Monitoring and Diagnosis of Cytomegalovirus Infection in Renal Transplantation

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Abstract. In this study, the utility of the cytomegalovirus antigen (CMV-AG) and the shell vial (SV) tests in the diagnosis and monitoring of posttransplant CMV infection were compared. Previous retrospective studies from the authors’ center suggested that the CMV-AG test, which uses monoclonal antibodies to detect viral antigen in circulating peripheral blood leukocytes (PBL) may be both a more sensitive and specific test. A cohort of 32 renal transplant recipients was followed-up prospectively with serial CMV-AG testing, as well as conventional culture and SV for blood and urine and tests for immunoglobulin M (IgM) antibody. It was discovered that the CMV-AG test was not only more sensitive than the SV test in detecting CMV infection, but that the degree of antigenemia as expressed by the number of positive cells per 50,000 PBL correlated with the likelihood and degree of symptomatic infection. All patients with a count > 10 positive cells/50,000 PBL developed clinical symptoms; therefore, this threshold could be useful in deciding clinically whether fever is related to CMV infection. Alternatively, if antigenemia were monitored serially after transplant, the same threshold could be used as a trigger for instituting antiviral therapy, because it was often reached prior to the onset of symptoms and had a high specificity for subsequent symptomatic infection. Such an approach could obviate unnecessary treatment of patients not destined to become symptomatic. Based upon the findings in this study, the CMV-AG test is superior to the SV assay because the actual count helps determine the likelihood that symptoms are a result of the virus and the processing time is shorter, it can be used to monitor the response to therapy and as a guide to the institution of preemptive therapy. (J Am Soc Nephrol 8: 1448–1457, 1997)

As many as 70 to 80% of recipients of solid organ transplants show laboratory evidence of cytomegalovirus (CMV) infection after transplant, and a significant number (10 to 30%) still develop tissue invasive disease (e.g., pneumonitis, enteritis, retinitis), with considerable morbidity and occasional mortality despite current prophylactic regimens (1–5). Seronegative recipients of an allograft from a seropositive donor (primary infection) as well as seropositive patients treated with anti-T cell agents (reactivation/superinfection) appear to be particularly at risk. Ideally, management of CMV after transplant requires a sensitive and reliable test capable not only of detecting infection early in the presymptomatic phase, but also of accurately identifying those patients who will progress to symptomatic infection. This could permit an alternative approach to blanket prophylaxis of CMV infection, namely “preemptive therapy” (1). Antiviral agents could initially be withheld until there is laboratory evidence of incipient CMV infection, at which point therapy would be instituted in an attempt to prevent full-blown infection. The last few years have seen the development of a number of candidate techniques that permit the more rapid diagnosis of CMV infection, including the shell vial (SV) modification of the conventional buffy coat culture technique (6), the detection of CMV antigenemia (7), and the detection of viral genetic material by the polymerase chain reaction (8).

The study presented here was designed to monitor renal transplant recipients prospectively and compare the utility of the cytomegalovirus antigen (CMV-AG) test and the SV test in the diagnosis and monitoring of CMV infection as well as their ability to identify a subgroup of patients liable to progress to symptomatic infection and, therefore, at high risk of tissue-invasive disease.

Materials and Methods

This was a prospective study involving all renal transplant recipients at the Erie County Medical Center, Buffalo, New York, from January 1992 onwards. Starting at the time of transplant, blood and urine samples were obtained at least every two weeks up to 6 months after transplant and tested for CMV-AG level, presence of anti-CMV IgM antibody, CMV viremia by both the SV and conventional culture techniques, and viruria by conventional culture of urine. Patients were seen at least biweekly in the renal transplant clinic, were questioned about the presence of symptoms, and were examined for signs of CMV infection. Complete blood counts, and serum creatinine and serum transaminase levels were measured at least every 2 weeks. Additional viral studies were obtained if the patient became febrile or if there was any reason to suspect clinical CMV infection. Posttransplant immunosuppression consisted of induction with antilymphocyte...
globohin (Minnesota ALG [University of Minnesota, Minneapolis, MN] until 8/92, ATGAM [Upjohn, Kalamazoo, MI] thereafter), steroids, and azathioprine. Cyclosporine was added once a patient’s serum creatinine concentration fell below 3 mg/dl, and once therapeutic cyclosporine levels were achieved antilymphocyte antibody treatment was discontinued. No specific anti-CMV prophylaxis was administered except to seronegative recipients of a kidney from a seropositive donor who received prophylaxis with CYTOGAM® (MedImmune, Gaithersburg, MD) according to the protocol of Snyderman et al. (2).

