Late Cytomegalovirus Disease in Marrow Transplantation Is Predicted by Virus Load in Plasma


Late occurrence of cytomegalovirus (CMV) disease after day 100 after bone marrow transplantation has become an increasing problem: whether a quantitative measurement of CMV DNA in plasma by polymerase chain reaction (P-PCR) could be predictive of such disease was investigated. In a prospective study, 117 subjects undergoing allogeneic marrow transplantation were followed for 120 days with weekly CMV blood cultures, with day 35 bronchoalveolar lavage CMV cultures, with weekly CMV P-PCR, and with clinical follow-up for an additional 1–2 years. Despite preemptive ganciclovir, CMV disease occurred in 9% of subjects, with a median time of onset of 176 days. Quantitative CMV P-PCR was associated with the late development of CMV disease \( (P = .01) \). Of 43 subjects with positive P-PCR results, 23% developed CMV disease, but no disease occurred in the 74 subjects with negative P-PCR \( (P < .001) \), despite the fact that 22% had CMV isolated from lung lavage fluid and 32% had CMV isolated from blood.

Risk factors for the occurrence of human cytomegalovirus (CMV)–related morbidity after allogeneic bone marrow transplantation (BMT) can be used to guide antiviral therapy [1–5]. The most important of these factors is the occurrence of asymptomatic CMV reactivation in blood or lung [2], and infection of urine or throat has not been as predictive of later disease [1, 2]. In addition, the occurrence of graft-versus-host disease (GVHD), and other factors predisposing to this, such as age >20 years and HLA mismatch of donor and recipient, are important risks for CMV infection and disease [6].

With the ability to determine CMV DNA in plasma by polymerase chain reaction (PCR) [7, 8], the concentration of viral DNA can be expressed as genome copies per milliliter and can be an indication of CMV virus load. It is not clear whether the amount of measurable CMV DNA in blood after BMT is predictive of disease. In human immunodeficiency virus–infected persons, however, it has been reported that the CMV DNA concentration is significantly higher in plasma in those who eventually develop CMV retinitis [9] and in cerebrospinal fluid in those who develop CMV-related polyradiculopathy [10]. The quantity of CMV DNA in bronchoalveolar cells correlates with the presence of pneumonitis in immunosuppressed persons with CMV pulmonary infection [11]. In allogeneic BMT, the presence of CMV DNA in plasma has been associated with risk for disease [3, 4, 12] and pulmonary CMV infection [13], but the significance of the quantity of CMV DNA in plasma has not been studied. We evaluated whether quantitative plasma PCR is predictive of late CMV disease.

Methods

Study population. During 1993–1994, 117 consecutive CMV-seropositive recipients of allogeneic BMT for hematologic malignancy or severe aplastic anemia were followed for the occurrence of CMV infection and disease in a prospective study. CMV infection was determined by twice-weekly blood cultures between days 28 and 56 (day 0 = day of marrow infusion) and weekly blood cultures until day 120; by plasma PCR (P-PCR) for CMV DNA on these same blood specimens, and by bronchoalveolar lavage (BAL) on day 35 after transplant. After day 120, CMV assays were performed only on patients with clinical indications suggestive of possible CMV infection.

Ganciclovir was given preemptively, as previously described [14], to a total of 71 subjects (62%), based on CMV culture positivity in day 35 BAL fluid (collected prospectively on all subjects), on two consecutive positive blood cultures prior to day 35, or on a single positive blood culture after day 35 but before day 120. No clinical decision was made on the basis of CMV P-PCR result. Thirty-six patients (31%) were treated because BAL fluid was positive for CMV; among the 81 BAL fluid CMV-negative subjects, an additional 35 were treated with ganciclovir for positive CMV blood cultures. The 117 subjects analyzed had a median number of plasma PCR tests/subject of 9, with a range of 2 to 37 samples/subject, and all subjects had at least 1 assay at or near the onset of disease (1 patient had 2 tests, 1 had 3 tests, and all the others had ≥4 tests). CMV disease was diagnosed at City of Hope as defined by the presence of CMV in patients showing evidence of pneumonitis or gastroenteritis [14].

Blood and BAL fluid culture. Blood leukocytes and BAL fluid were cultured as previously described on MRC-5 shell vials [1,
Table 1. Relationship of CMV genome copies in plasma to CMV infection and disease after marrow transplantation.

