Qualitative Plasma PCR Assay (AMPLICOR CMV Test) versus pp65 Antigenemia Assay for Monitoring Cytomegalovirus Viremia and Guiding Preemptive Ganciclovir Therapy in Allogeneic Stem Cell Transplantation

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The performances of a commercially available qualitative plasma PCR assay (AMPLICOR CMV test; Roche Diagnostics) and the pp65 antigenemia assay (AG) were evaluated for the monitoring of cytomegalovirus (CMV) viremia in 43 allogeneic stem cell transplant recipients. In addition, the suitability of both assays for triggering the initiation of preemptive ganciclovir therapy were assessed. A total of 37 CMV viremic episodes were detected in 28 patients. Positivity of plasma PCR testing in one or more consecutive specimens was the only marker of CMV viremia in 18 of the 37 episodes (PCR positive and AG negative, n = 50 specimens). Five episodes were diagnosed on the basis of a single positive AG result (AG positive and PCR negative, n = 5 specimens); both assays were eventually positive (PCR positive and AG positive, n = 27 specimens) for 14 viremic episodes; for these episodes, conversion of the PCR assay result to a positive result occurred an average of 1 week before conversion of the AG result. Overall, the concordance between the two methods was 90%, and the sensitivities of the plasma PCR assay and AG for the detection of CMV viremic episodes were 86.5 and 51.3%, respectively. Two patients who tested positive by both assays simultaneously progressed to CMV end-stage organ disease, despite the initiation of preemptive ganciclovir therapy. Conversion of the AG result to a negative result upon administration of preemptive ganciclovir therapy occurred a median of 7.5 days earlier than conversion of the plasma PCR assay result. Nineteen of the 28 patients with CMV viremia received AG-guided preemptive ganciclovir therapy; had the positivity of the plasma PCR assay triggered the initiation of preemptive therapy, 9 additional patients would have been unnecessarily treated since none of them developed CMV end-stage organ disease. Although the AMPLICOR CMV assay is more sensitive than AG, the latter appears to be more suitable both for guiding the initiation of preemptive therapy and for monitoring a patient’s response to antiviral therapy.
Patients. Forty-three consecutive patients undergoing allogeneic stem cell transplantation at the Bone Marrow Transplantation Unit of the Department of Hematology and Medical Oncology of the University Clinic Hospital in Valencia, Spain, between June 1997 and December 2000 were included in the study. All patients received stem cells from related donors. The CMV serostatus (as determined by a commercial immunoassay [Biokit, Barcelona, Spain]) of the transplant recipients and donors were as follows: donor positive and recipient positive, n = 35; donor positive and recipient negative, n = 3; donor negative and recipient positive, n = 3; donor negative and recipient negative, n = 2. From the time of hospital admission onwards, patients were given standard prophylaxis for bacterial (ciprofloxacin at 500 mg twice daily orally [p.o.]), fungal (fluconazole at 200 mg twice daily p.o.), and viral (acyclovir at 800 mg three times daily p.o.) infections. In addition, all patients received immunoglobulins intravenously (i.v.) at a dose of 400 mg/kg of body weight weekly until day +100 and then monthly until day +360.

Monitoring and management of CMV viremia. Patients were monitored weekly (in some cases the patients were monitored twice a week after a positive AG result) by AG for evidence of CMV viremia. Preemptive ganciclovir therapy was initiated at the time of a single positive AG result. Preemptive therapy consisted of the i.v. administration of ganciclovir at 5 mg/kg two times daily for 15 days or until the patient was negative by AG, followed by 1 month of maintenance therapy (ganciclovir at 5 mg/kg/day for 5 days/week). In addition, plasma specimens were aliquoted and frozen at -70°C. Plasma specimens collected over a 1-week period were tested by a commercially available CMV DNA PCR assay (Roche Diagnostics) at the end of the week (in a single run). Patients with CMV end-stage organ disease were treated with i.v. ganciclovir (at 5 mg/kg; two times daily for 21 days, followed by 5 mg/kg/day for 5 days/week for 1 month) and i.v. human immunoglobulin (at 400 mg/kg/day every 48 h for 15 days and weekly thereafter for 1 month).

