Effect of Cytomegalovirus Infection on 1-Year Mortality Rates Among Recipients of Allogeneic Bone Marrow Transplants

Atul Humar, Serena Wood, Jeff Lipton, Hans Messner, Jacinta Meharchand, Allison McGeer, Kelly MacDonald, and Tony Mazzulli

The effect of cytomegalovirus (CMV) infection on 1-year mortality rates among allogeneic bone marrow transplant recipients who are receiving a standard protocol as prophylaxis for CMV infection is unclear. We determined the risk factors for death within 1 year among 103 bone marrow transplant recipients by performing a multivariate analysis. The results of donor and recipient CMV serologies did not predict 1-year mortality, although there was a trend towards higher mortality among CMV-seropositive recipients who received marrow from seronegative donors (P = .077). Multivariate analysis revealed that the factors independently associated with 1-year mortality were the development of CMV antigenemia (relative risk [RR] = 2.74; confidence interval [CI] = 1.28–5.86), bone marrow transplantation (BMT) from unrelated donors (RR = 3.20; CI = 1.30–7.92), and severe acute graft-versus-host disease (RR = 3.50; CI = 1.50–8.17). Although significant on univariate analysis, advance underlying disease before BMT and the development of active CMV disease after BMT were not independent risk factors. In conclusion, the development of CMV antigenemia after BMT was associated with increased 1-year mortality, while the development of active CMV disease was not. Reactivation of CMV infection may represent a marker of poor immune reconstitution or may contribute to further immunosuppression after BMT.

Allogeneic bone marrow transplantation (BMT) is increasingly being performed for the treatment of hematologic diseases. The development of numerous infectious and noninfectious complications after BMT results in significant mortality despite therapeutic and prophylactic measures. Determination of risk factors that are predictive of increased mortality would help in identifying patients who are candidates for closer monitoring and more aggressive prophylactic or therapeutic interventions.

Disease caused by cytomegalovirus (CMV) has been shown to be a significant cause of morbidity and mortality among bone marrow transplant recipients [1, 2]. The risk of CMV disease has been related to seropositivity for CMV before BMT, the presence of graft-versus-host disease (GVHD), and older age [3, 4]. Pretransplantation CMV serostatus has been correlated with 1-year mortality among liver transplant recipients [5]. Numerous strategies have been used in studies evaluating prophylactic therapy with ganciclovir in bone marrow transplant recipients [6, 7]. Schmidt et al. [6] demonstrated a significant reduction in the incidence of active CMV disease with prophylactic ganciclovir therapy for patients with CMV-positive cultures of bronchoalveolar lavage (BAL) fluid obtained on day 35 after BMT. However, no improvement in survival was demonstrated, possibly because of the adverse effects of ganciclovir. Ganciclovir is currently used as prophylaxis for CMV infection at our institution. However, the overall effect of this intervention on 1-year mortality has not been examined in a multivariate analysis.

Studies examining risk factors for death after BMT have focused on demographic data for recipients (specifically age), the presence of acute GVHD, conditioning and GVHD prophylaxis regimens, and specific underlying disease variables [8–12]. The effects of pretransplantation CMV serostatus and the development of CMV infection and CMV disease after transplantation on 1-year mortality have not been fully elucidated in a multivariate analysis. Although one study demonstrated a correlation between the development of CMV antigenemia and death, the results of this study were confounded by the fact that patients received CMV prophylaxis on the basis of antigenemia assay results [13]. Therefore, we performed a multivariate analysis to evaluate the correlation between pretransplantation CMV serostatus, posttransplantation CMV infection and disease (including CMV antigenemia), and 1-year mortality for a cohort of allogeneic bone marrow transplant recipients who did not receive therapy based on the results of CMV antigenemia assays.

