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# NOTES

## Comparison of Plasma PCR and Bronchoalveolar Lavage Fluid Culture for Detection of Cytomegalovirus Infection in Adult Bone Marrow Transplant Recipients

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**Plasma PCR for human cytomegalovirus (CMV) DNA was compared with bronchoalveolar lavage (BAL) fluid culture as an indicator for disseminated CMV infection. Thirteen (32.5%) of 40 consecutive bone marrow transplant (BMT) recipients were BAL fluid culture positive for CMV on day 35 post-BMT, and 9 (69%) of the 13 had positive plasma PCRs between days 28 and 49. Of the 27 with negative BAL fluid cultures, 2 (7%) had positive plasma PCRs ($P < 0.0001$). Plasma CMV DNA in BMT recipients is a useful clinical marker for serious infection.**

Cytomegalovirus (CMV)-associated interstitial pneumonia is the most common life-threatening infectious complication occurring after allogeneic bone marrow transplantation (BMT) (13, 22). Strategies using acyclovir, ganciclovir, or foscarnet which lessen the rate of CMV infection and disease either by suppression of virus reactivation (1, 9, 16, 17, 23, 26) or by early treatment of infection prior to disease onset (10, 19) have been reported. In the latter method, the presence of CMV in blood or in bronchoalveolar lavage (BAL) fluid, but not in urine samples or samples from the throat, has been shown to predict the subsequent occurrence of CMV-associated interstitial pneumonia (10, 14, 15, 19) and to be a reliable guide for institution of preemptive antiviral therapy (10, 19). This approach has the advantage over general prophylactic therapy of treating only those at highest risk for disease after BMT, but it places the burden on the microbiology laboratory for early CMV diagnosis. CMV can be detected in blood by routine culture (27), by rapid shell vial assay (8), by CMV pp65 antigen staining of polymorphonuclear leukocytes (2, 21), and by PCR for CMV-specific DNA in either cellular specimens (6, 7) or cell-free material such as urine (5), cerebrospinal fluid (11, 24), serum (3, 12), or plasma (20). Recently, Spector et al. (20) and Wolf and Spector (25) have reported that the CMV plasma PCR assay can be used to detect CMV disease in AIDS patients and in allogeneic BMT recipients. The present report shows that the presence of CMV DNA in plasma correlates with the occurrence of asymptomatic pulmonary CMV infection in allogeneic marrow recipients and suggests that this could be a useful guide for preemptive antiviral management.

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From April to October 1992, 40 consecutive, adult patients received allogeneic BMT and underwent routine surveillance BAL on day 35 after BMT (day 0 = day of marrow infusion). Plasma samples were collected between days 28 and 49 and frozen at −20°C for later PCR analysis, and each subject had at least two plasma specimens available for evaluation. All subjects received a BAL on day 35, and aliquots of the clarified (600 × *g* for 10 min) BAL fluid were inoculated by centrifugal inoculation into 96-well microtiter plates containing a monolayer of human foreskin fibroblasts or directly into human foreskin fibroblast culture tubes (19). Microtiter cultures were examined at 18 h for CMV major immediate early antigen (mIE) by immunoperoxidase staining, and two culture tubes were held for 4 weeks for evidence of CMV-like cytopathic effect. In addition, the leukocyte fraction of blood was cultured in human foreskin fibroblast tubes and assayed for CMV by rapid stain for mIE and for cytopathic effect. Blood samples were collected once weekly, and the plasma fraction was separated from the cellular fraction by Ficoll-Hypaque density gradient centrifugation (700 × *g* for 30 min) followed by passage of the plasma layer through a 0.45-μm-pore-size syringe filter. The plasma DNA was extracted by a modification of the method of Ishigaki et al. (12). A 100-μl solution of filtered plasma was added to a 100-μl solution containing (final concentrations) 100 mM KCl, 20 mM Tris-HCl (pH 8.3), 5 mM $MgCl_2$, 0.2 mg of gelatin per ml, and 0.9% Tween 20 solution. After proteinase K (Sigma) digestion (final concentration, 120 μg/ml) at 55°C for 1 h, the reaction was inactivated at 95°C for 10 min and microcentrifuged (12,000 × *g* for 5 min), and then 10 μl of supernatant was used for PCR amplification. Two different pairs of CMV primers (5, 28) were used for DNA amplification, as follows (4): for mIE (UL123, exon 4; positions 171009 to 172274), primer 1 was 5′-CCA AGC GGC CTC TGA TAA CCA AGC C-3′, primer 2 was 5′-CAG CAC CAT CCT CCT CTT CCT CTG G-3′, and the probe was 5′-GAG GCT ATT GTA GCC TAC ACT TTG G-3′; for pp65 (UL83;