The presence of CMV infection was defined as the detection of either a significant level of IgM antibody, a new positive blood culture and a!.

andman administered except to seronegative recipients of a kidney from a

steroids, and azathioprine. Cyclosporine was added once a patient’s

MN] until 8/92, ATGAM [Upjohn, Kalamazoo, MI] thereafter), ste-

globulin (Minnesota ALG [University of Minnesota, Minneapolis,

CMV disease was considered to be present when any of the above-

mentioned laboratory tests were positive for CMV infection and was

associated with one or more of the following: leukopenia (<3000

and a CMV-AG count >50, they were treated with 14 days of

patients with laboratory evidence of CMV infection developed symp-

toms or a CMV-AG count >50, they were treated with 14 days of

intravenous ganciclovir 5 mg/kg iv (adjusted for renal function)

to 7.2) and incubated at 36°C. The first vial was fixed and stained on day 2; the

incubated at 36°C. The first vial was fixed and stained on day 2; the

second vial was processed on day 6, or 7. Shell vials were stained

using the Monofluoro Cytomegalovirus IFA Test Kit (Sanofi Diagnos-

tics, Bellevue, WA). The inoculum remained on the monolayer for 1 h

before 1 ml of maintenance medium (see above) was added. Cultures

were incubated in stationary racks at 36°C. Cultures were examined

cytotoxic effect daily during the first week and twice weekly

thereafter for a total of 30 days. Maintenance medium was changed

weekly. Uninoculated culture tubes served as negative controls.

Detection of Anti-CMV Immunoglobulin M

Levels were determined using an enzyme-linked capture assay

(CMV CAP-M, Biowhittaker). The capture IgM assay methodology

minimizes false positive results resulting from high levels of CMV-

IgG and rheumatoid factor interference. Tests were performed in
duplicate according to the manufacturer’s package insert. In brief, a
low positive standard (LPS) is assayed in triplicate and a mean

absorbance value is calculated. A ratio or index is then prepared for
each test sample by dividing its absorbance by the mean of the LPS.
Index values are interpreted as follows: <0.9, negative; 0.9 to 1.1, equivocal; >1.1, positive.

Results

Clinical Manifestations of CMV Disease

Demographics of the 32 patients are shown in Table 1. The

majority were male, Caucasian, and undergoing a first cadav-
eric transplant. Just over one fourth were diabetic and approx-
umimately one third had experienced at least one rejection episode in
the year after transplant. Five grafts were lost (one patient
death, three to rejection and one to hemolytic-uremic syn-
drome) and two patients died (one of Pneumocystis carinii
pneumonia, one of cardiac arrest). Twenty-one of the 32 pa-
tients followed-up showed evidence of CMV infection after
transplant when the conventional tests outlined in the Methods
section were used. Because eight of these patients (D–R–)
were at minimal risk of CMV, the incidence of CMV was 21
of 24 at risk recipients (D+R–, D+R+, D–R+). Ten of these in

in turn developed clinical symptoms that were believed to be
compatible with CMV infection, whereas the remaining pa-

tients with laboratory evidence of CMV remained asymptomatic.

Of the symptomatic patients, six exhibited features of CMV
disease (transaminemia in six, early retinitis in two).

