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Use of Laboratory Assays To Predict Cytomegalovirus Disease in Renal Transplant Recipients

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Eight laboratory assays, viz., the pp65 direct antigenemia test, a quantitative cytomegalovirus (CMV)-specific immunoglobulin G (IgG) assay (Biomerieux VIDAS), a CMV-specific IgM assay (Biomerieux VIDAS), the Hybrid Capture system (Murex), an in-house PCR with plasma (P-PCR) and leukocytes (L-PCR), and a commercial PCR (Roche AMPLICOR) with plasma (P-AMP) and leukocytes (L-AMP), were compared for their abilities to predict CMV disease before the onset of illness in a prospective study of 37 renal transplant recipients. By using an expanded criterion for active infection (two or more of the markers positive) and a clinical definition of disease, 22 (59%) patients were identified as having active CMV infection and 13 (35%) were identified as having CMV disease. Of the 13 CMV-seronegative recipients who received seropositive kidneys (R− group), 8 had active infection and disease. All assays were 100% specific and 100% predictive of CMV disease in the R− group. The leukocyte PCRs (L-PCR and L-AMP) were the most sensitive assays, had positive results an average of between 8 and 13 days before the onset of illness, and were the assays of choice. The performance of the assays was less satisfactory for the 24 patients who were CMV seropositive before transplantation (R+ group). A negative result was more useful for this group. Overall, P-AMP had the best results, and it could be the assay of choice for monitoring R+ patients. The non-PCR-based methods generally had high specificities but often gave late positive results and were not sensitive enough for use as prediction tools for either group of patients.

Human cytomegalovirus (CMV) infection is a major infectious complication of renal transplantation (12). Although CMV disease in renal transplant recipients is generally less severe compared to that in the recipients of other organs, CMV disease in renal transplant recipients has a significant impact on morbidity and graft survival, decreasing the cost-effectiveness of transplantation by 2.9-fold (11). Although effective chemotherapy such as ganciclovir therapy is available, the best result is observed when treatment is started early, and preemptive therapy, in which therapy is initiated at the first indication of active infection, is now practiced in many centers (13).

Traditionally, laboratory tests are used for the diagnosis of active CMV infection after the onset of symptoms. Advances in technology with the availability of more sensitive tests means that it is possible to have positive laboratory assay results well before the onset of disease (3). However, laboratory diagnosis of active CMV infection is not necessarily always associated with symptomatic CMV disease (15). To target treatment specifically to problem patients, it is necessary to identify markers that can predict the onset of CMV disease. Here, we describe a comparison of eight assays: the pp65 direct antigenemia test (DAT), a quantitative CMV-specific immunoglobulin G (IgG) assay (CMVG; Biomerieux VIDAS, Lyon, France), a CMV-specific IgM assay (CMVM; Biomerieux VIDAS), the Hybrid Capture system (HCS; Murex, Dartford, United Kingdom), and four versions of PCRs, viz., an in-house PCR with plasma (P-PCR) and leukocytes (L-PCR) and a commercial PCR (AMPLICOR; Roche Diagnostics, Basel, Switzerland) with plasma (P-AMP) and leukocytes (L-AMP).

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MATERIALS AND METHODS

Patients. Thirty-seven consecutive patients who underwent renal transplantation at the Royal Liverpool University Hospital (RLUH) and who were at risk of active CMV infection were prospectively enrolled in this study. Apart from one patient who received acyclovir for ophthalmic zoster just before transplantation, none of the patients received immunoglobulins or chemoprophylaxis against CMV. Preemptive therapy was not practiced at the time of the study. Patients were followed at weekly intervals for 12 weeks or until the loss of the graft or death. A whole-blood sample with EDTA as the anticoagulant and a clotted blood sample were taken during each visit. The medical team looking after the patients was not involved in the study procedure and was not aware of the study results. The medical team was free to conduct its own investigations whenever there was a clinical suspicion of CMV disease. Only DAT, CMVG, and CMVM were available to the medical team as routine diagnostic tests. Full blood counts (hemoglobin, leukocytes with differential, and platelets) and the results of renal function tests (urea, creatinine, and electrolytes) were monitored at each visit. The decision to administer ganciclovir treatment was based on the clinical picture and the investigation results available to the medical team. This study was approved by the RLUH Ethics Committee, and all participating patients gave informed consent.

