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# Low total IgM values and high cytomegalovirus loads in the blood of newborns with symptomatic congenital cytomegalovirus infection

## Abstract

**Aims:** Neurological outcomes differ considerably between symptomatic and asymptomatic infants with congenital cytomegalovirus (CMV) infection. Our objective was to characterize laboratory markers in symptomatic newborns in comparison with asymptomatic newborns with congenital CMV infection.

**Methods:** Ten newborns with symptomatic and 13 newborns with asymptomatic congenital CMV infection were included in this 3-year prospective cohort study. Total immunoglobulin M (IgM), CMV-IgM, CMV antigenemia, and CMV-DNA in blood and urine were measured and their positive rates and quantitative values compared between the symptomatic and asymptomatic groups.

**Results:** Fifty percent of newborns in the symptomatic group were positive based on total IgM; this was significantly lower than in the asymptomatic group (100%).

Quantitative total IgM values were significantly lower, and there were significantly more copies of CMV-DNA in the blood of symptomatic newborns than in asymptomatic newborns (median values for total IgM: 14 vs. 43 mg/dL and blood CMV-DNA:  $3.2 \times 10^2$  vs.  $3.5 \times 10^1$  copies/ $10^6$  white blood cells). CMV-IgM, CMV antigenemia, and urine CMV-DNA did not differ significantly between groups.

**Conclusion:** Low total IgM values and high blood CMV loads were associated with the presence of symptoms in newborns with congenital CMV infection.

**Keywords:** Antigenemia; immunoglobulin; laboratory markers; serology; viral load.

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## Introduction

Cytomegalovirus (CMV) is the main cause of congenital infection in developed countries [12]. In Japan, 0.31% of live newborns are infected with CMV [10]. Approximately 10%–15% of newborns with congenital CMV infection have clinical symptoms at birth, whereas the remaining 85%–90% of infected newborns are asymptomatic [12]. Approximately 85%–90% and 10%–15% of the symptomatic and asymptomatic newborns, respectively, develop neurological sequelae including sensorineural hearing loss and developmental disabilities [12]. Thus, neurological outcomes differ substantially between symptomatic and asymptomatic newborns with congenital CMV infection.

Newborn total immunoglobulin M (IgM) is the gold-standard diagnostic marker of mother-to-child infection. Additional laboratory markers of CMV infection in blood and urine include CMV-IgM, CMV antigenemia, and CMV-DNA copy number [4, 5, 7, 26]. However, few studies

have assessed these markers concurrently in newborns with congenital CMV infection.

Our objectives were to assess the positive rates and quantitative values for these markers in symptomatic and asymptomatic newborns with congenital CMV infection and to find laboratory markers that characterize symptomatic newborns.

## Methods

### Study design

This study was conducted under the approval of the Ethical Committee of Kobe University Graduate School of Medicine with informed consent of the parents of the patients. In this 3-year prospective cohort study, 4364 newborns received routine urine screening for CMV-DNA from November 2009 to March 2013 at Kobe University Hospital and its affiliated hospitals. Twenty-three Japanese newborns with congenital CMV infection whose urine samples were positive for CMV-DNA within 1 week after birth [10, 23] were enrolled in the study. All had undergone extensive medical examination during their neonatal period at Kobe University Hospital and were classified as having symptomatic ( $n=10$ ) or asymptomatic ( $n=13$ ) infections. Total blood IgM, CMV-IgM, CMV antigenemia, and CMV-DNA in blood and urine were measured and then, 1) positive rates for these markers were calculated in all patients, 2) positive rates and quantitative values were compared between the symptomatic and asymptomatic groups, 3) values associated with the symptoms were scrutinized using receiver operating characteristic curve (ROC) analyses, and 4) the correlations between the values of each laboratory marker and the gestational ages of the newborns were assessed.

