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PCR Detection of Cytomegalovirus DNA in Serum as a Diagnostic Test for Congenital Cytomegalovirus Infection

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PCR detected cytomegalovirus (CMV) DNA in the serum of 18 of 18 infants with symptomatic congenital CMV infection, 1 of 2 infants with asymptomatic congenital CMV infection, and 0 of 32 controls. Serum CMV PCR provided a rapid, sensitive, and specific method for diagnosis of congenital CMV infection in infants who were symptomatic at birth.

Approximately 1% of all children born each year in the United States, or 40,000 newborns, are congenitally infected with cytomegalovirus (CMV). Ten percent of these infants are born symptomatic, manifesting various neurologic, hematologic, and developmental abnormalities (3). The remainder are asymptomatic at the time of delivery, but 5 to 17% of these infants will develop progressive sensorineural hearing loss or other neurodevelopmental difficulties within the first 4 years of life (12). The standard diagnostic test for congenital CMV infection is a viral culture of urine obtained from a newborn within the first 21 days of life. CMV-specific immunoglobulin M (IgM) also has been utilized in the evaluation of these infants. However, the role of molecular biologic techniques in the diagnosis of congenital infection with CMV remains largely unexplored. We performed PCR on serum from infants with congenital CMV infection to determine if CMV DNA could be amplified and detected, and we compared the results with those for urine viral culture and serum CMV IgM antibody determination.

(These results were presented at the Society for Pediatric Research Annual Meeting [7a].)

Samples. Serum samples were obtained from each patient prior to the 21st postdelivery day of life from July 1989 to December 1994 at Texas Children's Hospital in Houston and stored at −70°C.

Serology. Anti-CMV IgM was measured by an enzyme immunosorbent assay (CMV Stat M; BioWhittaker, Inc., Walkersville, Md.), which was performed according to the manufacturer's instructions (4). An enzyme immunosorbent assay index of ≥0.30 was considered positive.

Viral culture. Conventional tube viral culture was performed on urine specimens obtained within the first 21 days of life. CMV was identified by production of its typical cytopathic effect on human foreskin fibroblast cell culture, and identification was confirmed by immunofluorescence (Microtrak CMV culture identification test, Syva Co., San Jose, Calif.).

Clinical definitions. Symptomatic congenital CMV (SCCMV) infection was confirmed by using the case definition set forth by the National Cytomegalovirus Disease Registry, which stipulates detection (by tissue culture, shell vial assay, or histopathologic study) of CMV in urine, saliva, secretions, or tissue obtained during the first 3 weeks of life from a newborn with one or more of several signs, symptoms, or laboratory abnormalities (3, 7). The total number of signs, symptoms, and abnormal laboratory findings for each patient was designated as that patient's illness score (IS), with scores of 1 through 16 possible. Asymptomatic congenital CMV infection (ACCMV) was defined as detection of CMV during the first 3 weeks of life in the absence of any abnormal signs, symptoms, or laboratory findings.

Control patients. Serum and urine samples were obtained within the first 21 days of life from infants being evaluated for alternate diagnoses. Thirty-two infants from whose urine CMV could not be isolated after 28 days of incubation on human foreskin fibroblast cell culture were used as control patients.

PCR. Serum from each patient was prepared in 25-μl aliquots for PCR by the GeneClean (Bio 101, Inc., La Jolla, Calif.) method (2). Primers used in this study amplify a 209-bp segment of the gene that encodes the major capsid protein of CMV. The PCR was carried out in a total volume of 50 μl, and the amplified PCR product was detected by both 5% polyacrylamide gel electrophoresis (PAGE) and liquid hybridization as previously described (11). Measures to reduce the risk of contamination included the use of aerosol-resistant pipette tips, latex gloves, and completely separate laboratory facilities for pre- and postamplification procedures.

Statistical methods. Serum CMV IgM antibody determination and serum PCR results were compared with urine viral culture results with regard to sensitivity, specificity, negative predictive value, and positive predictive value, and 95% confidence intervals (CI) were determined for each calculated value. Differences between groups were analyzed by the one-tailed Student's *t* test.