None of the 11 patients who were negative by conventional

study to detect CMV antigenemia. Initially, CMV-AG was detected
by the immunoperoxidase (IP) technique as originally described by
Van der Bij et al. (7). After October 15, 1993, CMV-AG was detected
using an immunofluorescent (IF) staining method, using a mixture of
monoclonal antibodies (CMV10, CMV11; Biotest, Dreieich, Ger-

}
Prevalence of Posttransplant CMV Antigenemia

A total of 683 CMV-AG tests were performed in the 32 patients, of which 228 were positive. Of these 228, accompanying blood cultures were positive in 96 (SV-only, 43; conventional culture–only, 9; SV and conventional culture, 44). The higher the CMV-AG count, the more likely the accompanying blood culture was positive (Table 2). There were five samples in which the SV test was positive although the accompanying CMV-AG was negative. In four of these, conventional culture was also positive. The five samples were obtained in asymptomatic patients 57 to 155 days after transplant.

Positive tests for CMV-AG were detected at some point in 22 of the 32 patients. Independent evidence of CMV infection was found by conventional testing in 18 of these patients: conventional blood culture, 11; urine culture, 18; positive IgM antibody, 11. Figure 1 (top panel) shows the time after transplant at which CMV-AG was first detected in each patient. The majority were noted between 15 to 65 days (mean, 42 ± 5 days), which corresponds to the known time of occurrence of posttransplant CMV infection. Figure 1 (bottom panel) shows the overall prevalence of CMV antigenemia after transplant by plotting the percentage of at-risk patients who exhibited CMV antigenemia at some time during a particular 4-wk period. The peak prevalence of CMV antigenemia again occurred 4 to 8 weeks after transplant. Interestingly, antigenemia persisted, albeit often at low values, up to 6 months in as many as 20% of patients. In all of these patients, persistent viremia could also be detected by blood culture. An even higher percentage (32%) showed evidence of urinary shedding.

Table 3 shows the distribution of the peak CMV-AG counts seen in each of the 32 patients studied, in accordance with the pretransplant serostatus of the donor and recipient. As expected, the greatest risk of high counts (>50/50,000 PBL) and symptomatic infection occurred in seronegative recipients of a kidney from a seropositive donor (66%). This was despite the fact that all but one recipient received CYTOGAM® prophylaxis. In patients seropositive for CMV pretransplant, CMV-AG was detected just as frequently, but the peak counts tended to be lower, as was the incidence of counts >50 (28%). A surprising finding was the high incidence of antigenemia (37%) in seronegative recipients of a seronegative kidney, but these patients all had very low counts (<1) and likely represented false positives related to the use of the peroxidase technique. In asymptomatic patients, the CMV-AG tended to remain <10 (mean peak 3.1 ± 3.5) and usually disappeared spontaneously after a variable period of time. In contrast, the CMV-AG rose above ten in all ten symptomatic patients, with a mean peak value of 290 ± 191. CMV-AG was detected initially an average of 10 days before the onset of clinical symptoms in six of the ten symptomatic patients. The other two patients were found to have positive counts 7 and 14 days after the onset of fever, which happened to be the next CMV-AG count obtained in both cases.

Sensitivity of Various Tests in the Diagnosis of Posttransplant CMV Infection

Table 4 details the clinical sensitivity and specificity of various diagnostic tests in the diagnosis of posttransplant CMV infection. Sensitivity was defined as the percentage of patients with documented CMV infection with a positive test result among all infected patients tested. Specificity was defined as the percentage of subjects with negative test results among all noninfected subjects. Positive predictive value was calculated as (true positive tests/true positive and false positives) and negative predictive value as (true negatives/true negatives and false negatives). These four values were calculated for each test with regard to its ability to detect and/or predict all CMV infections (asymptomatic and symptomatic) and also just symptomatic infection alone.

Urine culture by conventional technique proved to be the
Figure 1. Top panel, time to initial detection of CMV-AG after transplant. Bottom panel, prevalence of CMV antigenemia in the 24 wk after transplant.

