Monitoring of cytomegalovirus infection in solid-organ transplant recipients by an ultrasensitive plasma PCR assay

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Abstract

Early and accurate monitoring of cytomegalovirus (CMV) infection in solid-organ transplant recipients is of major importance. We have assessed the potential benefit of an ultrasensitive plasma-based PCR assay for renal transplant recipients. The pp65 CMV antigen (pp65 Ag) assay using leukocytes was employed as a routine test for the monitoring of CMV in 23 transplant recipients. We compared the pp65 antigenemia with the CMV load quantified by an ultrasensitive PCR (US-PCR) with a limit of detection of 20 CMV DNA copies/ml of plasma. CMV infection was detected in 215 (67%) of 321 plasma samples by the US-PCR compared with 124 (39%) of 321 samples by the pp65 Ag assay. The US-PCR assay permitted the detection of CMV infection episodes following transplantation a median of 12 days earlier than the pp65 Ag assay. Moreover, during CMV infection episodes, DNA detection by the US-PCR was consistently positive, whereas false negative results were frequently observed with the pp65 Ag assay. We found a good correlation between the two assays, and the peak viral loads were significantly higher in patients with CMV-related [...]
Monitoring of Cytomegalovirus Infection in Solid-Organ Transplant Recipients by an Ultrasensitive Plasma PCR Assay

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Cytomegalovirus (CMV) remains a leading cause of complication in solid-organ transplant recipients (SOTR) and has a significant impact on transplant outcome (3, 25, 26, 28). CMV disease is associated with high viral loads and is effectively prevented by a prophylactic antiviral strategy (7, 20, 29). Low CMV replication levels may also have deleterious effects on the long-term allograft function and on the incidence of opportunistic infections (11). The availability of a sensitive test capable of detecting CMV replication at an early stage is thus of importance. The pp65 antigen (pp65 Ag) assay is a reference test for the monitoring of CMV viremia and has been validated in clinical trials with immunosuppressed patients (10, 18). However, this assay has several limitations, including its dependence on the leukocyte count and its inability to quantify low viral loads. PCR assays have been developed to improve the detection and the monitoring of CMV infection. Qualitative PCR assays have not provided insight into the dynamics of viral replication and are of limited utility for the prevention of clinical complications (2, 15). In contrast, quantitative PCR assays, whether performed with whole blood, leukocytes, or plasma, are highly sensitive for CMV load determination and are effective tools for establishing the suitability of the initiation of preemptive therapy (14, 22, 24). However, most of these assays lack standardization and have not been validated with large cohorts of transplant patients (32). Recently, a standardized and commercially available assay, the Cobas Amplicor CMV Monitor test, has been developed for plasma DNA quantification (4, 5, 21). However, previous studies have shown a limited sensitivity of PCR performed with plasma compared to that of assays performed with whole blood or with leukocytes (12, 23, 34). For this reason, we have improved the sensitivity of the Amplicor CMV Monitor test by lowering its detection limit from 400 to 20 DNA copies/ml (17). This ultrasensitive PCR (US-PCR) has been validated by a previous study performed with hematopoietic stem cell transplant recipients (17).

The aim of the present study was to evaluate the US-PCR as an alternative to the routinely used pp65 Ag assay for the monitoring of CMV infection in SOTR. Both methods were compared for detection sensitivity and the dynamics of CMV load on prospectively collected blood samples.

MATERIALS AND METHODS

Patients. In our institution we routinely monitor SOTR for CMV infection on a weekly basis for a 3-month period after transplantation. Follow-up is extended for high-risk patients and those in which antiviral therapy has been initiated. Twenty-three unselected consecutive SOTR were enrolled in the study (19 kidney recipients, 2 pancreas and kidney recipients, 1 islet and kidney recipient, and 1 liver recipient) irrespective of the time of transplantation. Mean age was 52 years (range, 33 to 73 years), and 14 were male. All patients were either CMV immunoglobulin G (IgG) positive before transplantation (R+/n = 15) or recipients of an organ from a CMV IgG-positive donor (D+; n = 8). Eleven patients were D+R+, eight were D+ but CMV IgG negative before transplantation (R−), and four were R+ but received an organ from a CMV IgG-negative donor (D−). Induction therapy consisted of anti-CD25 antibodies, and five patients received rabbit antithymocyte globulin for delayed graft function. Baseline immunosuppression was achieved by using either cyclosporine A or tacrolimus and mycophenolate mofetil. Patients received rabbit antithymocyte globulin for delayed graft function. Base-
A pellet was suspended in 600 μl of lysis buffer containing a proportion of quan-

titation standard similar to that introduced in the unmodified PCR assay. After

prefection by ethanol, the pellet was suspended and used for the PCR. Further

steps were performed according to the manufacturer’s recommendations, and
determination of the number of copies per milliliter was adjusted for plasma
input. These modifications allowed the limit of detection to be lowered from 400

to 20 DNA copies/ml and have been extensively validated in previous experi-
ments with healthy blood donors and bone marrow transplant recipients (17).

