

Evaluation of the AMPLICOR CMV Test for Direct Detection of Cytomegalovirus in Plasma Specimens

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We evaluated the AMPLICOR CMV test (PCR) for the direct detection of cytomegalovirus in plasma. Sixty-eight specimens were involved for the comparison between the AMPLICOR test and the antigenemia assay. The sensitivities, specificities, and positive and negative predictive values were 97.1, 100, 100, and 97.1%, respectively, for the AMPLICOR test and 79.4, 100, 100, and 82.9%, respectively, for the antigenemia assay.

Recently, cytomegalovirus (CMV) has been an important pathogen in immunocompromised patients. CMV disease requires immediate treatment, and laboratory diagnostic tests should be predictive of CMV disease.

CMV antigen detection (by antigenemia assay) is widely used for the diagnosis of CMV disease, and an antigenemia assay kit is available (6).

PCR is one of the promising methods in laboratory testing. The detection of CMV DNA in plasma by PCR has been reported for immunocompromised hosts (1, 2, 5, 8, 9, 12), and plasma seems to be a convenient material for detecting CMV (1, 2, 5, 8, 9, 12). On the basis of these thoughts, a new PCR-based test (AMPLICOR CMV, Roche Diagnostics Systems, Branchburg, N.J.) was developed for the detection of CMV.

We evaluated the AMPLICOR test by comparing it with the antigenemia assay, using specimens from immunocompromised patients, such as bone marrow transplantation (BMT) or renal transplantation (RT) patients, and other patients.

The AMPLICOR PCR assay was performed according to the manufacturer's instructions. Fifty microliters of plasma was mixed with 500 μ l of extraction reagent and incubated at 100°C for 30 min. Fifty microliters of the mixture was transferred into a PCR tube containing all the components necessary for PCR amplification plus an internal control (IC). The PCR tube was placed in a device (GeneAmp System 9600; Perkin-Elmer) for PCR amplification. After amplification, the nucleotide sequences were detected by an enzyme immunoassay technique. The absorbances at 450 nm were measured. Specimens having absorbance values of ≥ 0.25 were considered positive, and those with absorbance values of < 0.25 were considered negative.

The antigenemia assay was done as follows. A 5-ml aliquot of whole blood was mixed with 1.5 ml of 6% dextran solution in saline. After sedimentation for 30 min at 37°C, the polymorphonuclear leukocyte-enriched supernatant was centrifuged (500 $\times g$, 10 min). The leukocytes were resuspended at a concentration of 10×10^5 /ml in phosphate-buffered saline.

Then, 200 μ l of the suspension was spotted onto a slide with a cytocentrifuge, dried, and fixed in a solution containing 5% formaldehyde and 2% sucrose in phosphate-buffered saline. The slides were stained by an immunofluorescence assay with a monoclonal antibody (MAb) against the CMV pp65 antigen, MAb C7 (7) or MAb C10/C11 (4, 10, 11). The slides for each specimen were prepared in duplicate, and the positive cells were counted by two persons. The presence of one or more CMV-positive cells per 5×10^4 cells was considered a positive result; the presence of zero CMV-positive cells was considered a negative result.

Active CMV infection was defined by positive results of CMV detection (CMV isolation, CMV antigen detection, or CMV DNA detection) and/or positive results of the serological test (seroconversion or a greater-than-fourfold rise in immunoglobulin G antibody titer) (3).

CMV disease was defined by the occurrence of unexplained fever for 3 or more days in addition to evidence of active CMV infection plus at least one of the following signs: leukopenia, thrombocytopenia, atypical lymphocytosis, hepatitis, gastrointestinal bleeding or ulceration, interstitial pneumonia, CMV retinitis, or neuropathy (3).

Sixty-eight whole-blood specimens were provided with consent by 22 patients (16 post-BMT patients, 3 post-RT patients, 1 acute leukemia patient, 1 adult T-cell leukemia patient, and 1 immunocompromised patient with unknown disease). Each specimen was divided in two parts; one part was used for plasma preparation (aliquots were frozen at -80°C for the AMPLICOR test), and the other part was used for the antigenemia assay. The results of the AMPLICOR CMV test were compared to those of the antigenemia assay. Seven of 68 specimens were AMPLICOR test positive and antigenemia assay negative. One of 68 specimens was AMPLICOR test negative and antigenemia assay positive. These eight specimens with discrepant results were analyzed (Fig. 1).