Table 3. Distribution of peak CMV-AG counts and the incidence of CMV infection/disease according to donor/recipientserostatus

<table>
<thead>
<tr>
<th>Serostatus of Donor/Recipient</th>
<th>n</th>
<th>Peak CMV-AG Count</th>
<th>CMV Infection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>≤1</td>
</tr>
<tr>
<td>Negative/negative</td>
<td>8</td>
<td>5 (63%)</td>
<td>3 (37%)</td>
</tr>
<tr>
<td>Positive/negative</td>
<td>6</td>
<td>1 (17%)</td>
<td>1 (17%)</td>
</tr>
<tr>
<td>Positive/positive</td>
<td>13</td>
<td>4 (31%)</td>
<td>2 (16%)</td>
</tr>
<tr>
<td>Negative/positive</td>
<td>5</td>
<td>0 (0%)</td>
<td>1 (20%)</td>
</tr>
<tr>
<td>Total</td>
<td>32</td>
<td>10</td>
<td>7</td>
</tr>
</tbody>
</table>

most sensitive (95%), but because many of the patients with positive urine cultures were asymptomatic, it was not predictive of symptomatic infection. Conventional blood cultures were relatively insensitive in detecting overall CMV infection (58%), but as has been the experience elsewhere, such viremic patients were usually symptomatic. Thus conventional blood culture had a sensitivity of 90% and a specificity of 91% in the diagnosis of symptomatic CMV. However, both conventional blood (58 ± 5 days after transplant) and urine cultures (65 ± 4 days after transplant) took a long time to become positive, limiting their clinical utility. Although the presence of IgM antibody could be detected at an average of 47 ± 3 days, this test was relatively insensitive, being detected in only about 58% of patients with other laboratory evidence of CMV. Both the SV technique and the CMV-AG test increased the ability to detect evidence of virus in the blood. The CMV-AG test was, however, more sensitive (89%) than the SV test (84%) in detecting CMV infection. Furthermore, in symptomatic patients, the CMV-AG count usually started low and then rose to a peak that corresponded to the symptomatic phase (Table 5), subsequently falling to low levels again as symptoms resolved. Most of the positive CMV-AG tests that were not accompanied by positive blood cultures showed low counts and were drawn in the pre- or postsymptomatic phases. Another cause of negative cultures occurring at relatively high CMV-AG counts was the use of antiviral therapy.
Table 4. Sensitivity (Sens), and specificity (Spec), positive predictive value (PPV), and negative predictive value (NPV) of various tests in the diagnosis of symptomatic and asymptomatic CMV infection after transplant

<table>
<thead>
<tr>
<th>Test</th>
<th>All CMV Infection</th>
<th>Symptomatic CMV Infection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sens</td>
<td>Spec</td>
</tr>
<tr>
<td>Urine culture</td>
<td>0.95</td>
<td>1</td>
</tr>
<tr>
<td>Blood culture—conventional</td>
<td>0.58</td>
<td>1</td>
</tr>
<tr>
<td>Blood culture—shell vial</td>
<td>0.84</td>
<td>0.92</td>
</tr>
<tr>
<td>IgM antibody</td>
<td>0.58</td>
<td>1</td>
</tr>
<tr>
<td>CMV-AG positive</td>
<td>0.89</td>
<td>0.69</td>
</tr>
<tr>
<td>CMV-AG &gt;10</td>
<td>0.58</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 5. Patients experiencing symptomatic episodes of CMV infection: symptoms and CMV-AG counts before (PRE) and after (INITIAL) the onset of symptoms, as well as the highest CMV-AG count (PEAK) reached

<table>
<thead>
<tr>
<th>Patient</th>
<th>Episode</th>
<th>Symptoms</th>
<th>Pre-CMV-AG</th>
<th>Initial CMV-AG</th>
<th>Peak CMV-AG</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>Fever, myalgias</td>
<td>14</td>
<td>34</td>
<td>34</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>Fever, myalgias</td>
<td>0</td>
<td>48</td>
<td>48</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>Fever, myalgias, fatigue, hepatitis</td>
<td>10</td>
<td>65</td>
<td>71</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>Fever, myalgias, fatigue, hepatitis</td>
<td>3</td>
<td>24</td>
<td>74</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>Fever, fatigue, diarrhea, hepatitis, retinitis</td>
<td>13</td>
<td>1722</td>
<td>1722</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>Fever, myalgias</td>
<td>5</td>
<td>119</td>
<td>119</td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>Fever, hepatitis</td>
<td>0</td>
<td>29</td>
<td>105</td>
</tr>
<tr>
<td>8</td>
<td>1</td>
<td>Fever, myalgias</td>
<td>0</td>
<td>54</td>
<td>150</td>
</tr>
<tr>
<td>9</td>
<td>1</td>
<td>Fever, myalgias, hepatitis, retinitis</td>
<td>35</td>
<td>74</td>
<td>183</td>
</tr>
<tr>
<td>10</td>
<td>1</td>
<td>Hepatitis</td>
<td>67</td>
<td>186</td>
<td>186</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td></td>
<td>12</td>
<td>211</td>
<td>328</td>
</tr>
<tr>
<td></td>
<td>SEM</td>
<td></td>
<td>±8</td>
<td>±169</td>
<td>±173</td>
</tr>
</tbody>
</table>