Although the US-PCR assay can detect less than 20 copies/ml, it has been

formally validated to detect 20 copies or more/ml. In the present study, we have

also reported the few positive results below the validated limit of detection in

order to show that the assay provides consistent results when low levels of

viremia are detected.

Statistical analysis. Comparisons between independent variables were as-

sessed by the Mann-Whitney U test. The Wilcoxon signed rank test was applied

for comparison of the different subgroups, and linear regression was used to

compare the two assays. The difference was considered significant when \( P < 0.05 \). Statistical analysis was performed with SPSS 9.0 (SPSS Inc., Chicago, Ill.)

and with Epi Info 5.01b (Centers for Disease Control, Atlanta, Ga.).

RESULTS

Assay correlation. A total of 321 blood samples were col-

clected from 23 SOTR. The pp65 Ag assay and the US-PCR
detected CMV loads in 124 (39%) and 215 (67%) of 321

samples, respectively (\( P < 0.001 \)). All pp65 antigenemia-posi-
tive samples were also positive by the US-PCR. However, 91

(42%) of the samples positive by the US-PCR were negative by

the pp65 Ag assay (Table 1). Of these 91 samples, 11 gave

duplicate positive results by the US-PCR a median of 12 days (range, 6
to 21 days) before positive results were obtained with the pp65

Ag assay; 47 remained positive after results of the pp65 Ag

assay were negative for a median of 16 days (range, 7 to 42
days); 33 were consistently positive by the US-PCR during

episodes of CMV viremia, whereas results of the pp65 Ag assay

were intermittently negative (Fig. 1). The samples positive by

the US-PCR and negative by the pp65 Ag assay were associ-

ated with low levels of CMV DNA in plasma, i.e., a mean of 87
copies/ml (range, 5 to 1,655 copies/ml).

Monitoring of CMV infection and disease. CMV DNA was
detected at least once during the follow-up period in all pa-

tients. Concomitantly or subsequently, all patients were also

positive by the pp65 Ag assay. Among patients monitored

routinely from the first month of transplantation (Fig. 1), CMV

DNA in plasma was detected by US-PCR before the pp65 Ag

assay in 8 of 14 cases. In these patients, the first positive results

were observed a median of 32 days (range, 18 to 137 days) after

transplantation with the US-PCR compared with 43 days

(range, 25 to 150 days) after transplantation with the pp65 Ag

assay (\( P < 0.01 \)). In 6 of 14 cases, both assays were positive

simultaneously (Fig. 1). All 8 D+R− patients developed primary infections, and

all 15 D+R+ and D−R+ patients developed reactivations.

Asymptomatic infection, CMV syndrome, and CMV disease

were diagnosed in 18 (78%), 4 (17%), and 1 (5%) patient,

respectively. CMV disease occurred in a D+R− patient (pa-

TABLE 1. Comparison of results of the US-PCR and the pp65 Ag assay for detection of CMV in 321 blood samples

<table>
<thead>
<tr>
<th>Result of pp65 Ag assay (no. of samples)</th>
<th>No. found by US-PCR to be:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive (124)</td>
<td>Positive (n = 215)</td>
</tr>
<tr>
<td>Negative (197)</td>
<td>Negative (n = 106)</td>
</tr>
</tbody>
</table>
Patient 1, D+R-

Patient 2, D+R+

Patient 3*, D+R-

Patient 4*, D+R+

Patient 5*, D+R+

Patient 7, D+R-

Patient 8, D+R-

Days

Log_{10} CMV DNA copies/mL

Number of positive pp65 cells/25x10^6 leukocytes
Patients 9, 11, 13, and 15 are shown in the left column, and patients 10, 12, 14, and 16 are shown in the right column. Each graph represents the number of positive pp65 cells/25x10^6 leukocytes and CMV DNA copies/mL over time. The graphs show the variability in response to antiviral therapy among different patients.

Days

FIG. 1—Continued.
FIG. 1—Continued.
patient 7) at day 88, despite a primary prophylaxis of 20 days. Three D+R− patients (patients 1, 9, and 12) developed late-onset CMV infections at days 146, 222, and 150 posttransplantation. Two of them (patients 1 and 12) had received primary prophylaxis for 65 and 31 days, respectively. The median peak DNA level in asymptomatic SOTR was 1,660 copies/ml (range, 15 to 5,000 copies/ml) compared with 5,000 copies/ml (range, 1,285 to 5,000 copies/ml) in patients who presented with CMV syndrome or disease (P = 0.048), as shown in Table 2. The median peak viremia level in asymptomatic SOTR was 16 pp65-positive cells/2.5 × 10⁵ leukocytes (range, 1 to 300 positive cells) compared with 170 pp65-positive cells/2.5 × 10⁵ leukocytes (range, 30 to 300 positive cells) in patients who presented with CMV syndrome or disease (P = 0.036).

