagnosed with pCMV infection. In 129 cases (92.8%), both CMV tests were negative.

CMV, cytomegalovirus; GA, gestational age; PMA, postmenstrual age

Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web site:

Data S1. Data set of this study