Sample preparation. Between 15 and 20 ml of EDTA-anticoagulated whole blood was obtained from each patient per visit, and the samples were processed immediately. An aliquot of 3.5 ml was removed for HCS. The remaining sample was layered onto an equal volume of Histopaque 1119 (Sigma, St. Louis, Mo.), and the mixture was centrifuged at 700 × *g* for 20 min. The plasma at the top of the gradient was collected and stored at −70°C in multiple aliquots. The diffuse band of peripheral blood leukocytes (PBLs) at the interface of the plasma and the Histopaque was harvested, resuspended in 10 ml of phosphate-buffered saline (PBS), and washed. Residual erythrocytes were lysed osmotically by exposing the pellet to sterile distilled water for 20 s before the pellet was resuspended in 10× concentrated PBS to restore tonicity. The washed PBLs were finally counted with a hemocytometer and were adjusted to 10^6 cells per ml with PBS. A 100-μl (100,000-cell) aliquot of the PBLs were immediately processed for DAT. The remaining cells were divided into 0.5-ml aliquots and were stored at −70°C. Serum for CMVG and CMVM was separated from the clotted blood and was stored at −70°C.

DAT. A standard indirect immunofluorescent technique was used for DAT. The first antibody was a monoclonal antibody to CMV pp65 (monoclonal anti-

body IC3; Tissue Culture Service, Buckingham, United Kingdom), and the second antibody was fluorescein isothiocyanate-conjugated anti-mouse IgG with Evan's Blue counterstain (Syva, Maidenhead, United Kingdom). The fixation and staining procedures recommended by the manufacturers were followed. Positive cells containing CMV pp65 antigen were recognized by the characteristic nuclear fluorescence of polymorphonuclear leukocytes under a fluorescence microscope. The number of positive cells in each smear was counted, and the result was expressed quantitatively as the number of positive polymorphonuclear leukocytes per 10^5 PBLs.

HCS. The assay with HCS (version 1.0) was performed according to the manufacturer's instructions. Briefly, 3.5 ml of whole blood was lysed with the manufacturer's lysis solution, denatured, and hybridized with an RNA probe specific for CMV DNA. The RNA-DNA hybrid that was formed was captured with anti-RNA-DNA antibodies in capture tubes, and an alkaline phosphatase-conjugated antibody specific for RNA-DNA hybrids was used for detection with a chemiluminescent substrate. The number of relative light units (RLUs) for each sample was read with a luminometer. A negative control and three positive standards were used in each assay in duplicate. The positive cutoff value was calculated as twice the mean number of RLUs for the negative control, according to the manufacturer's recommendation. Specimens with RLU values greater than the positive cutoff value were considered positive, and specimens with RLU values lower than but within 75% of the positive cutoff value were considered equivocal by the manufacturer.