Criteria for the diagnosis of CMV viremia and CMV end-stage organ disease. A patient was diagnosed as having an episode of CMV viremia when either AG or the plasma PCR assay (or both) proved positive. CMV pneumonitis was diagnosed on the basis of the clinical condition, the presence of interstitial infiltrates on chest X-rays, and the histological demonstration of CMV inclusions in tissue samples obtained at biopsy or necropsy.

Virological assays. Blood samples were drawn into EDTA-treated tubes and were processed within 2 h. Polymorphonuclear leukocytes (PMNLs) and plasma were separated by the standard dextran sedimentation method. AG was carried out by a recent optimization of a standard immunofluorescence procedure (11). Briefly, PMNLs containing supernatants were transferred to a 15-ml conical centrifuge tube and were centrifuged at 300 × g for 10 min. Supernatants were discarded and cell pellets were resuspended in phosphate-buffered saline (PBS) (105 PMNLs) were spotted onto a glass slide by using a cytocentrifuge for a final concentration of 10.0 × 106 PMNLs/ml; then, 0.2 ml of the cell suspensions (2 × 106 PMNLs) were spotted onto a glass slide by using a cytocentrifuge (500 × g for 3 to 4 min). Two slides were prepared per specimen. The slides were air dried, fixed in a solution containing 5% formaldehyde and 2% sucrose in PBS (10 min at room temperature), and then washed twice with PBS. The cells were permeabilized by immersing the slides in a PBS solution containing 2% sucrose and Nonidet P-40 (50 min at room temperature). The slides were then washed in PBS, rinsed in distilled water, and air dried. The cells were incubated with pp65-specific monoclonal antibody and then with an anti-mouse immunoglobulin G–fluorescein isothiocyanate conjugate (both immunoglobulin G and fluorescein isothiocyanate were obtained from Chemicon International, Temecula, Calif.). The presence of one or more pp65-positive cells (≥105 PMNLs) was considered a positive result. Qualitative detection of CMV DNA in plasma was carried out by the AMPLICOR PCR assay (Roche Diagnostics), according to the instructions of the manufacturer. Standard precautions for avoiding PCR contamination were adopted.

Data analysis. Comparison of data was performed by the nonparametric Mann-Whitney U test with the assistance of commercially available software (Instat, San Diego, Calif.). By this test, the average rank of two independent samples is statistically compared. Two-tailed P values are given, and those of <0.05 were considered to be of statistical significance.

RESULTS

Detection of CMV viremia by plasma CMV DNA PCR assay and AG. Sequential blood samples from 43 patients were analyzed in the study. A total of 543 blood specimens were tested by both methods; 5 additional samples were tested only by PCR due to severe neutropenia. A total of 37 episodes of CMV viremia were detected in 28 patients, and most of these episodes (n = 30) occurred before day +100. Seven of the 28 patients had several consecutive episodes of viremia (5 patients had two episodes and 2 patients had three episodes). A positive plasma PCR result was the only evidence of CMV viremia in 18 episodes (10 of the 18 episodes were defined by the positivity of a single specimen; for the remaining 8 episodes, two or more sequential samples [up to four sequential samples] were found to be positive). To rule out a false-positive result of the plasma PCR due to cross-contamination, a number of these specimens were retested by using a different aliquot. All of these samples were found to be repeatedly positive. AG was the only test positive for five viremic episodes (only one sample from each episode was positive). One or more sequential blood specimens drawn during 14 episodes of CMV viremia were found to be positive by both assays; for 8 of these episodes, the PCR test and AG became positive simultaneously, while for the remaining 6 episodes the onset of positivity of the plasma PCR test preceded that of AG by an average of 7.1 days.

The performances of the two assays are summarized in Table 1. Overall, the concordance between the methods was 90%, and the sensitivities of the PCR assay and AG for the detection of episodes of CMV viremia were 86.5 and 51.3%, respectively.