<table>
<thead>
<tr>
<th>PCR result</th>
<th>Negative (n = 74)</th>
<th>&lt;10^4 copies/mL (n = 27)</th>
<th>≥10^6 copies/mL (n = 16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time to positive PCR, days</td>
<td>—</td>
<td>34* (21–83)</td>
<td>41* (21–144)</td>
</tr>
<tr>
<td>CMV-positive in BAL fluid</td>
<td>16 (22%)</td>
<td>16 (59%)</td>
<td>4 (25%)</td>
</tr>
<tr>
<td>CMV-positive blood culture</td>
<td>7</td>
<td>11</td>
<td>4</td>
</tr>
<tr>
<td>CMV disease</td>
<td>0</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>CMV-negative in BAL fluid</td>
<td>58 (78%)</td>
<td>11 (41%)</td>
<td>12 (75%)</td>
</tr>
<tr>
<td>CMV-positive blood culture</td>
<td>17</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>CMV disease</td>
<td>0</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>Total CMV disease</td>
<td>0</td>
<td>3 (11%)</td>
<td>7 (44%)</td>
</tr>
</tbody>
</table>

NOTE. PCR, polymerase chain reaction. BAL, bronchoalveolar lavage. Data are no. of subjects (%).
* Significantly different by Wilcoxon rank sum test: P = .04.
† Significantly different by Fisher’s exact test: P = .02.

Results

Occurrence of CMV disease. CMV disease occurred in 10 (23%) of 43 PCR-positive marrow transplant recipients and none of 74 PCR-negative subjects (P < .001 by Fisher’s exact test). There were 3 cases of CMV disease in patients positive for CMV in BAL fluids and 7 in patients with negative BAL fluid results (table 1). There were 8 cases of CMV pneumonitis, 1 on day 56 after transplant in a person not qualified to receive preemptive ganciclovir and 7 cases occurring after day 150 (days 156, 171, 181, 182, 345, 408, and 623). Of these late cases, all patients had received preemptive ganciclovir, 3 on the basis of positive result in BAL fluids and 4 on the basis of CMV-positive blood culture. In addition, there were 2 cases of CMV gastroenteritis, both occurring in persons not qualified for early ganciclovir, and the onsets of these diseases were days 50 and 79. The median time to CMV disease was day 176 after transplant. In summary, most CMV pneumonitis occurred late after marrow transplant in persons who had already been treated preemptively with ganciclovir. Some early disease occurred due to failure of culture methods to detect infection, as has been previously described [1, 2].

CMV load and clinical infection. As shown in figure 1A, the CMV P-PCR measurement correlated with the occurrence of disease. When the highest level of CMV DNA was compared for the group with and without CMV disease, there was a significant difference between the median values (6.0 × 10^4 vs. 0.3 × 10^4 genome copies/mL of plasma; P = .01 by Wilcoxon rank sum test). Table 1 shows the relationships between CMV P-PCR results and asymptomatic CMV infection in lung, CMV-positive blood culture, and CMV disease. The median time to first positive PCR result (34 vs. 41 days) was significantly shorter in the group with lower levels of CMV plasma DNA (<10^4 vs. ≥10^6 genome copies/mL of plasma; P = .04 by Wilcoxon rank sum test). The rate of occurrence of CMV-positive result in BAL fluid was significantly higher in those with positive than in those with negative P-PCR results (47% vs. 22%), which confirms a previous observation [13]. There was no disease in the 74 subjects negative by CMV P-PCR, despite the fact that 22% had detectable CMV in BAL fluid on day 35 after transplant and 32% had positive blood cultures. Since it was the trigger for early ganciclovir therapy, progressive CMV infection may have been aborted before it could be detected in the plasma. The group with positive PCR results
and <10^4 genome copies/mL of plasma had a significantly reduced rate of disease compared with that of subjects with 
\( \geq 10^4 \) genome copies of CMV DNA/mL of plasma (11% vs. 44%; \( P = .02 \) by Fisher’s exact test). This result remained the same after adjusting for preemptive ganciclovir treatment by use of the Mantel-Haenszel \( \chi^2 \) test. Interestingly, 21 subjects had a maximum number of genome copies per milliliter before the start of ganciclovir treatment and 19 during treatment. In the 7 subjects with \( \geq 10^4 \) genome copies/mL who eventually became ill, all had CMV pneumonitis or gastroenteritis despite preemptive ganciclovir, at a median time of 181.5 days.

When the results were summarized in a contingency table, with the presence of CMV disease used as the reference standard, the sensitivity of any positive CMV P-PCR result was 100%, with specificity of 69%, positive predictive value of 23%, and negative predictive value of 100%. With a cutoff for CMV P-PCR positivity of \( \geq 10^4 \) genome copies/mL, the sensitivity of the CMV P-PCR assay was 70%, the specificity 73%, the positive predictive value 44%, and the negative predictive value 89%. As noted above, the occurrence of CMV DNA in plasma was significantly associated with eventual CMV disease. However, the rate of positive culture in BAL fluid or in blood was not significantly associated with CMV disease (\( P > .20 \) for both by Fisher’s exact test). The diminished value of these tests for prediction of disease was presumably due to the fact that both tests were used to trigger the start of antiviral therapy, which would decrease disease occurrence.