P-PCR and L-PCR. The in-house PCR method was a modification of the procedure described by Gozlan et al. (6). The major capsid protein gene was used as the target, and the same primers and internal probe were used for the amplification and detection of PCR products. Both PBLs and plasma were used in this study. DNA was extracted with the QIAamp blood kit (Qiagen, Hilden, Germany) according to the manufacturer's instruction. The PCR procedure was modified so that the PCR products could be detectable in an enzyme-linked immunosorbent assay format. The forward primer (CMCP1) was biotin labelled (Life Technologies, Paisley, United Kingdom), and 10 μl of extracted DNA was used per reaction mixture with 20 mM Tris HCl (pH 8.4), 50 mM KCl, 3.2 mM $MgCl_2$, deoxynucleoside triphosphates at a concentration of 200 μM, 12.5 pmol each of the forward primer (biotin-CMCP1) and the reverse primer (CMCP2), and 0.3 U of *Taq* polymerase (Life Technologies) in a final volume of 25 μl. The Omnigene thermocycler with a heated lid (Hybaid, Ashford, United Kingdom) was used for PCR, and the program consisted of 95°C for 10 min, followed by 40 cycles of 94°C for 1 min, 58°C for 1 min, and 72°C for 1 min. The reverse sequence of the internal probe (CMCP3) described by Gozlan et al. (6) was labelled at the 3′ end with digoxigenin with a commercial oligonucleotide 3′ end labelling kit (Boehringer Mannheim, Mannheim, Germany). The PCR products were denatured with an equal volume of 1.6% NaOH–1 mM EDTA at room temperature for 20 min. Then, 5 μl of the denatured PCR product was mixed with 100 μl of hybridization solution (Boehringer Mannheim) and 1 pmol of the digoxigenin-labelled probe in a streptavidin-coated microtiter plate (Labsystem, Helsinki, Finland), and the mixture was incubated at 37°C for 1 h. The plate was then washed five times with 25 mM Tris HCl (pH 7.5), 0.15 M NaCl, 2 mM $MgCl_2$, and 0.1% Tween 20. Anti-digoxigenin-peroxidase conjugate (Boehringer Mannheim) was added to each well (75 mU in PBS–1% skimmed milk), and the plate was incubated at 37°C for 30 min, followed by five washes with PBS–0.1% Tween 20; 100 μl of 3,3′,5,5′ tetramethylbenzidine (Sigma) was used as the substrate, and the reaction was stopped with 0.5 M sulfuric acid after 10 min. The optical density (OD) at 450 nm was read with a spectrometer. An evaluation of the spread of OD readings among known positive and negative samples showed that positive and negative results were distinguished by a cutoff of 0.5, and under optimal conditions, a single copy of purified CMV DNA could be amplified and the products could be detected by using this cutoff. Hence, an OD reading of 0.5 or more was considered a positive result in this study. All samples were tested in duplicate, with one negative and two positive standards tested in each run. Discrepant results between duplicates were repeated until a consensus result was obtained.

P-AMP and L-AMP. The AMPLICOR assay is a commercial PCR in which the DNA polymerase gene (UL54) of CMV is used as the amplification target. Both PBLs and plasma were used in this study, and the manufacturer's instructions were followed. For PBLs, each 0.5-ml aliquot was centrifuged in a microcentrifuge at 13,000 rpm for 10 min to pellet the cells. It was then resuspended with 50 μl of the supplied extraction reagent. In the case of plasma, 50 μl was directly mixed with 500 μl of the extraction reagent. The sample reagent mixtures for both PBLs and plasma were then incubated at 100°C for 30 min, and 50 μl of the mixture was transferred to amplification tubes containing 50 μl of the working PCR master mixture plus an internal control (IC). The dUTP and uracil DNA glycosylase system was incorporated into the AMPLICOR assay to control carryover contamination (9). The GeneAmp system 9600 thermocycler (Perkin-Elmer, Norwalk, Conn.) was used for the amplification with the recommended program. The PCR product was detected in an enzyme immunoassay format by using the microtiter plate supplied with the assay kit. The ODs at 450 nm were measured, and readings of 0.35 or more were considered positive according to the manufacturer's recommendation. Detection of the IC amplification product was performed for all amplifications, and a negative CMV amplification result was considered invalid if the IC amplification result was also negative (OD, <0.35).

CMVG. A commercial automated enzyme-linked fluorescent assay (Biomerieux VIDAS) was used for determination of the CMV-specific IgG titer in serum. Briefly, a solid-phase receptacle served both as an antigen-coated solid phase and the pipetting device. Test serum (100 μl) was introduced into a sealed strip containing all the reagents and was placed in the VIDAS instrument together with a solid-phase receptacle and was tested by using the CMVG program. When the assay was completed, the fluorescence was read and analyzed by computer and the result was expressed in arbitrary units (AU) per milliliter. Readings of less than 4 AU/ml were considered negative according to the manufacturer's recommendations. Samples with readings of more than 400 AU/ml were retested after a 1 in 10 dilution with a negative serum sample to obtain a reliable quantitative result.