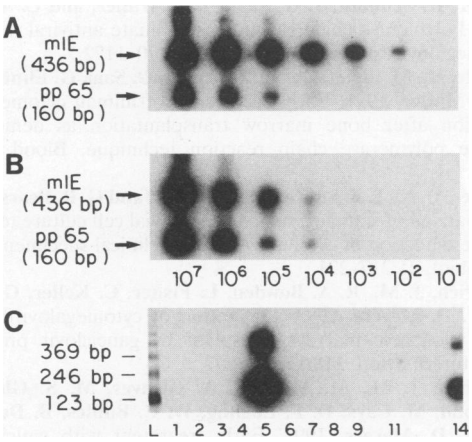


FIG. 1. Plasma PCR assay. Serial 10-fold dilutions of CMV DNA standard were made in water (A) or in CMV-seronegative plasma (B) and assayed by PCR using primers for CMV mIE and CMV lower matrix protein (pp65) sequences. (C) Typical Southern blot shown for nine clinical plasma specimens from the BAL fluid culture-negative group (lanes 1 through 9), with a standard negative (lane 11) and positive (lane 14) sample. The lengths of the PCR DNA products (in base pairs) are shown, and the numbers of CMV genome copies per 10-μl sample are given below the lanes in panel B. L, molecular weight ladder.

positions 121094-119355), primer 1 was 5′-AAA GAG CCC GAC GTC TAC TAC ACG T-3′, primer 2 was 5′-CCA GGT ACA CCT TGA CGT ACT GGT C-3′, and the probe was 5′-GTT CTC CAT GGA GCA AAC CAG CTC GTG CGC-3′. The extracted DNA (10 μl) was amplified in a reaction mixture containing (final concentrations) 1 μM each primer, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM (each) deoxynucleoside triphosphate, and 2.5 U of *Taq* polymerase (Perkin-Elmer Cetus, Norwalk, Conn.) in a total volume of 50 μl. Samples were covered with 100 μl of mineral oil and amplified for 25 cycles in a DNA thermal cycler (Perkin-Elmer) as follows: 1 min at 94°C for denaturation, 2 min at 55°C for annealing, and 2 min at 72°C for extension. Aliquots (10 μl) of each amplification product were characterized by Southern analysis as described previously (28). Each plasma PCR was run with $10^3$ and $10^4$ genome copies of CMV DNA control and a negative control consisting of distilled water and reaction mix. The presence of CMV DNA in the positive plasma samples was determined by visual comparison with the control standards.

To estimate the sensitivity of this assay, Towne strain CMV DNA, extracted from purified virions plus dense bodies, was diluted serially 10-fold in water and each dilution was then subjected to amplification by PCR. In addition, PCR was performed on DNA extracts at the same dilutions in CMV-seronegative plasma. As shown in Fig. 1, the sensitivity of CMV DNA detection in distilled water was $10^1$ to $10^2$ genome copies per 10 μl, and, after plasma extraction, it was reduced to $10^3$ genome copies per 10 μl.

Of the 40 subjects studied, 13 (32.5%) had BAL fluid cultures positive for CMV by rapid assay or long-term culture on day 35 after BMT, and 27 (67.5%) were CMV negative by BAL fluid culture evaluation. As shown in Table 1, of the 13 subjects with positive BAL fluid cultures, 9 (69%) had positive plasma PCRs at some time between days 28 and 49 ($P = 0.001$ by the chi-square test.) Of these 9, 7 were positive by day 35 and 2 became positive by PCR during the subsequent 2 weeks

TABLE 1. Detection of CMV DNA by plasma PCR

| Day post-BMT | No. of positive plasma samples/no. analyzed[a] (%) from patients with day 35 BAL fluid culture result | | Cumulative no. of positive subjects |
|---|---|---|---|
| | Positive[b] | Negative[c] | |
| 28 | 3/10 (30) | 0/22 (0) | 3 |
| 35 | 6/10 (60) | 0/14 (0) | 7 |
| 42 | 4/10 (40) | 1/10 (10) | 8 |
| 49 | 5/9 (55) | 1/8 (16) | 9 |

[a] Per week.
[b] Total, 13 subjects.
[c] Total, 27 subjects.

while receiving ganciclovir. Of the 27 BAL subjects with negative BAL fluid cultures, 2 had positive plasma PCR.

