Cytomegalovirus (CMV) Virus Load Kinetics to Predict Recurrent Disease in Solid-Organ Transplant Patients with CMV Disease

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Despite standard therapy, cytomegalovirus (CMV) disease recurs in a significant proportion of organ transplant recipients. The kinetics of CMV load in response to therapy may allow early prediction of recurrence. CMV loads were obtained at regular intervals after starting ganciclovir therapy in 52 transplant recipients with CMV disease. Virus load kinetics were analyzed using decay curves to assess viral dynamics, including half-life and time to viral clearance. Recurrent CMV disease occurred in 12 (23%) of 50 patients. The time period to viral clearance was longer among patients with recurrence of CMV disease (P = .002), and lack of clearance was also associated among patients with disease recurrence (P < .001). Viral kinetics followed a logarithmic decay curve expressed by the equation \( y = y_0 e^{-kt} \). Virus load half-life was 8.8 days among patients with recurrence versus 3.17 days among patients without recurrence (P = .001). This was not explainable by detectable ganciclovir resistance. CMV load kinetics are useful for identifying, at a very early stage, patients who are more likely to have recurrence.

Cytomegalovirus (CMV) disease continues to remain an important cause of morbidity among solid-organ transplant recipients. Symptomatic CMV disease is generally treated with a 2–4 week course of intravenous ganciclovir. However, the risk of recurrent CMV is estimated to be 25%–30% [1–3]. The optimal duration of antiviral treatment for CMV disease is difficult to determine in individual patients, because the risk of relapse must be weighed against the toxicity and expense of antiviral therapy. Factors such as pretransplant donor (D) and recipient (R) CMV serostatus and intensity of immunosuppression, specifically use of antilymphocyte therapy, may assist in determining the likelihood of relapse [1, 2]. More recently, however, testing of CMV load has been proposed as a useful tool for monitoring response to therapy [4, 5]. High virus loads at the onset of disease and persistent detectable viral DNA at the end of therapy may be useful markers for determining which patients are at a higher risk of relapse [5].

Recently, the application of virus load kinetics (i.e., rate of change in virus load) has been found to be a useful early predictor of patients at imminent risk of developing CMV disease [6]. Patients who have a faster rate of increase in virus load have been shown to be at a higher risk of developing subsequent CMV disease. Similarly, we hypothesized that the use of virus load kinetics among patients who commence treatment for CMV disease may allow for very early identification of patients most likely to relapse. In this setting, the rate of decline in virus load with the commencement of therapy may predict relapse and therefore may help guide clinicians to the optimal duration of antiviral therapy. In the present study, we prospectively assessed the use of virus load kinetics for predicted relapse of CMV disease among solid-organ transplant recipients following antiviral treatment.

Patients and Methods

Patients, study design, and definitions. From July 1997 to July 2001, solid-organ (liver, kidney, heart, and lung) transplant recipients with symptomatic CMV disease were eligible for inclusion in the study. Immunosuppression protocols and ganciclovir prophyl-
Ganciclovir resistance studies were performed among patients who relapsed. This was done by assessing changes in the viral load at onset of CMV disease relapse, and failure to clear DNAemia was based on last available virus load measurement. Decay curves for CMV load clearance and best-fit lines were determined by plotting virus loads versus time using Excel 2000 (Microsoft).

**Laboratory testing.** Virus load testing was done on plasma samples using the Roche Cobas Amplicor system using methods described elsewhere [7]. Results were recorded in log_{10} copies/mL.

Results

Fifty-two patients with CMV disease were enrolled. Organ transplant types included liver (n = 35), kidney (n = 7), lung (n = 7), and other (n = 3). CMV disease presentations were viral syndrome (n = 38), colitis (n = 6), hepatitis (n = 4), and pneumonitis (n = 4). All patients had resolution of symptoms by the end of their induction course of intravenous ganciclovir.

Recurrence of CMV disease occurred in 12 (23.1%) of 52 patients. Recurrence occurred at a mean of 60 days from the onset of the first episode (median, 40 days; range, 24–150 days). The type of CMV disease relapse included viral syndrome (n = 9), pneumonitis (n = 1), colitis (n = 1), and retinitis (n = 1). Type of transplant, D/R, CMV serostatus pretransplant, and previous antilymphocyte globulin therapy were not significant risk factors for recurrent CMV (table 1). Relapse occurred in 3 (21.4%) of 14 patients with tissue invasive disease versus relapse occurring among 9 (23.7%) of 38 patients with viral syndrome (P, not significant).