CMVM. For CMVM we used the same assay format that we used for the IgG assay, except that the CMVM strips and the CMVM program were used instead and the result was qualitative rather than quantitative. The manufacturer considered a fluorescent signal index of 0.9 or more to be a positive result and an index of between 0.7 and 0.9 to be an equivocal result, and these criteria were adopted in this study.

Clinical data. Clinical records were analyzed retrospectively by one of us (A.B.) who was not aware of the study results from the laboratory. All episodes of unexplained fever for 2 or more days, unexplained general systemic symptoms that could be attributable to CMV disease, episodes of unexplained sustained neutropenia (fewer than 3×10^9 neutrophils/liter), and episodes of unexplained sustained thrombocytopenia (defined as a persistent drop in platelet count to below 75% of the baseline level) were recorded. The date of onset of symptoms in relation to the transplantation date and the outcome for each patient at 6 months posttransplantation were noted.

Definitions. To avoid sporadic false-positive PCR results, sporadic nonconsecutive PCR positive results were excluded (5, 20). CMVG was considered positive when there was either seroconversion or a fourfold rise in the antibody level. A single positive result was considered positive for DAT, HCS, and CMVM. In order to enhance identification, an expanded "gold standard" criterion was used in the definition of active CMV infection. A patient was considered to have active CMV infection whenever two or more methods used in this study were positive. CMV disease was considered definite when there was histological evidence of organ involvement and possible when two or more of the clinical features listed above were temporally associated with laboratory evidence of active CMV infection by the above definition (2). To evaluate the ability to predict CMV disease, patients were analyzed according to the serological status of the recipients before transplantation, and samples that were taken after the onset of CMV disease were excluded. Sensitivity was defined as the proportion of patients with CMV disease who had positive laboratory assay results; specificity was defined as the proportion of patients without CMV disease who had negative laboratory assay results. The positive predictive value (PPV) was defined as the proportion of patients with positive results who had CMV disease; the negative predictive value (NPV) was defined as the proportion of patients with negative results who had no CMV disease.

RESULTS

Patients. Sixty-three patients underwent renal transplantation at RLUH during the study period. Thirteen patients were CMV-seronegative recipients of seronegative kidneys and therefore were not at risk of CMV infection or disease. These patients were excluded from the study for ethical reasons. Of the remaining 50 patients, 13 declined to provide consent for participation in the study. Of the 37 patients enrolled in the study, 13 were CMV seronegative recipients of seropositive kidneys (D+R−) and 24 were CMV seropositive (R+) before transplantation; of the patients in the latter group 4 received CMV-seronegative kidneys (D−R+) and 20 received CMV-seropositive kidneys (D+R+). The mean age of the 37 enrolled patients was 45 years (range, 24 to 71 years) and the male-to-female ratio was 25:12. A total of 425 sets of samples were received for analysis (average, 11 samples per patient). Two patients left the study prematurely: one died at day 45 posttransplantation and one had a graft nephrectomy at day 35 posttransplantation. The patients were followed for a mean period of 78 days (range, 35 to 89 days).

CMV infection and disease. Twenty-two patients (59%) had two or more positive laboratory assay results during the study and therefore by definition had active CMV infection. Four patients had only one positive laboratory assay result (Table 1) and was not considered to be actively infected (HCS, $n = 1$; L-AMP, $n = 1$; CMVG, $n = 2$). One episode of definite CMV

TABLE 1. Frequency of laboratory assays with positive results according to donor and recipient serological status