It has been shown that asymptomatic CMV infection of the lung, as ascertained by BAL fluid culture, progresses to interstitial pneumonitis in 67 to 70% of cases (18, 19). The sensitivity and specificity of culture of fluid from a day 35 BAL, using incidence of CMV-associated interstitial pneumonia as the basis for comparison, have been reported to be 0.50 and 0.88, respectively, with positive and negative predictive values of 0.68 and 0.77, respectively (19). These values are similar to blood culture results evaluated in other BMT populations at risk for CMV disease (15, 25). With the advent of preemptive ganciclovir therapy in this population, it is no longer possible to compare new CMV assays for efficiency relative to CMV disease occurrence. But the plasma PCR assay for CMV DNA can be evaluated relative to the CMV culture of BAL fluid, a standard for disseminated infection. As shown in Table 2, the sensitivity of the PCR assay was 0.69 and the specificity was 0.93. The predictive power of a positive PCR was 0.82 and the predictive power of a negative PCR was 0.86. It is possible that the use of ganciclovir in the population positive by culture of fluid from BAL beginning on day 35 could have altered the sensitivity and specificity of subsequent analyses in this group. Seven of 13 (54%) from the group with CMV-positive BAL fluid cultures also had positive blood cultures within 84 days of BMT, compared with 8 of 27 (30%) from the group with no detectible pulmonary infection at day 35. Compared with BAL fluid-culture as a standard, the blood culture assay had a

TABLE 2. Correlation of plasma CMV DNA detection and blood culture with BAL fluid culture for CMV

| Test and result | No. of samples with BAL fluid culture result[a] | |
|---|---|---|
| | Positive | Negative |
| Plasma PCR[b] | | |
| Positive | 9 | 2 |
| Negative | 4 | 25 |
| Blood culture[a] | | |
| Positive | 7 | 8 |
| Negative | 6 | 19 |

[a] CMV was cultured as described in the text from BAL fluid on day 35 after marrow transplantation and weekly from blood on days 28 through 84. A positive assay was defined either by the presence of CMV mIE at 24 h postinoculation (rapid assay) or by cytopathic effect confirmed with immunologic staining using anti-CMV mIE antibody after 4 weeks (long-term culture).
[b] Positive and negative PCR results were defined by comparison with results obtained with control CMV DNA or no DNA.

sensitivity of 0.54, a specificity of 0.70, and positive and negative predictive powers of 0.47 and 0.76, respectively.

The 13 patients with positive BAL fluid cultures received ganciclovir treatment, and none developed CMV pulmonary disease during a 12-month period of follow-up. The two subjects with negative day 35 BAL fluid cultures, who had positive plasma CMV DNA assays on days 42 and 49, respectively, had persistently negative blood cultures, no treatment, and no CMV-associated disease. As indicated by the study of Schmidt et al. (19), it is known that in this high-risk population, sequential BAL will yield positive cultures in as many as half of those who are negative on day 35. It is possible that the use of sequential plasma PCR for detection of CMV DNA will improve and possibly eliminate the need for a prospective BAL in assessing risk for CMV infection in this population.

Thus, plasma CMV DNA detection by PCR correlates well with CMV detection by BAL fluid culture, and this confirms the observation of Spector et al. (20) and of Wolf and Spector (25) that plasma from persons with AIDS and from BMT recipients with advanced CMV infection has detectible CMV DNA. It should be noted that the sensitivity of our assay is 10-fold lower than that described by Spector et al. (20) and yet it was able to detect the CMV DNA in plasma during virus dissemination. The origin of CMV DNA in plasma is not understood, but this suggests an aspect of CMV biology, namely, the extracellular presence of virions or nucleocapsids in plasma, which deserves further characterization. Regardless of the explanation for the presence of CMV DNA in plasma, this appears to be a marker for progression of virus infection. In this study, the plasma PCR assay appeared to have a higher predictive value for determining progressive CMV infection than conventional blood cultures. Subsequent studies are warranted to determine whether the plasma PCR assay can be used to efficiently institute and monitor ganciclovir therapy for prevention of CMV disease. With the addition of methods which would facilitate the detection of PCR products, the plasma PCR assay may become a useful tool in the clinical management of the immunocompromised patient.

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