Sixteen patients (70%) received ganciclovir therapy based on the results of the pp65 Ag assay. Ganciclovir was administered as a preemptive therapy in 11 of these patients and as a treatment for CMV syndrome or disease in 5. The reasons for the administration of deferred therapy were as follows: one patient was lost from follow-up (patient 7) and reappeared later with CMV disease, two patients developed late-onset CMV syndrome during a period of infrequent outpatient visits (patients 1 and 9), and the remaining two (patients 11 and 16) developed clinical symptoms during the period of observation after the first pp65 Ag assay results. The median peak viral load assessed by the US-PCR was significantly higher in the 16 SOTR who received antiviral therapy than in the 7 patients who did not: 4,703 copies/ml (range, 1,140 to 5,000 copies/ml) versus 36 copies/ml (range, 15 to 2,050 copies/ml) (P < 0.001). Nine (56%) of the treated patients had persistent detectable CMV DNA (median, 149 copies/ml; range, 12 to 5,000 copies/ml) for an average of 53 days (range, 8 to 154 days) after termination of ganciclovir treatment.

**DISCUSSION**

This study assessed whether a quantitative and ultrasensitive plasma-based PCR assay with a detection limit of 20 CMV DNA copies/ml could improve the monitoring of posttransplantation CMV infections.
plant CMV infection. Results demonstrated an increased sensitivity of the US-PCR for the detection of CMV load compared with that of the pp65 Ag assay (67% versus 39% positive samples). This led to an earlier identification of CMV infection episodes in the majority of cases, and CMV DNA was detectable for a longer period of time than pp65 antigenemia in either treated or untreated patients. These results are consistent with those of a previous study with hematopoietic stem cell transplant recipients showing that the US-PCR was much more sensitive than the standard Cobas Amplicor Monitor test and the pp65 assay (17). The US-PCR also provided consistent positive results during each CMV infection episode, whereas the pp65 Ag assay gave frequent false negatives (Fig. 1). This supports the need for a test that can quantify low viral loads and accurately describe the dynamics of CMV load.

Ganciclovir was given to more than 70% of our patients based on the results of the pp65 Ag assay only. Since we observed an excellent correlation between the two assays (Fig. 2) and since episodes of significant DNA-emia without subsequent antigenemia were not observed (Fig. 1), it is unlikely that the remaining cases would have been treated based on the US-PCR results. In addition, the patients with CMV-related symptoms or disease had higher peak viral loads (median, 5,000 copies/ml) than those without symptoms (median, 1,660 copies/ml). Moreover, patients that were treated (based on the pp65 assay) had much higher viral loads (median, 4,703 copies/ml; >1,140 copies/ml in all patients) than those that did not require treatment (median, 36 copies/ml; <2,050 copies/ml in all patients). This suggests that in SOTR viral loads higher than 5,000 copies/ml are associated with a high risk of CMV disease and that viral loads below 2,000 copies/ml are unlikely to be associated with CMV-related complications. If used appropriately, these cutoff levels could prevent unnecessary ganciclovir treatment.

Treatment initiation is based not only on the viral load itself but also on a combination of different criteria, including the kinetics of the viral load and the patient’s immune condition. Early detection of low viral loads in kidney recipients at high risk for complications (e.g., D+R– patients and those receiving anti-T-cell antibody or high doses of methylprednisolone) is certainly of interest (9) and may lead to a timely initiation of therapy. The ability to detect persistent low-level CMV replication after termination of antiviral therapy is also of interest. The viral load at the completion of antiviral therapy has been shown to predict the risk of relapsing (8, 16, 19, 27, 33), and an ultrasensitive assay may secure treatment termination for high-risk renal transplant subjects. But most importantly, a negative result from an ultrasensitive assay can rule out CMV replication and may prevent unnecessary treatment. Of note, new drugs such as oral valganciclovir with improved, but still limited, bioavailability compared with that of their intravenous formulations will be increasingly prescribed. The detection of low CMV loads during prophylactic or preemptive therapy may lead to treatment adjustment and prevent the risk of emergence of resistant strains (1, 6).

In conclusion, our observations showed that the implementation of an US-PCR assay that can reproducibly quantify low levels of CMV DNA in plasma would improve the monitoring of CMV infection or reactivation in SOTR. In particular, this assay could improve the suitability of the initiation and termination of antiviral therapy during CMV infection. To our knowledge, this is the first validation of a plasma-based PCR assay’s showing superiority over the pp65 assay for SOTR. Our observations suggest also that it is unlikely that the implementation of a test with higher sensitivity for the detection of low CMV loads will lead to unnecessary antiviral therapies.

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REFERENCES