No. of laboratory assays with positive results	No. of patients (no. of patients with disease) in the following groups:		
	D+R−	R+	Total
0	5 (0)	6 (0)	11 (0)
1	0 (0)	4 (0)	4 (0)
2	0 (0)	4 (1)	4 (1)
3	0 (0)	2 (0)	2 (0)
4	0 (0)	0 (0)	0 (0)
5	1 (1)	1 (1)	2 (2)
6	0 (0)	3 (1)	3 (1)
7	0 (0)	1 (1)	1 (1)
8	7 (7)	3 (1)	10 (8)
Total	13 (8)	24 (5)	37 (13)

disease was identified in a patient with biopsy-proven CMV gastritis. Twelve episodes of possible CMV disease were identified in 12 patients by using the definition described above. Of the 13 patients with confirmed or possible CMV disease, 10 had one or more positive laboratory test results before the onset of illness and 2 patients had one or more positive laboratory test results simultaneously with the onset of clinical disease. One patient had a protracted nonspecific illness, but none of the CMV assay results were positive until 15 days after the initial onset of symptoms. Eight patients were treated with ganciclovir by the medical team; all of these patients had definite or possible CMV disease when they were analyzed retrospectively. When the patients were followed up at 6 months, one patient had died, one patient had lost the graft, one patient had moderate graft function, and three patients had nonfunctioning grafts. All patients with adverse outcomes had CMV infection and disease.

Within the D+R− subgroup, 5 of 13 (38%) patients were not infected with CMV and none of these five patients had any positive laboratory assay results or CMV symptoms. In contrast, all eight patients in this subgroup who were infected had CMV disease (Table 2) and seven of eight of these patients had positive results by all the laboratory assays. Positive laboratory assay results preceded disease in seven of the eight patients in this group. A much broader range of combinations of laboratory assay results was seen for the R+ group (Table 1); 14 of 24 had active CMV infection and 5 of 24 had CMV disease. Three patients had positive results by all laboratory assays, but only one of the three patients had CMV disease. Positive laboratory assay results preceded CMV disease in three of five patients in this group.

Laboratory assays. Of the 425 sets of samples received, there were adequate samples for the processing of 422 by DAT, 410 by CMVG, 410 by CMVM, 413 by HCS, 411 by P-PCR, 413 by P-AMP, 412 by L-PCR, and 413 by L-AMP. Six (1.5%) samples had invalid L-AMP results because the IC failed to amplify. For all samples, the IC was successfully amplified by P-AMP. Sporadic nonconsecutive positive PCR results were noted for four patients by P-PCR, two patients by P-AMP, six patients by L-PCR, and seven patients by L-AMP.

Test results were analyzed according to the pretransplantation CMV serostatus of the recipients (R− or R+). Of the R− group, all eight laboratory assays were highly specific (100%) and 100% predictive of CMV disease. The two leukocyte PCRs had the highest sensitivities (L-PCR, 88%; L-AMP, 75%), were positive an average of 8 to 13 days before the onset of disease, and had the highest proportion of positive results before the onset of illness (Table 3). L-PCR was significantly more sensitive than DAT, CMVG, and CMVM in predicting CMV disease (by Fisher's exact test, $P < 0.05$). The performance of the laboratory assays was more variable and less satisfactory for the R+ group. The PPVs of CMVG and all PCR-based assays were significantly lower than the corresponding value for the R− group (by Fisher's exact test, $P < 0.05$). However, there were no differences in the NPVs of any of the assays between the R+ and R− groups (by Fisher's exact test, $P > 0.05$). P-AMP had the overall best results because it had the highest sensitivity, specificity, PPV, and NPV. However, even with the best PPV, a positive P-AMP result was predictive of CMV disease for only 38% of the patients tested by this assay. The NPVs may thus be of greater use as negative predictive tools for this group (Table 3). The non-PCR-based methods (DAT, CMVG, CMVM, and HCS) generally had high specificities for both the R− (specificities, 100%) and R+ (specificities, 68 to 90%) groups. HCS was usually positive at about the time of illness (Table 3), but DAT, CMVG, and CMVM often gave late positive results.

DISCUSSION

The current management of active CMV infection in organ transplant recipients emphasizes the identification of patients at risk of CMV disease and the provision of preemptive therapy (13). This relies on the availability of laboratory assays that can predict CMV disease (17). It is necessary to separate the analysis of the R− group from the analysis of the R+ group because the meaning of a positive laboratory assay result may not be the same.