### Definition of symptomatic congenital CMV infection

Newborns were categorized as having symptomatic infection if at least one of the following clinical manifestations was evident at their initial medical examination: small for gestational age (SGA), hepatosplenomegaly/hepatitis, thrombocytopenia, brain abnormality, chorioretinitis, or an abnormal auditory brainstem response (ABR). SGA was defined as a birth weight (BW)  $\leq 1.5$  standard deviations from the mean BW of Japanese newborns of the same gestational age. Hepatosplenomegaly was confirmed by ultrasound examination and/or abdominal X-ray. Hepatitis was defined as a serum alanine aminotransferase level  $>100$  U/L, and thrombocytopenia as a platelet count  $<1 \times 10^5/\mu\text{L}$  [9, 14, 19]. Brain abnormality was defined as intracranial calcifications, ventricular dilation, cortical dysplasia, or subependymal cyst detected by ultrasound, computed tomography, or magnetic resonance imaging. A pediatric ophthalmologist diagnosed chorioretinitis. ABR abnormalities were diagnosed using a Neuropack S1 (Nihon Kohden Co., Tokyo, Japan) according to the manufacturer's recommended protocol. A lack of response either unilaterally or bilaterally to a noise  $>40$  dB for infants with a post-conceptual age of  $\geq 37$  weeks and 50 dB for infants with a post-conceptual age of 34–36 weeks was defined as abnormal [1, 14, 28].

### Measurement methods and cut-off values

Total IgM and CMV-IgM were measured with commercially available kits (Siemens Healthcare Diagnostics Corp., Tokyo, Japan) using the nephelometry method [8] and enzyme immunoassay [24, 30], respectively. CMV antigenemia was measured at a commercial laboratory (Special References Laboratories, Inc., Tokyo, Japan) by a direct immunoperoxidase technique using the monoclonal antibody C7 (Teijin, Tokyo, Japan) as previously described [13, 16, 25]. The number of positive cells per  $5 \times 10^4$  white blood cells (WBCs) was counted. For measurement of CMV-DNA in blood and urine, DNA was extracted with a QIAamp DNA Minikit (Qiagen Corp., Tokyo, Japan) and real-time quantitative PCR was performed as previously described [27]. The results were expressed as the number of CMV-DNA copies per  $10^6$  WBCs in blood, and per mL in urine. The intra-day coefficients of variation for these assays were 0.76% for total IgM ( $n=20$ ), 6.1% for CMV-IgM ( $n=20$ ), 13.6% for CMV antigenemia ( $n=8$ ), 8.8% for blood CMV-DNA ( $n=10$ ), and 10.1% for urine CMV-DNA ( $n=10$ ).

Based on previous reports [24, 29] and the assay manufacturer's criteria, the following threshold values were defined as positive: total IgM  $\geq 20$  mg/dL, CMV-IgM  $\geq 0.9$  IgM index value (negative 0–0.89, borderline 0.90–1.99, and positive  $\geq 2.0$ ), CMV antigenemia  $\geq 1$  CMV antigen-positive cell per  $5 \times 10^4$  WBCs, CMV-DNA in blood  $\geq 1 \times 10^2$  copies per  $10^6$  WBCs, and CMV-DNA in urine  $\geq 3 \times 10^3$  copies per mL.

### Statistical analysis

Data are expressed as median (range) or number (%). Univariate analyses were performed using the Mann-Whitney nonparametric rank test or the Fisher exact test as appropriate for between-group comparisons. When blood markers were significantly different between the groups in the quantitative analyses, the cut-off levels associated with the presence of symptoms were determined by ROC analysis [15]. Sensitivity, specificity, negative predictive value, positive predictive value, and the likelihood ratios for positive and negative results were calculated. Linear regression was performed to determine the correlations between the values of each laboratory marker and the gestational ages of the newborns, and correlation coefficients ( $R^2$ ) were calculated. Differences were considered statistically significant if  $P < 0.05$ .

## Results

### Patient clinical characteristics

The median gestational age of the symptomatic infants was significantly lower than that of the asymptomatic infants (36 [31–38] vs. 38 [35–41] weeks,  $P < 0.05$ ). The median BW also differed significantly between the groups (2188 [1378–3160] vs. 2758 [2060–3840] g for symptomatic and asymptomatic, respectively;  $P < 0.05$ ). Furthermore, symptomatic newborns underwent clinical examination earlier than asymptomatic newborns (1 [0–27] vs. 19 [0–28] days of age,  $P < 0.01$ ). Ten newborns with congenital CMV

infection had typical manifestations at the time of initial examination; of these, two had SGA, four had hepatosplenomegaly/hepatitis, five were thrombocytopenic, seven had brain abnormalities, seven had ABR abnormalities, and four had chorioretinitis.