Patient characteristics. Eighteen patients (11 males and 7 females) had SCCMV infection confirmed by tube viral cell culture. ISs varied from a single abnormality in one infant (small for gestational age) to multiple abnormalities in several infants (IS range, 1 to 13; mean, 7). Central nervous system involvement was seen in 14 of 18 (78%) infants. A significant difference was observed between the ISs of patients with neurological involvement (IS range, 4 to 13; mean, 7.9) and patients without evidence of neurological involvement (IS range, 1 to 8; mean, 3.75; $P = 0.0095$).

Of the two patients with ACCMV infection, one was identified through a previously published epidemiological study and the other was born to a mother with a documented primary CMV infection during pregnancy (14).

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TABLE 1. Results of urine CMV culture, detection of CMV DNA in serum by PCR, and detection of serum CMV IgM antibody in congenitally infected and uninfected neonates

Laboratory evaluation	No. (%) of patients		
	With SCCMV (n = 18)	With ACCMV (n = 2)	Uninfected (n = 32)
Urine CMV culture positive	18 (100)	2 (100)	0 (0)
CMV IgM antibody positive	4 (22)	1 (50)	0 (0)
Serum PCR positive by:			
PAGE	12 (67)	0 (0)	0 (0)
LH[a]	18 (100)	1 (50)	0 (0)

[a] LH, liquid hybridization.

Serology and serum PCR. Results of urine CMV culture, serum CMV PCR, and CMV IgM antibody determination for all patients are shown in Table 1. Compared with the diagnosis of SCCMV infection by urine culture, CMV IgM antibody determination had a sensitivity of 22% (95% CI, 6.4 to 47.6), specificity of 100% (CI 95%, 89.1 to 100), positive predictive value of 100% (95% CI, 39.8 to 100), and negative predictive value of 70% (95%) CI, 54.3 to 82.3). A significant difference was observed between the ISs of patients who were CMV IgM positive (IS range, 8 to 13; mean, 10) and the ISs of patients who were CMV IgM negative (IS range, 1 to 11; mean, 6.1; $P = 0.0165$).

Compared with urine culture diagnosis of SCCMV infection, serum CMV PCR by liquid hybridization had a sensitivity of 100% (95% CI, 81.5 to 100), specificity of 100% (95% CI, 89.1 to 100), positive predictive value of 100% (95% CI, 81.5 to 100), and negative predictive value of 100% (95% CI, 89.1 to 100). No CMV DNA was amplified in the serum of any of the control patients.

The use of serum PCR to detect CMV DNA has been reported for patients with solid organ as well as bone marrow transplants (1, 5, 6, 8). Brytting et al. found CMV DNA by PCR in the sera of five of five newborns with proven or probable congenital CMV infection and detected CMV IgM in three of five (60%) of these infants (1). Our CMV IgM antibody results are similar to those previously published, suggesting a limited role for this test in the diagnosis of infants with SCCMV infection (10). However, the presence of detectable CMV IgM in our patients did correlate with an average IS higher than that for patients who were CMV IgM negative.

Although CMV was previously thought to infect the host only by cell-to-cell transmission, it is now evident through the use of PCR that CMV DNA is present in the serum and/or plasma of selected groups of patients infected with CMV (1, 5, 6, 8, 9, 13). On the basis of our results and those of Brytting et al., this also appears to be true for infants with SCCMV infection.

In summary, serum PCR for CMV provides a rapid, sensitive, and specific method to diagnose congenital CMV infection in infants who are symptomatic at birth. The role of commercially available CMV IgM assays in the diagnosis of congenital infection with CMV needs further study using larger populations of affected infants.