Patients in the R− group had primary infection with CMV, and any positive laboratory assay result at any time is significant. Our results confirmed that not all R− patients who received a seropositive kidney had CMV infection, but all who acquired CMV infection had CMV disease and all eight assays evaluated were 100% specific. The choice of a laboratory assay for the prediction of CMV disease in the R− group therefore depends on the sensitivity of the marker and the time from the first positive result to the time of onset of illness. The leukocyte PCRs (L-PCR and L-AMP) appeared to be the tests of choice for the R− group because they were highly sensitive and were positive, on average, 8 to 13 days before the onset of illness. This will provide an adequate window for preemptive therapy.

Laboratory assays were more likely to be positive for those in the R+ group without clinical disease because asymptomatic reactivation could occur in immunosuppressed patients. The

TABLE 2. CMV infection, disease, and outcome by donor and recipient serological status

Serological status	No. (%) of patients with the following:				
	Total	CMV infection	CMV disease[a]	Ganciclovir treatment	Good outcome at 6 mo[b]
D+R−	13	8 (62)	8 (62)	7 (54)	11 (85)[c]
D−R+	4	1 (25)	1 (25)	0 (0)	4 (100)
D+R+	20	13 (65)	4 (20)	1 (5)	16 (84)[d]
Total	37	22 (59)	13 (35)	8 (22)	31 (84)

[a] All patients with CMV disease had CMV infection.
[b] All patients with adverse outcome had CMV infection and disease.
[c] One patient had graft nephrectomy and one patient had a nonfunctioning graft.
[d] One patient died, two patients had nonfunctioning graft, and one patient had moderate graft function.

TABLE 3. Performance of the eight laboratory assays in the prediction of CMV disease according to pretransplantation serological status

Patient group and test	Prediction of CMV disease[a]				Mean time from positive result to disease onset (days)[b]	Proportion of positive tests occurring before disease onset[a]
	Sensitivity	Specificity	PPV	NPV		
Seronegative recipients						
DAT	1/8 (13)	5/5 (100)	1/1 (100)	5/12 (42)	+4.1	1/8 (13)
CMVG	2/8 (25)	5/5 (100)	2/2 (100)	5/11 (46)	+4.1	2/8 (25)
CMVM	2/8 (25)	5/5 (100)	2/2 (100)	5/11 (46)	+9.1	2/8 (25)
HCS	4/8 (50)	5/5 (100)	4/4 (100)	5/9 (56)	+1.4	4/7 (57)
P-PCR	4/8 (50)	5/5 (100)	4/4 (100)	5/9 (56)	−6.3	4/7 (57)
P-AMP	5/8 (63)	5/5 (100)	5/5 (100)	5/8 (63)	−2.4	5/7 (72)
L-PCR	7/8 (88)	5/5 (100)	7/7 (100)	5/6 (83)	−8.0	7/8 (88)
L-AMP	6/8 (75)	5/5 (100)	6/6 (100)	5/7 (71)	−13.4	6/8 (75)
Seropositive recipients						
DAT	0/5 (0)	17/19 (90)	0/2 (0)	17/22 (77)	+7.0	0/3 (0)
CMVG	0/5 (0)	13/19 (68)	0/6 (0)[c]	13/18 (72)	+10.5	0/2 (0)
CMVM	0/5 (0)	17/19 (90)	0/2 (0)	17/22 (77)	+7.5	0/2 (0)
HCS	2/5 (40)	14/19 (74)	2/7 (29)	14/17 (82)	−2.0	2/4 (50)
P-PCR	3/5 (60)	10/19 (53)	3/12 (25)[c]	10/12 (83)	−10.8	3/4 (75)
P-AMP	3/5 (60)	14/19 (74)	3/8 (38)[c]	14/16 (88)	−5.5	3/4 (75)
L-PCR	2/5 (40)	11/19 (58)	2/10 (20)[c]	11/14 (79)	+1.8	2/4 (50)
L-AMP	3/5 (60)	11/19 (58)	3/11 (27)[c]	11/13 (85)	−6.8	3/5 (40)