**Virus load and recurrence.** CMV load at onset of CMV disease was 4.73 log_{10} copies/mL among patients with recurrence versus 4.82 log_{10} copies/mL among patients without recurrence (P, not significant) (table 1). Failure to clear CMV DNAemia during initial treatment occurred in 8 (15.4%) of 52 patients. Lack of viral clearance with initial treatment was significantly associated with recurrence (P < .001). Among patients who did clear CMV, the mean time to viral clearance was significantly longer among patients with recurrence versus those without (mean, 33.8 vs. 17.2 days, respectively; P = .002) (table 1). Of note, some patients cleared CMV even after completion of induction therapy. Virus loads in these patients were usually slightly above the lower limit of detection of the assay at the time of completion of induction therapy.

<table>
<thead>
<tr>
<th>Table 1. Risk factors for cytomegalovirus (CMV) disease relapse in 52 patients.</th>
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<tr>
<td><strong>Factor</strong></td>
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<tr>
<td>Virus load at onset, log_{10} copies/mL</td>
</tr>
<tr>
<td>Time to clear CMV, days</td>
</tr>
<tr>
<td>Virus load half-life, days</td>
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<tr>
<td>Virus load cleared (%)</td>
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<tr>
<td>Received steroid bolus for rejection (%)</td>
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<tr>
<td>D/R CMV serostatus pretransplant (%)</td>
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<td>Received antilymphocyte globulin therapy (%)</td>
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NOTE: Data are mean ± SD (median; range) for “virus load at onset,” “time to clear CMV,” and “virus load half-life.” NS, not significant.

a Time to clearance calculated only for subgroup of patients who cleared CMV load (i.e., 44 patients total).

b D/R, donor positive/recipient negative χ^2 or Fisher's exact tests were used for categorical variables and Mann-Whitney U tests for continuous variables.
**Virus load kinetics.** The kinetics of virus load clearance were determined by plotting virus loads versus time, where 0 was the start of ganciclovir therapy (figure 1). Best-fit lines were used to derive equations describing the decay curve. Virus load kinetics followed a logarithmic decay curve in the majority of patients, expressed by the equation \( y = y_0 e^{-ax} \), where \( y_0 \) is the initial virus load, \( x \) is time from start of treatment, and \( a \) is the decay constant [4]. Virus load half-life was then calculated using the equation \((\ln 2)/a\). In the entire cohort of 52 patients, virus load half-life was only 4.5 days (median, 2.5 days; range, 0.72–19.7 days), reflecting a relatively rapid clearance of virus with the commencement of therapy. However the mean half-life was significantly longer among patients with CMV disease recurrence (mean, 8.8 days; median, 6.6 days; range, 1.2–19.7 days) versus only 3.17 days (median, 1.94 days; range, 0.72–18.2 days) among patients without recurrence \((P = .001)\) (table 1). The mean half-life was not significantly different among patients with tissue invasive disease versus viral syndrome (4.72 vs. 4.39 days, respectively; \(P\), not significant) and among patients who had received previous ganciclovir prophylaxis versus those who had not (4.67 vs. 4.12 days, respectively; \(P\), not significant). Risk of recurrence for individual patients could be determined on the basis of half-life or virus load. For example, patients with a viral half-life of \(\approx 3\) days had only a 6.7% chance of recurrence, whereas patients with a half-life of \(\approx 7\) days had a 55.7% chance of recurrence. Similarly, patients who achieved a 1-log reduction (10-fold) in virus load by day 7 had only an 8.7% chance of recurrence versus 34.5% in those that did not achieve such a reduction.

**Resistance testing.** Isolates from patients with recurrent CMV disease were tested for UL97 gene mutations to detect ganciclovir resistance. Mixed virus populations (mutant and wild type) were detected in 1 patient. This patient had a UL97 mutation conferring ganciclovir resistance detected (Leu595Phe). At the time of relapse, this patient did not respond clinically to ganciclovir and was treated with foscarnet. No other significant mutations were detected, and the remaining patients all responded clinically to ganciclovir at the time of their relapse.