Methods

Consecutive patients undergoing allogeneic BMT at the Princess Margaret Hospital (Toronto) between 1 December 1995
and 5 May 1996 were eligible for inclusion in this study. After informed consent was obtained, specimens for antigenemia assays and blood, urine, and throat cultures for CMV were collected weekly from day 0 to day 100 after BMT. This has been shown to be the peak period for reactivation of CMV infection after BMT [1]. As part of our standard BMT protocol, all CMV-seropositive recipients and all recipients whose donors were CMV seropositive underwent screening bronchoscopy on day 35 to obtain BAL fluid for culture. All patients with BAL fluid positive for CMV were treated prophylactically with intravenous ganciclovir (10 mg/[kg · d] for 2 weeks) and received maintenance therapy (6 mg/[kg · d] for 8 weeks). These patients received blood and blood products that had not been screened for CMV.

Seronegative recipients of seronegative donor marrow did not undergo screening bronchoscopy on day 35 and received only CMV-negative blood and blood products. Patients did not receive immunoglobulin for the prevention of CMV disease. The results of cultures of blood, urine, and throat specimens and the antigenemia assays were not disclosed, and none of the patients received prophylaxis for CMV infection on the basis of the results of these assays. Symptomatic patients with CMV infection or disease (see below for definitions) were treated with ganciclovir or foscarin, with or without immunoglobulin, at the discretion of the primary physician.

Demographic data, results of donor and recipient CMV serologies, disease status at BMT, data on GVHD, and 1-year survival outcomes were gathered prospectively. All tests were performed prospectively on freshly obtained samples. Data on development of active CMV disease were obtained by retrospectively reviewing patients’ charts. Pretransplantation donor and recipient CMV serologies were determined by using the Abbott AxSYM enzyme immunoassay (Abbott Laboratories, Abbott Park, IL). Posttransplantation antigenemia assays and shell vial and tube cultures of throat, urine, blood, and BAL specimens were processed according to standard methods, as previously described [14].

CMV infection was defined as recovery of CMV in any shell vial or tube culture or as a positive antigenemia assay. CMV disease was defined as evidence of CMV infection with associated signs and symptoms. Criteria for the diagnosis of CMV disease were used as previously described [15]. CMV pneumonia was defined as the presence of compatible clinical symptoms and chest radiographic findings, together with the detection of CMV in BAL fluid or a lung biopsy specimen. Gastrointestinal disease was defined as the presence of symptoms and the detection of CMV in biopsy specimens. CMV viral syndrome was defined as unexplained fever in association with leukopenia or thrombocytopenia [16]. Acute GVHD was defined according to current clinical criteria [2].

GVHD prophylaxis consisted of cyclosporin A and methotrexate [2]. Recipients of transplants from unrelated donors received cyclosporin A, methotrexate, and corticosteroids. T cell depletion of donor bone marrow was not performed. Conditioning protocols included cyclophosphamide with or without busulfan or cyclophosphamide with or without ara-C plus total body irradiation, depending on the underlying diagnosis and disease status. Patients were classified as having advanced or early disease on the basis of pretransplantation status. Patients with relapsed acute leukemia after the first complete remission, chronic leukemia in the accelerated phase, multiple myeloma, non-Hodgkin’s lymphoma, or myelodysplastic syndrome were classified as high-risk (advanced disease), and all other patients were classified as low-risk (early disease).

Patients

A total of 103 bone marrow transplant recipients were evaluated over the study period. Underlying diagnoses included chronic myelogenous leukemia (n = 35), acute myelogenous leukemia (n = 22), myelodysplastic syndrome (n = 11), acute lymphocytic leukemia (n = 13), non-Hodgkin’s lymphoma (n = 9), multiple myeloma (n = 5), chronic lymphocytic leukemia (n = 4), aplastic anemia (n = 3), and myelofibrosis (n = 1). The overall mortality at 1 year was 30% (31 of 103 patients). The mean age of recipients was 40.1 years (range, 17–61 years) and did not differ between survivors and nonsurvivors (39.4 years vs. 42.0 years; P = not significant). Seventy recipients were male, and 33 were female. Since the purpose of this study was to examine the effect of CMV infection and disease on outcome, four patients were excluded from the analysis of posttransplantation variables because samples collected for evaluation for CMV infection were inadequate. Three of these patients died of causes unrelated to CMV infection in the immediate weeks following transplantation and the fourth patient was followed up at another institution after discharge from the BMT unit.