In cases in which both SV and CMV-AG tests were positive, the CMV-AG was detected on average at 39 ± 5 days after transplant, compared with 42 ± 2 days for the SV test. Most of this difference resulted from the longer processing time required for the culture process because in 14 of the 16 cases, the initial positive samples for both tests were drawn on the same day. In one case, the first positive CMV-AG was obtained on a sample drawn 10 days before the first positive culture, whereas in the remaining case a positive culture specimen was obtained 7 days before the first sample showing antigenemia. Both tests were relatively nonspecific in diagnosing symptomatic CMV infection because they were positive in a significant number of asymptomatic individuals. We therefore attempted to see if the quantitative nature of the CMV-AG test could be used to increase specificity by the use of different threshold values. We found that a CMV-AG count of >10 was both highly sensitive and specific for the diagnosis of symptomatic infection.

All patients who developed symptomatic infection developed a CMV-AG count of >10 during that bout of infection. Indeed, as shown in Table 5, the initial CMV-AG count drawn after the onset of symptoms was >10 in nine of the ten patients, and in the final patient (Patient No. 9), in whom it was 6, it rose to >10 shortly thereafter. In contrast, the test was specific in so far that no patient whose CMV-AG count remained persistently less than 10 developed symptoms of CMV infection. However, not all individual CMV-AG counts >10 were associated with symptoms. Indeed only 20 of 72 (mean value, 274 ± 94) were drawn on days when patients were actually symptomatic. Another 33 (mean value, 85 ± 32) were obtained during antiviral therapy after clinical symptoms had resolved. Seventeen (mean value, 10 ± 50) developed after completion of a course of antiviral therapy and represented asymptomatic recurrence of antigenemia. It was interesting to note that in the only patient who had recurrent symptomatic infection (Patient No. 8), symptoms appeared to develop at a higher CMV-AG level than during the initial infection. The last two CMV-AG samples not associated with symptoms (values of 14 and 34, respectively) were obtained in patient No. 1 before the onset of symptoms.
Finally, Figure 2 shows that although the peak CMV-AG tended to be higher in patients with CMV disease manifestations, it did not appear to clearly differentiate between these groups.

Comparison of Utility of Various Diagnostic Tests as Guides to Instituting Preemptive Therapy

The utility of these various tests as guides to instituting preemptive antiviral therapy was also assessed. Table 6 compares the number of patients who would have been identified for empiric therapy by a number of potential parameters, as well as the time after transplant at which they would have been identified by that particular test. Positive urine and blood cultures could not be used because positive results were not detected until—on average—14 and 15 days, respectively, after the onset of clinical symptoms. The presence of IgM antibody was detected in only seven of ten symptomatic patients at an average of 2 days after onset of symptoms. Both a positive SV test or a positive CMV-AG test could be detected before onset of clinical symptoms in many cases (SV, four of ten; CMV-AG, six of ten), respectively. However, use of either of these criteria would have led to institution of preemptive therapy in a considerable number of patients who never subsequently developed symptoms (SV, six; CMV-AG, 11). The quantitative nature of the CMV-AG test allows one to select different threshold values as a trigger for preemptive therapy. Raising the threshold of intervention to 10 positive cells per 50,000 PBL would have resulted in treatment of all ten symptomatic patients with therapy instituted an average of 8 days before onset of symptoms (range, 46 days before to 16 days after initial detection of unexplained fever).