**Discussion**

We have shown that virus load measurements are useful for monitoring response to therapy among patients with CMV disease. Delays in clearance of virus and failure to clear virus are important predictors of relapse. However, more interesting is the novel finding that virus load kinetics in the first few days of treatment is predictive of relapse. Patients who did
not relapse had a very short half-life (mean, 3.17 days), whereas patients who did relapse had a significantly longer half-life (mean, 8.8 days). Therefore, using virus load to assess initial response to therapy has practical implications for patient treatment by allowing clinicians to identify very early which patients require more prolonged antiviral therapy to prevent recurrences. For example, a virus load could be measured on day 7 of treatment. From our data, a patient who reduced their virus load by $\geq 1\log_{10}$ (10-fold reduction) would have only an 8.7% chance of recurrence. However, a patient without such a reduction would have a 34.5% risk of recurrence. Similarly, a patient who did not have at least a 2-fold reduction ($0.3\log_{10}$) in virus load by day 7 of treatment would have a 56% risk of relapse. Measurement of weekly CMV loads is common practice at many centers and represents a clinically reasonable time frame in which to repeat a virus load. These patients could then be targeted for longer-term or perhaps combination antiviral therapy, along with a more intensive reduction in immunosuppression.

Differing virus load kinetics were not explainable by detectable ganciclovir resistance. Genotypic resistance testing among patients who relapsed demonstrated only a single UL97 mutation conferring ganciclovir resistance. It is possible that some cases of resistance were not detected because the RFLP method we used will only detect the most common UL97 mutations and because we did not assess for UL54 mutations. However, in their first episode of CMV disease, all patients responded clinically to ganciclovir. At the time of relapse, all patients except 1 responded to ganciclovir (the patient with the confirmed ganciclovir resistance responded only to foscarnet). More likely, differences in rates of viral clearance reflect differing degrees of host immune responses to CMV. Although no observable difference in the degree of exogenous immunosuppression were seen, this is a crude measure of overall immunity to CMV. Measurement of anti-CMV T cell populations may provide more accurate information about host immunity to CMV, but it is not as readily available as virus load measurements [3].

Clinical characteristics of patients have been assessed for their utility for predicting recurrence. Specific risk factors for relapse have included D/R pretransplant serostatus, use of more potent immunosuppression, and presence of tissue invasive disease [1–3, 5]. We did not find that CMV D/R status was a risk factor for relapse, but this may, in part, be because of the relatively small sample size. Also, studies analyzing clinical risk factors for relapse have been conflicting and have identified different characteristics of significance. Different patient populations and immunosuppressive protocols may account for this. A reproducible, commercially available measurement such as CMV load may represent a clinically more useful alternative to identifying patients most likely to relapse. A limitation of our study was that treatment duration for CMV disease was not standardized and was left up to the treating physician. However, this should not affect the early phase of viral clearance and most accurately reflects clinical practice.

CMV load measurements have been recognized as being useful for both predicting CMV disease and following response to therapy [5, 10, 11]. Currently, it has become standard of care at many centers to treat patients with CMV disease until the virus load becomes undetectable. Sia et al. [5] assessed virus load at baseline and after 2 weeks of ganciclovir treatment in 24 patients with CMV disease. They demonstrated that persistent viral DNA at the end of therapy was an important risk factor for recurrence. Unlike our study, however, they found that pretreatment virus load, when measured in leukocytes (but not in plasma) was also predictive of recurrence. Recently, Emery et al. [6], studied virus load kinetics and found that the rate of change in virus loads in the early phases of CMV replication was also useful for predicting the first episode of CMV disease (i.e., patients who had a faster rate of increase in virus load were at higher risk of developing CMV disease). Roberts et al. [4] were the first to assess virus load kinetics in response to treatment in 6 transplant patients with CMV disease. Kinetics in these 6 patients also followed an exponential decay curve with a mean half-life of 3.3 days. We have confirmed that virus load kinetics do indeed follow an exponential decay curve and have also found that they provide an early predictor of CMV disease relapse.

Assessment of early changes in virus load may also be useful for assessing new anti-CMV therapies. Response to therapy could be determined early in the treatment course, allowing for rescue therapy with standard antivirals among patients who are failing. In summary, virus load kinetics provide a practical and useful early predictor of relapse and can help guide intensity and duration of antiviral therapy.

References