### Positive rates of laboratory markers in newborns with congenital CMV infection

Of all the newborns with congenital CMV, 78, 52, 44, 96, and 100% tested positive based on total IgM, CMV-IgM, CMV antigenemia, and blood and urine CMV-DNA, respectively. The positive rate for total IgM in the symptomatic group was significantly lower than in the asymptomatic group. There were no significant between-group differences in CMV-IgM, CMV antigenemia, and blood and urine CMV-DNA (Table 1).

### Comparison of laboratory values between symptomatic and asymptomatic newborns

Quantitative analysis revealed that the total IgM values were significantly lower and the viral loads in blood were

significantly higher in the symptomatic infants than in the asymptomatic infants ( $P < 0.01$ ). The values for CMV-specific antigenemia and IgM, and for urine CMV-DNA, did not differ significantly between the groups (Figure 1).

### Total IgM and blood CMV-DNA values associated with symptoms

Total IgM and blood CMV-DNA values in symptomatic or asymptomatic newborns with congenital CMV infection were analyzed by ROC analysis. Cut-off values of 25 mg/dL for total IgM and  $1.8 \times 10^2$  copies per  $10^6$  WBCs for blood CMV-DNA were identified as associated with symptomatic status in infected newborns. The areas under the curve for low total IgM and high blood CMV-DNA were 0.854 and 0.842, respectively.

The test performance characteristics were as follows: a cut-off value of 25 mg/dL for total IgM had a sensitivity of 80%, a specificity of 85%, a negative predictive value of 15%, a positive predictive value of 80%, and likelihood ratios for positive and negative results of 5.20 and 0.24, respectively. A cut-off value of  $1.8 \times 10^2$  copies per  $10^6$  WBCs for blood CMV-DNA had a sensitivity of 80%, specificity of

**Table 1** Positive rates of laboratory markers in congenital cytomegalovirus-infected newborns.

|              | n  | Total IgM            | CMV-IgM  | CMV antigenemia | Blood CMV-DNA | Urine CMV-DNA |
|--------------|----|----------------------|----------|-----------------|---------------|---------------|
| All          | 23 | 18 (78%)             | 12 (52%) | 10 (44%)        | 22 (96%)      | 23 (100%)     |
| Symptomatic  | 10 | 5 (50%) <sup>a</sup> | 6 (60%)  | 4 (40%)         | 10 (100%)     | 10 (100%)     |
| Asymptomatic | 13 | 13 (100%)            | 6 (46%)  | 6 (46%)         | 12 (92%)      | 13 (100%)     |

Data are expressed as number (%), <sup>a</sup> $P < 0.01$  compared to asymptomatic newborns. CMV=cytomegalovirus, IgM=immunoglobulin M.

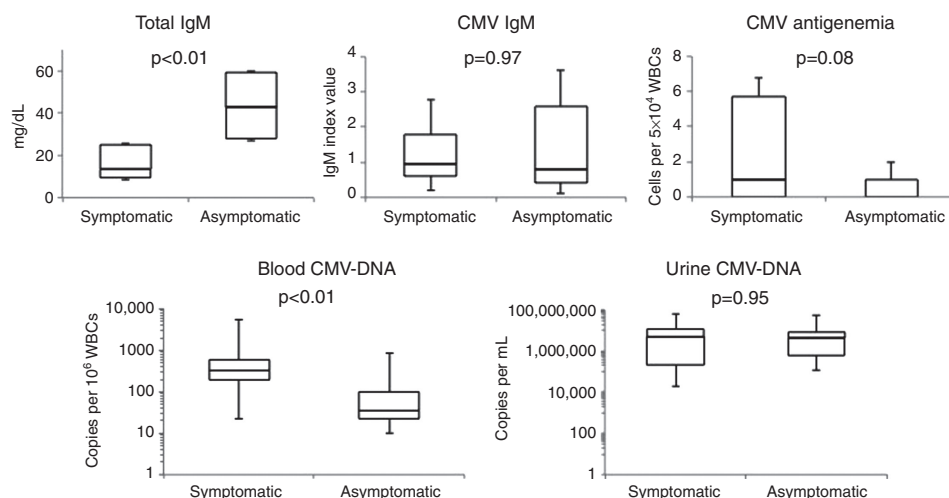


**Figure 1** Comparison of laboratory marker values between symptomatic and asymptomatic newborns with congenital cytomegalovirus infection. Data are expressed as median and range. IgM index value: negative 0–0.89, borderline 0.9–1.99, and positive  $> 2$ . CMV=cytomegalovirus, IgM=immunoglobulin M, WBC=white blood cell.