Discussion

Diagnosis and treatment of CMV infection has traditionally been hampered by the lack of suitable tests permitting prompt and accurate diagnosis. In the last few years, a number of newer diagnostic tests have been introduced, which permit the diagnosis of CMV infection to be made more rapidly and with greater sensitivity. The SV technique (6) uses monoclonal antibodies to early viral antigens to detect the presence of such viral products in cell culture preparations as early as 16 h after inoculation. The CMV antigen test (7) uses similar technology to detect early viral antigens directly in circulating peripheral blood leukocytes of the patient. Finally, the polymerase chain reaction can be used to detect CMV DNA in the plasma or leukocytes of the patient (8). In many cases, these tests are so sensitive that they can detect the presence of viremia even before the onset of symptoms (11). This has opened the possibility of preemptive therapy of posttransplant CMV infection in the presymptomatic phase. Unlike prophylaxis, which is administered to all patients beginning at the time of or shortly after transplantation, preemptive therapy can be restricted to those patients exhibiting early evidence of infection and is timed to coincide with the period of early viral replication. In this way, it is hoped that such treatment will shorten the duration of infection and prevent hospitalization while limiting widespread use of antivirals that could encourage resistance. This study was designed to assess the utility of serial monitoring of renal transplant recipients with both the CMV antigen test and the SV culture technique and to compare their effectiveness in diagnosis of symptomatic CMV infection. A secondary goal was to determine if either technique could be used to detect presymptomatic infection and thus guide preemptive therapy.
institution of antiviral therapy before serious complications such as pneumonia and retinitis ensued.

CMV antigenemia was detected in a high percentage (76%) of renal transplant recipients in this study. As shown in Figure 1, CMV-AG was usually first detected 4 to 8 wk after transplant, which is in agreement with the known natural history of posttransplant CMV infection and previous reports of other investigators (1). The incidence of positive SV tests (50%) and positive conventional cultures (33%) was much lower. The difference could be explained either if the CMV-AG test was more sensitive than the other two tests in detecting circulating virus at a lower level of viral burden or, alternatively, if it was less specific, with low counts being detected in the absence of virus. Without an independent reference standard, it is impossible to resolve this issue definitively, but we believe that the discrepancy is due to the greater sensitivity of the CMV-AG test for the following reasons: First, the higher the CMV-AG count, the more likely it was that the cultures were positive, suggesting that a positive culture requires a higher viral load. Second, many of the positive CMV-AG tests that were not accompanied by positive blood cultures were low counts seen before the onset of clinical symptoms or during the recovery phase after the resolution of clinical illness. Finally, positive CMV-AG tests were extremely rare in seronegative recipients of a kidney from a seronegative donor. Of the 86 CMV-AG tests done in this group, only five were positive, all with very low counts (≤1/50,000 PBls). These five positives occurred in three patients who did not exhibit any other positive laboratory tests for CMV. Although we cannot rule out inadvertent exposure, these patients were at low risk of CMV, and together with the low counts and lack of symptoms these tests probably represent false positives. Such false positives were identified only during the period when the peroxidase technique was in use and may have been the result of interference by endogenous peroxidase activity. This was one reason why we switched to the immunofluorescence technique for CMV-AG detection, which we feel carries an even greater specificity. In doing this, our experience was similar to that of Landry and Ferguson (12). Finally, this data suggests that the incidence of CMV infection, symptomatic or asymptomatic, in the group of seronegative-seronegative transplants is too low to warrant prophylactic monitoring.

The CMV-AG test proved sensitive in detecting posttransplant CMV infection (89%) compared with urine culture (95%), SV blood culture (84%) or development of a new IgM antibody (58%). Only ten of the 21 patients with laboratory evidence of infection developed symptoms, and all ten were positive by CMV-AG, and urine and blood culture, although only seven (70%) developed detectable IgM antibody, suggesting that the latter assay is not sufficiently sensitive for diagnosis of posttransplant CMV infection. Positive CMV-AG counts were detected in the majority of asymptomatic patients (nine of 11), albeit at low levels, whereas positive SV blood (six patients) and conventional urine cultures (nine patients) were also not uncommon, reducing the specificity of these tests in confirming the diagnosis of symptomatic CMV. Conventional blood cultures again proved to be highly specific for symptomatic CMV, but unfortunately the long delay in obtaining the results reduces their clinical utility. Although the CMV-AG was more sensitive than the blood culture by SV technique, most of this enhanced sensitivity consisted in the detection of asymptomatic cases. However, as in our previous study (9), the quantitative nature of the CMV-AG test proved helpful in differentiating symptomatic from asymptomatic illness in that only patients with CMV-AG counts of >10 were symptomatic.