The seven AMPLICOR test-positive, antigenemia assay-negative specimens were reexamined by the AMPLICOR test using a different aliquot of frozen plasma, and all of them were repeatedly positive by both the AMPLICOR CMV test and IC PCR. These specimens were obtained from five different patients, and their clinical backgrounds were reviewed to determine whether active CMV infection or CMV disease could be diagnosed on the day when the specimen was taken. Specimens

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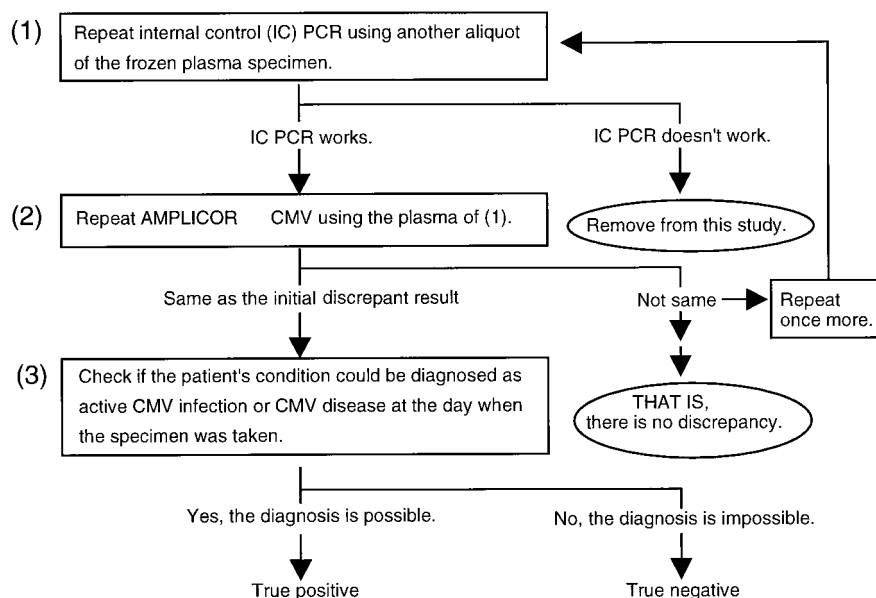


FIG. 1. Schema of analysis for discrepant results.

1 and 2 were obtained on separate days from the same patient, who suffered from CMV disease (gastrointestinal bleeding) after BMT. Specimen 3 was obtained from a patient who suffered from CMV disease plus graft-versus-host disease after BMT. Specimens 4 and 5 were obtained from the same immunocompromised patient on separate days. The patient suffered from multiple organ failure and CMV disease. Specimen 6 was obtained from an RT patient. He presented unexplained continuous fever and was considered to be suffering from active CMV infection. Specimen 7 was from another RT patient, who presented almost same episode as that of the patient from whom specimen 6 was obtained.

One AMPLICOR test-negative, antigenemia assay-positive specimen (specimen 8) was reexamined by the AMPLICOR test using another aliquot of frozen plasma. The result from this repetition was negative by the AMPLICOR CMV test and positive by IC PCR. This specimen was obtained from a BMT patient who was suffering from CMV disease (hepatitis) plus graft-versus-host disease on the day when the specimen was taken. As a result, these eight specimens with discrepancies were considered true positives for CMV disease. The final

results were summarized (Table 1). The sensitivities and specificities were 97.1 and 100%, respectively, for the AMPLICOR test and 79.4 and 100% for the antigenemia assay, respectively.

The AMPLICOR CMV test was designed as a qualitative assay. To evaluate the level of CMV in plasma semiquantitatively, a dilution series of each plasma sample collected sequentially from several patients was prepared with pooled plasma, which had, in advance, been proved with the AMPLICOR kit to be CMV negative and IC positive. Each dilution was tested with this kit. Figure 2 shows the clinical course of an 18-year-old female who underwent BMT due to acute leukemia. We could monitor the increasing level of CMV in plasma during CMV disease. The CMV antigenemia values on days 108 and 130 were lower than those on neighboring days because the lower leukocyte counts ($<10^9$ /liter) on days 108 and 130 affected the antigenemia assay.