92%, a negative predictive value of 14%, a positive predictive value of 89%, and likelihood ratios for positive and negative results of 10.4 and 0.22, respectively.

## Correlation between values of each laboratory marker and gestational age

No significant correlations were found between the values of each laboratory marker and the gestational age of symptomatic and asymptomatic newborns (total IgM:  $R^2=0.098$ ,  $P=0.38$  in symptomatic and  $R^2=0.00091$ ,  $P=0.92$  in asymptomatic newborns, CMV-IgM:  $R^2=0.0023$ ,  $P=0.97$  in symptomatic and  $R^2=0.00015$ ,  $P=0.97$  in asymptomatic newborns, CMV antigenemia:  $R^2=0.12$ ,  $P=0.32$  in symptomatic and  $R^2=0.072$ ,  $P=0.86$  in asymptomatic newborns, blood CMV-DNA:  $R^2=0.17$ ,  $P=0.24$  in symptomatic and  $R^2=0.017$ ,  $P=0.67$  in asymptomatic newborns, and urine CMV-DNA:  $R^2=0.0019$ ,  $P=0.90$  in symptomatic and  $R^2=0.059$ ,  $P=0.43$  in asymptomatic newborns).

## Discussion

Laboratory techniques for the diagnosis of CMV infection, such as CMV-IgM, CMV antigenemia, and CMV-DNA, have greatly improved during recent years. We assessed these markers concurrently in newborns with congenital CMV infection to evaluate their efficacy for the detection of congenital CMV and prediction of symptomatic status. Low total IgM values and high blood CMV loads were associated with the presence of clinical symptoms in newborns with congenital CMV infection. High CMV-DNA copy numbers in the blood of symptomatic newborns has been reported previously [6, 11, 21]; however, to our knowledge this is the first report of a relationship between low total IgM values and clinical symptoms in newborns with congenital CMV infection.

Interestingly, when total IgM rather than CMV-specific IgM was assessed, we found that the positive rates for total IgM, as well as the total IgM values in the symptomatic group were significantly lower than in the asymptomatic group. Because fetal CMV infection early in pregnancy tends to cause more severe symptoms than infection late in pregnancy [20], we speculate that maternal CMV infection during early pregnancy results in lower total IgM in symptomatic cases. It is plausible that the longer period since infection in symptomatic cases reduces the total IgM value at birth. Alternatively, symptomatic newborn patients who were infected early in pregnancy might

have a poor immunological response to infection. Further studies on the mechanisms underlying the low total IgM values at birth in symptomatic newborns are needed.

CMV-IgM had a low positive rate in both symptomatic and asymptomatic newborns with congenital CMV infection. This may have resulted from the insensitivity of the current method for CMV-IgM measurement in newborns. In addition, it may be that not all congenitally CMV-infected fetuses and newborns produce CMV-IgM, such as occurs in immunocompromised or reinfected patients [3, 17, 21].

CMV antigenemia tests, which involve direct detection of antigens in neutrophils using a monoclonal antibody against CMV, are also useful diagnostic tools for CMV infection in patients with immunodeficiencies or after immunosuppressive therapy [2]. However, in our population, a CMV antigenemia test using C7 detection was positive in only 40% and 46% of symptomatic and asymptomatic newborns, respectively; this difference was not significant.

Other studies have also reported high positive rates for CMV-DNA in blood (100% by Revello et al. [22], 95% by Nelson et al. [18], and 91% by Lanari et al. [11]). Thus, blood CMV-DNA may be more useful than CMV-IgM and CMV antigenemia for the diagnosis of congenital CMV infection; however, because blood CMV-DNA is not positive in 100% of congenitally infected newborns, we suggest that blood CMV-DNA should not be used to screen for congenital CMV infection.

A potential limitation of our study was that the gestational age significantly differed between symptomatic and asymptomatic newborns. This occurred because some of the symptomatic newborns were born preterm and subsequently admitted into neonatal intensive care units. However, the lack of correlations between total IgM or blood CMV-DNA values and gestational age suggests that the gestational age was not the factors attributing low total IgM and high blood CMV-DNA values in symptomatic newborns.

In conclusion, we propose that low total IgM values and high blood CMV loads are significant characteristics of newborns with symptomatic congenital CMV infection.

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