Previous studies have compared the CMV-AG test with the SV technique as early detectors of posttransplant CMV infection. Wunderli et al. (13) prospectively screened 104 renal transplant recipients and found evidence of CMV infection in 40. They detected antigenemia in only 25%, whereas SV cultures were positive in almost 70%, and they suggested that a combination of these tests was required for optimal diagnosis. However, most other studies that have compared the CMV-AG test and SV cultures have found the detection of antigenemia not only to be more sensitive than SV cultures but have also found that CMV-AG can be detected, on average, 1 week earlier. Erice et al. (14) found the CMV-AG test to be

### Table 6. Utility of various tests in guiding the institution of preemptive therapy for CMV infection

<table>
<thead>
<tr>
<th>Factor</th>
<th>CMV-AG &gt;0</th>
<th>CMV-AG &gt;10</th>
<th>CMV-AG &gt;50</th>
<th>Positive Blood Culture (Shell Vial)</th>
<th>Positive Blood Culture (Conventional)</th>
<th>Positive Urine Culture</th>
<th>Positive IgM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients identified</td>
<td>21</td>
<td>11</td>
<td>8</td>
<td>16</td>
<td>11</td>
<td>18</td>
<td>11</td>
</tr>
<tr>
<td>Number symptomatic</td>
<td>10</td>
<td>10</td>
<td>8</td>
<td>10</td>
<td>9</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>Days after transplant</td>
<td>42</td>
<td>46</td>
<td>43</td>
<td>42</td>
<td>58</td>
<td>72</td>
<td>47</td>
</tr>
<tr>
<td>Days before (−) or after (+) symptoms</td>
<td>−8</td>
<td>−8</td>
<td>−3</td>
<td>−6</td>
<td>+14</td>
<td>+15</td>
<td>+7</td>
</tr>
</tbody>
</table>
more sensitive than the SV assay in 170 recipients of solid
organ transplants. Boeckh et al. (15), in a study of 59 sero-
positive bone marrow transplant recipients, found that the
CMV-AG test carried a sensitivity of 95% in diagnosing post-
transplant CMV infection and was detectable an average of 8
days prior to shell vial culture. As outlined above, we did not
detect any difference in the time to the first positive SV result
versus the first positive CMV-AG result, although the longer
length of time between screening samples that we utilized (14
versus 7 days) may have accounted for our failure to detect
CMV-AG at an earlier time. We chose 2 weeks for logistical
reasons and to permit long-term (6-month) follow-up. How-
ever, in light of our experience in this study, we now favor
testing at least weekly, particularly during the highest risk
period (4 to 8 wk after transplant). On the other hand, if no
evidence of antigenemia develops by 12 weeks, subsequent
CMV infection is unlikely and surveillance antigen testing is
no longer cost-effective.

We also found that a CMV-AG level >10 was specific for
the development of symptomatic infection in that all patients
who developed a CMV-AG of greater than 10 either were
symptomatic at the time or developed symptoms shortly there-
after, usually within one week. On the other hand patients
whose counts remained persistently below 10 remained asym-
ptomatic. As shown in Table 5, symptoms tended to develop
between 12 and 211, the respective mean CMV-AG counts
immediately before and after the onset symptoms. The
CMV-AG count then rises to a peak that usually corresponds
to the time of maximum symptomatology. The peak achieved
tended to be higher in patients with disease manifestations, but
as shown in Figure 2 the CMV-AG count alone could not be
used to differentiate between patients with disease and the viral
syndrome alone. However, the number of patients with disease
was small, and the duration of disease manifestations short.
This may have been the result of the use of prophylactic
CYTOGAM® in high-risk positive-to-negative transplants, to-
gether with our policy of vigorous early therapy. Indeed, no
mortality was seen in any patient with symptomatic CMV
infection, and graft outcome was excellent (Table 7).