By use of either a univariate or stepwise logistic regression analysis, the only variable that significantly predicted the probability of developing CMV disease in this group was the number of CMV DNA genome copies per milliliter (\( P = .02 \), by use of a log transformation of the highest number of genome copies) (figure 1B). On the other hand, BAL positivity, blood culture positivity, total amount of preemptive ganciclovir treatment, and occurrence of acute or chronic GVHD were not significantly associated with late CMV disease. The logistic curve does not ascertain the risk of any single subject developing CMV disease when the CMV load is known, but it does illustrate the contribution of increasing CMV burden to the risk of CMV disease occurrence.

**Discussion**

The ability to measure virus load promises to change the way antiviral drug therapy is used. With regard to CMV infection after BMT, in which therapy is already based on risk assignment, there is the potential for virus load determination to further refine this treatment strategy. It has been demonstrated that increased CMV DNA in the plasma of persons with AIDS is associated with a risk for CMV disease of the central nervous system [9, 10]. Studies are in progress to determine whether early treatment based on risk might be a more precise method for prevention of CMV retinitis. In allogeneic BMT, although qualitative PCR assays have been used for patient

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**Figure 1.** CMV load and clinical disease. A, Highest value of CMV DNA in blood (expressed as genome copies/mL of plasma) for allogeneic marrow transplant recipients with and without clinical disease at any time after marrow transplantation. Medians and SEs are shown. There was a significant difference in median value of the highest number of genome copies between patients with and without CMV disease (\( P = .01 \)). B, Predicted probability of developing CMV disease, based on stepwise logistic regression model using log of genome copies/mL of plasma as predictor of CMV disease (\( P = .02 \)). 0 on x axis represents 10,000 genome copies/mL, at which point there is 0.2 probability of developing disease. Equation used is \( E(y|x) = \exp(-1.4164 + 1.1794x)/(1 + \exp[-1.4164 + 1.1794x]), \) where \( x = \log(\text{genome copy number}) \) and \( y = \text{CMV disease}. \)
management [3, 4], there has been no such evidence that quantitative plasma CMV DNA measurements can be used to guide antiviral prophylaxis. More importantly, there is no available risk factor for late-onset CMV disease in this population.

In the group studied here, early ganciclovir use based on blood or pulmonary CMV culture results was associated with disease in 9% of subjects, most of whom became sick late after discontinuation of ganciclovir. Disease occurred significantly more frequently in those with the highest CMV DNA plasma levels, providing further evidence that the ability of the virus to replicate in the host determines pathogenesis of disease. This is the first demonstration that the absolute value of plasma CMV DNA actually correlates with disease in this patient population. Presumably, then, the level of plasma CMV DNA is a surrogate marker for host resistance to infection. Of note, the highest levels of CMV DNA occurred at a median time of 41 days after marrow transplantation, despite the fact that the median time to all CMV disease was day 176. This discrepancy in time between peak CMV DNA in plasma and onset of disease was most likely due to the intervening use of ganciclovir, which cleared detectible infection, and to the recurrence of infection after cessation of therapy. Thus, the plasma CMV DNA level defines patients who not only have an early inabilty to limit CMV infection but also remain at continued high risk for disease despite ganciclovir therapy, presumably because of inability to develop CMV-specific immunity during the interval of treatment.

A mathematical model can be made that indicates the association of this increased CMV burden with the risk of eventual CMV disease. Of interest, in the era of early ganciclovir use, neither CMV blood cultures during days 28–120 after BMT, prospective day 35 BAL fluid CMV culture, nor preemptive ganciclovir treatment itself were predictors for late CMV disease. CMV positivity was detected in blood or BAL fluid in 22%–32% of P-PCR–negative subjects. We attribute these results to the fact that only a positive culture of BAL fluid or blood triggered ganciclovir therapy, reducing the risk of detecting CMV in plasma if the infection was at low levels. More than 40% (see table 1) of the blood culture–positive subjects never had a positive P-PCR test, suggesting that P-PCR might not be sufficiently sensitive for monitoring patients early after transplant. Prospective studies are needed to determine whether monitoring CMV DNA load in plasma could be used for risk assignments to begin or extend ganciclovir or other anti-CMV therapy in hopes of preventing late CMV disease after BMT. As these tests become more readily available, it is possible that guidance of treatment with CMV DNA plasma assays will lead to a more rational use of antiviral prophylaxis in this population.

Acknowledgments
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References