Clinical outcomes for patients with CMV viremia and performances of plasma PCR assay and AG in patients who developed CMV end-stage organ disease. Nineteen of the 28 patients with laboratory evidence of CMV viremia received AG-guided preemptive ganciclovir therapy. Of these, two patients progressed to CMV end-stage organ disease; one of the two patients developed biopsy-proven CMV pneumonitis by day +57. Blood specimens drawn from one of the patients 1 and 2 weeks before the clinical manifestations of CMV disease became apparent tested positive by both assays. The other patient developed necropsy-proven CMV pneumonitis by day +50. Both tests became positive 5 days before diagnosis of the disease. In both cases, the patients died (of respiratory failure) shortly after the diagnosis of CMV end-stage organ disease and despite the initiation of i.v. ganciclovir inductive therapy. Of the nine patients with CMV viremia (detected by plasma PCR assay) who were not receive preemptive ganciclovir therapy, none went on to develop CMV end-stage organ disease. Finally, 1 of the 19 patients who received preemptive ganciclovir therapy presented with a clinically silent secondary episode of CMV viremia (diagnosed on the basis of a single positive plasma PCR assay result).

Performances of plasma PCR assay and AG test in monitoring resolution of ongoing CMV viremia. The analysis of the 14 episodes of CMV viremia in which both assays eventually
TABLE 2. Performances of plasma CMV PCR assay and AG in monitoring clearance of CMV viremia after initiation of preemptive therapy

| Patient no. | Time (day after transplant) of initiation of preemptive therapy | Time (day) of negative conversion after start of preemptive ganciclovir therapy |
|-------------|---------------------------------------------------------------|--------------------------------------------------------------------------|
|             | Plasma PCR assay | AG     |                  |                  |                  |
| 7           | +67              | +7     | +7              |                  |                  |
| 11          | +47              | +14    | +7              |                  |                  |
| 14          | +39              | +57    | +29             |                  |                  |
| 21          | +59              | +20    | +13             |                  |                  |
| 25          | +187             | +12    | +12             |                  |                  |
| 26          | +447             | +7     | +7              |                  |                  |
| 27          | +48              | +21    | +21             |                  |                  |
| 30          | +60              | +23    | +13             |                  |                  |
| 31          | +48              | +7     | +7              |                  |                  |
| 36          | +34              | +20    | +11             |                  |                  |
| 37          | +82              | +19    | +19             |                  |                  |
| 38          | +37              | +22    | +22             |                  |                  |
| 40          | +48              | +25    | +25             |                  |                  |
| 42          | +55              | +45    | +5              |                  |                  |

became positive (Table 2) revealed that, overall, conversion to a negative result by AG upon administration of preemptive ganciclovir therapy occurred earlier than that by the plasma PCR test (a median of 12.5 days for AG versus a median of 20 days for the plasma PCR test), although the difference did not reach statistical significance ($P = 0.164$). The time of conversion to a negative result by the two assays was simultaneous in eight episodes; in the remaining six episodes, the plasma PCR test result continued to be positive after conversion of the AG result to a negative result. Earlier conversion of the PCR test result to a negative result with respect to the time of conversion of the AG result to a negative result was not observed.