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REFERENCES

1. **Brytting, M., W. Xu, B. Wahren, and V.-A. Sundqvist.** 1992. Cytomegalovirus DNA detection in sera from patients with active cytomegalovirus infections. J. Clin. Microbiol. **30:**1937–1941.
2. **Buffone, G. J., G. J. Demmler, C. M. Schimbor, and J. Greer.** 1991. Improved cytomegalovirus DNA amplification from urine after purification of DNA using glass beads. Clin. Chem. **37:**1945–1949.
3. **Demmler, G. J.** 1991. Infectious Diseases Society of America and Centers for Disease Control: summary of a workshop on surveillance for congenital cytomegalovirus disease. Rev. Infect. Dis. **13:**315–329.
4. **Demmler, G. J., H. R. Six, S. M. Hurst, and M. D. Yow.** 1986. Enzyme-linked immunosorbent assay for the detection of IgM-class antibodies against cytomegalovirus. J. Infect. Dis. **153:**1152–1155.
5. **Frickhofen, N., and N. S. Young.** 1991. A rapid method of sample preparation for detection of DNA viruses in human serum by polymerase chain reaction. J. Virol. Methods **35:**65–72.
6. **Ishigaki, S., M. Takeda, T. Kura, N. Ban, T. Saitoh, S. Sakamaki, N. Watanabe, Y. Kohgo, and Y. Niitsu.** 1991. Cytomegalovirus DNA in the sera of patients with cytomegalovirus pneumonia. Br. J. Haematol. **79:**198–204.
7. **Istas, A. S., G. J. Demmler, J. G. Dobbins, J. A. Stewart, and the National Congenital Cytomegalovirus Disease Registry Collaborating Group.** 1995. Surveillance for congenital cytomegalovirus disease: a report from the National Cytomegalovirus Disease Registry. Clin. Infect. Dis. **20:**665–670.

7a. **Nelson, C. T., A. S. Istas, M. K. Wilkerson, and G. J. Demmler.** 1995. Polymerase chain reaction (PCR) detection of cytomegalovirus (CMV) DNA in serum as a diagnostic test for congenital CMV infection. Pediatr. Res. **37:**296A.

8. **Patel, R., T. F. Smith, M. Espy, R. H. Wiesner, R. A. F. Krom, D. Portela, and C. V. Paya.** 1994. Detection of cytomegalovirus DNA in sera of liver transplant recipients. J. Clin. Microbiol. **32:**1431–1434.
9. **Spector, S. A., R. Merrill, D. Wolf, and W. M. Dankner.** 1992. Detection of human cytomegalovirus in plasma of AIDS patients during acute visceral disease by DNA amplification. J. Clin. Microbiol. **30:**2359–2365.
10. **Stagno, S., M. K. Tinker, C. Elrod, D. A. Fuccillo, G. Cloud, and A. J. O'Beirne.** 1985. Immunoglobulin M antibodies detected by enzyme-linked immunosorbent assay and radioimmunoassay in the diagnosis of cytomegalovirus infections in pregnant women and newborn infants. J. Clin. Microbiol. **21:**930–935.
11. **Troendle-Atkins, J., G. J. Demmler, W. D. Williamson, J. M. McDonald, A. S. Istas, and G. J. Buffone.** 1994. Polymerase chain reaction to detect cytomegalovirus DNA in the cerebrospinal fluid of neonates with congenital infection. J. Infect. Dis. **169:**1334–1337.
12. **Williamson, W. D., G. J. Demmler, A. K. Percy, and F. I. Catlin.** 1992. Progressive hearing loss in infants with asymptomatic congenital cytomegalovirus infection. Pediatrics **90:**862–866.
13. **Wolf, D. G., and S. A. Spector.** 1993. Early diagnosis of human cytomegalovirus disease in transplant recipients by DNA amplification in plasma. Transplantation **56:**330–334.
14. **Yow, M. D., D. W. Williamson, L. J. Leeds, P. Thompson, R. M. Woodward, B. F. Walmus, J. W. Lester, H. R. Six, and P. D. Griffiths.** 1988. Epidemiologic characteristics of cytomegalovirus infection in mothers and their infants. Am. J. Obstet. Gynecol. **158:**1189–1195.