To date, the antigenemia assay has been commonly used, because results of this test correlate with the clinical status of CMV disease (3). Here, we show that results of the antigenemia assay correlates with those of the AMPLICOR test as

TABLE 1. Comparison of the AMPLICOR CMV test and the antigenemia assay

| Method and result | No. of specimens with result for CMV disease or active CMV infection | | Sensitivity (%) | Specificity (%) | Positive predictive value (%) | Negative predictive value (%) |
|-------------------|--|----------|-----------------|-----------------|-------------------------------|-------------------------------|
| | Positive | Negative | | | | |
| Amplicor CMV test | | | | | | |
| Positive | 33 ^a | 0 | 97.1 | 100 | 100 | 97.1 |
| Negative | 1 ^b | 34 | | | | |
| Antigenemia assay | | | | | | |
| Positive | 27 ^b | 0 | 79.4 | 100 | 100 | 82.9 |
| Negative | 7 ^a | 34 | | | | |

^a Seven of 33 Amplicor CMV test-positive specimens are identical to 7 antigenemia assay-negative ones. That is, there are seven Amplicor CMV test-positive, antigenemia assay-negative specimens.

^b One Amplicor CMV test-negative specimen is identical to 1 of 27 antigenemia assay-positive ones. That is, there is one Amplicor CMV test-negative, antigenemia assay-positive specimen.

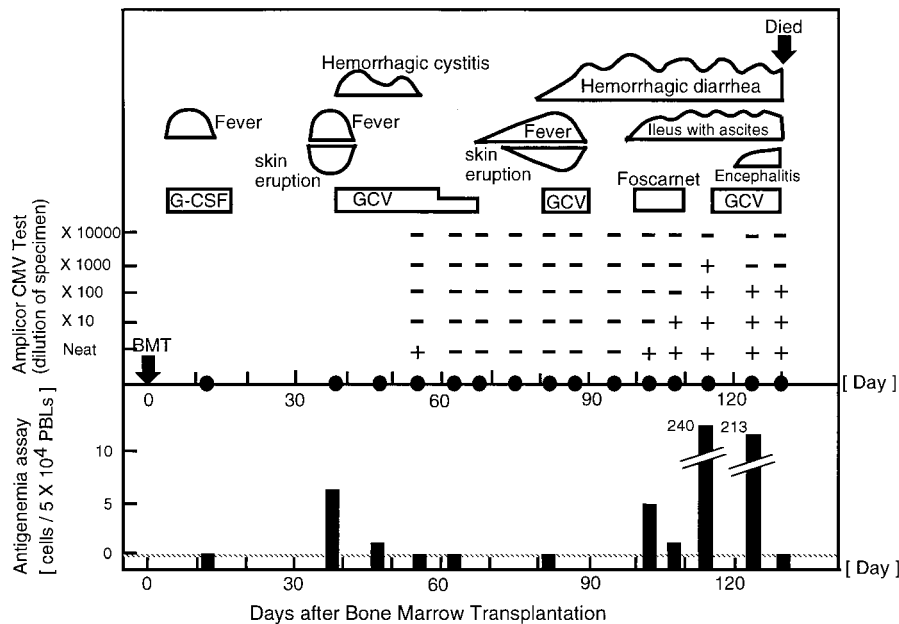


FIG. 2. Clinical course for an 18-year-old, female patient. The results of the antigenemia assay were expressed as numbers of positive cells per 5×10^4 peripheral blood leukocytes (PBLs). CMV was treated with intravenous ganciclovir (GCV) at 5 mg/kg of body weight twice a day and intravenous foscarnet at 200 mg/kg/day. G-CSF, granulocyte colony-stimulating factor; +, positive; -, negative.

well (Fig. 2). This suggests that the AMPLICOR test using plasma is suited to evaluate the clinical status of CMV disease.

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