With antiviral therapy, the classic symptoms (fever, chills,
sweats) and signs (transaminasemia) often resolved rapidly
within a few days even in the presence of persistent antigen-
emia of >10, and although some patients exhibited a dramatic

Table 7. Impact of CMV status on patient and graft survival

<table>
<thead>
<tr>
<th>CMV Infection</th>
<th>Peak CMV-AG</th>
<th>1-Yr Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Rejection</td>
</tr>
<tr>
<td>None (n = 11)</td>
<td>0</td>
<td>54%</td>
</tr>
<tr>
<td>Asymptomatic</td>
<td>4 ± 1</td>
<td>36%</td>
</tr>
<tr>
<td>(n = 11)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Symptomatic</td>
<td>357 ± 180</td>
<td>10%</td>
</tr>
<tr>
<td>(n = 10)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
possible, then our study would suggest that a suitable threshold might be a CMV-AG count >10. Use of this threshold would have resulted in earlier institution of therapy by approximately 4 days in the eight patients actually treated, and the two additional patients would have qualified for therapy. Both subsequently developed mild clinical symptoms and their CMV-AG counts peaked at 34 and 48, respectively, but then fell spontaneously to low levels as symptoms resolved. Use of a positive blood culture (SV) as the criterion would have resulted in treatment of an additional eight patients, six of whom were never symptomatic. Conventional blood and urine culture results were, in our opinion, obtained too late to be useful for guiding preemptive therapy. IgM antibodies were not seen in up to 30% of symptomatic patients, making it too insensitive for guiding preemptive therapy.

One problem with this study is the fact that we did not do a concurrent evaluation of detection of viral DNA in peripheral blood leukocytes by polymerase chain reaction (PCR). Furthermore, there is evidence that PCR techniques are even more sensitive and can detect evidence of CMV infection even earlier than the antigenemia test (11). However, PCR techniques remain poorly standardized and most are nonquantitative. Their very high sensitivity means that they are, therefore, more likely than the antigen test to identify infected patients who are not destined to become symptomatic, but prospective studies will be required to definitively resolve this issue.

The test as performed in our laboratory is not available commercially nor have we compared it directly with the Food and Drug Administration-approved kits currently available. Standardization thus remains a concern with the CMV-AG test, and it is not clear whether our results can be transferrred to other laboratories. However, our findings and the threshold for development of symptoms are similar to those reported by two other groups (11,17), and we were impressed by the correspondence between the values assayed simultaneously by the immunoperoxidase and immunofluorescent techniques. Finally, there is the question of cost. The test is somewhat labor-intensive, but we estimate that the test, including material and labor costs, can be done for approximately $40. Thus three months of weekly profiling would cost approximately $500 but might be cost-effective if they contributed to less need for blanket prophylaxis and, in particular, less hospitalization for CMV infection.

In conclusion, we followed-up renal transplant recipients prospectively with biweekly determinations of CMV-AG counts from peripheral blood samples. We confirmed our previous findings that the CMV-AG test was sensitive in detecting evidence of CMV infection and that the degree of antigenemia as expressed by quantification of the number of positive cells per 50,000 PBLs correlated both with the likelihood and degree of symptomatic infection. Thus all patients with a cell count >10/50,000 developed clinical manifestations of infection, and we suggest that this threshold could be useful in deciding clinically whether fever is related to CMV infection. This level of antigenemia could also be used as a threshold for instituting antiviral therapy, because it was often reached prior to the onset of symptoms and had a high specificity for symptomatic infection, thus avoiding unnecessary treatment of asymptomatic patients. We believe that the CMV-AG test is superior to the SV assay because the actual count seen helps determine the likelihood that symptoms are the result of the virus, the processing time is longer (48 to 72 h) for the SV, and use of the SV as a guide to preemptive therapy would result in the treatment of more asymptomatic patients.

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References

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