**DISCUSSION**

In the present study, the plasma PCR assay proved to be more sensitive than AG in detecting CMV viremia in allogeneic stem cell transplant recipients. In effect, by the plasma PCR assay, 32 of 37 episodes of CMV viremia could be diagnosed, while the AG test was able to detect only 19 episodes. If the specificities of both assays are considered to be 100%, the sensitivities of the plasma PCR test and AG were found to be 86.5 and 51.3%, respectively. In addition, the analysis of CMV viremic episodes in which the results of both tests eventually turned positive revealed that the time of onset of a positive plasma PCR test result preceded that of a positive AG result by an average of 1 week, indicating that a positive plasma PCR test result is an earlier marker of CMV viremia than a positive AG result. The sensitivity rates reported in the present study are lower than those published by Hiyoshi et al. (16) (97.1 and 79.4% for the plasma PCR test and AG, respectively), although the superior sensitivity of the plasma PCR test was also evident in the present study. Our data also seem to be in agreement with those published by Hebart et al. (15), who used an in-house-designed PCR test to detect CMV DNA in plasma in a number of allogeneic bone marrow transplant recipients. In that study, although the sensitivities of the assays were not calculated, the concordance between the two tests was reported to be 92% (versus 90% in our study); moreover, as in our own experience, most of the samples with discordant results were found to be plasma PCR assay positive and AG negative. Likewise, a superior sensitivity of plasma PCR assays in comparison with the sensitivity of AG has been reported in different transplant settings (26). Our data are nevertheless discrepant from those recently reported by Boivin et al. (6) for a comparable cohort of patients; in the latter study the number of subjects with a positive test result was significantly higher for AG than for the qualitative plasma PCR assay and the AG result tended to turn positive earlier than the PCR assay result did. The reasons for such a discrepancy are not clear since the protocol used to perform AG appeared not to be substantially different from that followed in our study and, moreover, the same commercial PCR was used. The sole difference between the two studies is the time during which plasma specimens were kept frozen: a few days in our case versus several months in the other study (6); it is not clear, however, whether this variation could account for such a discrepancy. Our data are also in contrast to those reported by Boeckh et al. (4), who found the sensitivity of the plasma PCR test to be similar to that of AG. That study, however, used an in-house-designed PCR assay whose sensitivity might not have been optimal. In agreement with previous reports (6), the AG result globally tended to turn negative earlier than the plasma PCR test result (a median of 7.5 days earlier) after the initiation of preemptive ganciclovir treatment. Since patients who continued to be CMV DNA PCR assay positive after conversion of the AG result to a negative result did not progress to CMV end-stage organ disease, the latter assay appears to be more suitable than PCR for monitoring of the efficiency of anti-CMV therapy.

In our cohort, the AG-guided strategy for the triggering of preemptive therapy resulted in an incidence of CMV end-stage organ disease before day +100 of 4.8%, which is comparable to that reported by Boeckh et al. (3), who also started preemptive therapy upon a positive (any level) AG result, and to that reported by Einsele et al. (9), who initiated ganciclovir treatment when two consecutive whole-blood samples became positive by PCR. Our CMV end-stage organ disease-preventing strategy also resulted in a low incidence of secondary episodes of CMV viremia (only one) and in a zero incidence of late CMV end-stage organ disease. In the two patients who progressed to CMV disease despite the initiation of preemptive ganciclovir therapy, the results of both tests turned positive concomitantly; therefore, PCR-guided preemptive therapy would not have resulted in more favorable clinical outcomes for these patients. In accordance with our CMV end-stage organ disease-preventing strategy, 19 of 43 (44.1%) patients studied received preemptive ganciclovir therapy. Had either a single positive plasma PCR test result or two consecutive positive plasma PCR test results triggered the initiation of preemptive therapy, nine and six additional patients, respectively, would have received preemptive ganciclovir therapy that would have been unnecessary, since all these patients remained free of CMV end-stage organ disease during the study period.

In summary, the AMPLICOR CMV commercial plasma PCR assay is more sensitive than AG for the detection of CMV viremia in allogeneic stem cell transplant recipients. Nevertheless, we find AG to be more suitable both for guiding the initiation of preemptive therapy and for monitoring the efficacy
of ganciclovir treatment. Several drawbacks are associated with the use of AG, however: the need for rapid processing in order not to lose sensitivity and the impossibility of using the test during severe neutropenia. Perhaps the quantitative version of the presently evaluated PCR assay (the COBAS AMPLICOR CMV MONITOR assay) will prove useful once the procedure is sufficiently evaluated and the threshold for the initiation of preemptive therapy is clearly defined. Several studies seem to support this view (6, 7).

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