[a] Data represent number of patients positive/total number of patients (percent).
[b] Time of CMV disease is time zero. +, positive test result after onset of illness; −, positive test result before onset of illness.
[c] Significant difference ($P < 0.05$) between R− and R+ groups by two-tailed Fisher's exact test.

more variable and less satisfactory performance of the laboratory assays for the R+ group is therefore not totally unexpected. Hence, the function of laboratory assays for this group of patients is more to exclude rather than to predict CMV disease. Overall, P-AMP had the best results for this group, with a high NPV (88%), and it thus appears to be most suitable for monitoring patients.

The higher sensitivity of the leukocyte assays compared to use of plasma in this study is consistent with previous findings (4, 19, 21). In this study, plasma was taken from the top of a gradient, and some dilution may have occurred. However, the effect of this on sensitivity may not be significant because there are many other factors that affect the sensitivity and specificity of PCR (7). From a technical point of view, the use of plasma had a significant advantage over leukocytes in PCR. It is easier to perform, and favorable results have previously been reported when P-AMP was compared with DAT (8). Whereas L-AMP was associated with PCR inhibition (indicated by the failure of IC amplification for 6 of 413 samples), we did not find any inhibition in P-AMP (0 of 413 samples). The use of EDTA as the anticoagulant is important because other studies that used heparin as the anticoagulant generally obtained poorer results that were probably related to PCR inhibition (4, 19). Another advantage of P-AMP is the fewer occurrences of sporadic, nonconsecutive positive results (two patients by P-AMP versus seven patients by L-AMP). Sporadic, nonconsecutive positive PCR results have been described in other studies that used PCR to monitor CMV infection (5, 20). It may be the result of PCR contamination, fluctuations in virus replication, or a viral load near the detection threshold of the test. Because of the presence of sporadic, nonconsecutive positive results, repeat testing of leukocytes is necessary to be certain of their significance. This, however, may not be necessary with plasma. In this study, leukocyte assays were not better than those with plasma as predictive tools for the R+ group. This may be related to the presence of latent virus in PBLs detected in leukocytes and the fact that the detection of CMV DNA in plasma is less likely to occur in the absence of CMV disease.

DAT was recognized in many previous studies as an effective tool for the diagnosis of CMV disease (1, 14, 18) and was positive before the onset of illness (16, 18). However, because of the variations in the sources of monoclonal antibodies and the preparation procedure, DAT results between centers may not be directly comparable. Our results suggested that its role in the prediction of disease is limited. Variation in technique may partly explain this discrepancy. However, we have followed strictly the method recommended by the manufacturer. Because chemoprophylaxis was not given in this study, both clinicians and patients were more aware of the threat of CMV disease. This increase in alertness could result in an earlier recognition of the insidious onset of CMV symptoms and signs compared to the time of recognition in other studies, and as a result the results of some of the less sensitive laboratory assays may become positive after instead of before the onset of illness.

HCS is another commercial assay with predictive potential (10). Our results suggested that it has higher sensitivities and predictive values than DAT, CMVG, and CMVM but that it is not as sensitive as the PCR tests. Also, it gave many equivocal results (14 of 413; 3%) which usually flanked the positive results, suggesting that they represented low-level positivity. A newer, more sensitive version of HCS (version 2) is now available, and it may provide better performance. The CMVG and CMVM results confirmed that serology is not generally useful for monitoring the progress of transplant recipients (12, 17).

To conclude, R− patients who receive CMV-seropositive kidneys should be monitored by sensitive assays such as L-PCR or L-AMP and should be given preemptive therapy when they test positive. None of the assays evaluated in the present study predicted CMV disease in the R+ group, but P-AMP was better than the other assays in both its overall performance and its test characteristics and is therefore the test of choice for the exclusion of CMV disease. Quantitative assays for CMV DNA will soon be available for routine use, and this may be a better predictive tool for the R+ group of patients.

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