Statistical Methods

Variables were analyzed for their association with death 1 year after transplantation. A log rank test from the Kaplan-Meier survival analysis was used for categorical pretransplantation variables. For continuous pretransplantation variables and posttransplantation variables, a Wald $\chi^2$ test from the Cox proportional hazards survival analysis was used. For the multivariate analysis, variables that were significantly associated with 1-year mortality ($P < .05$) on univariate analysis were included and analyzed by using a stepwise Cox proportional hazards regression model.

Results

Active CMV disease was present at the time of death in only three of 31 patients. CMV disease was not considered the sole cause of death in any of the cases. Death was attributed to disease relapse (n = 3), bacterial sepsis (n = 5), fungal infection (n = 4), multiple factors (n = 12), acute GVHD
<table>
<thead>
<tr>
<th>Variable</th>
<th>No. (%) who survived</th>
<th>No. (%) who died</th>
<th>RR (95% CI)</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pretransplantation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Advanced underlying disease</td>
<td>35 (48.6)</td>
<td>22 (71)</td>
<td>2.34 (1.08–5.08)</td>
<td>.049</td>
</tr>
<tr>
<td>Donor and recipient CMV status</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Donor negative, recipient negative</td>
<td>26 (36.1)</td>
<td>7 (22.6)</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>Donor positive, recipient negative</td>
<td>12 (16.7)</td>
<td>4 (12.9)</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>Donor positive, recipient positive</td>
<td>26 (36.1)</td>
<td>13 (41.9)</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>Donor negative, recipient positive</td>
<td>8 (11.1)</td>
<td>7 (22.6)</td>
<td>2.58 (0.90–7.37)</td>
<td>.077</td>
</tr>
<tr>
<td>Unrelated donor</td>
<td>7 (9.7)</td>
<td>8 (25.8)</td>
<td>2.43 (1.09–5.43)</td>
<td>.031</td>
</tr>
<tr>
<td><strong>Posttransplantation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CMV infection</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CMV antigenemia</td>
<td>14 (19.4)</td>
<td>13 (48.1)</td>
<td>2.72 (1.28–5.80)</td>
<td>.018</td>
</tr>
<tr>
<td>Positive CMV blood culture</td>
<td>10 (13.9)</td>
<td>6 (22.2)</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>Positive CMV urine culture</td>
<td>19 (26.4)</td>
<td>7 (25.9)</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>Positive CMV throat culture</td>
<td>13 (18.1)</td>
<td>6 (22.1)</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>Positive day-35 BAL fluid specimen</td>
<td>4 (8.2)</td>
<td>6 (33.6)</td>
<td>3.38 (1.26–9.04)</td>
<td>.015</td>
</tr>
<tr>
<td>CMV disease</td>
<td>8 (11.1)</td>
<td>8 (29.6)</td>
<td>2.30 (1.00–5.25)</td>
<td>.049</td>
</tr>
<tr>
<td>Severe acute GVHD (grade 3 or 4)</td>
<td>8 (11.1)</td>
<td>9 (33.3)</td>
<td>2.81 (1.26–6.29)</td>
<td>.012</td>
</tr>
</tbody>
</table>

NOTE. BAL = bronchoalveolar lavage; CMV = cytomegalovirus; GVHD = graft-versus-host disease; NS = not significant.

* Calculated by using the logrank test from Kaplan-Meier survival analysis or a Wald \( \chi^2 \) test from the Cox proportional hazard survival analysis (only \( P \) values of \( < .15 \) are shown).

1 Includes 72 patients who survived and 31 who subsequently died.

2 Includes 72 patients who survived and 27 who died.

3 For 67 patients who underwent screening bronchoscopy.

(n = 4), severe veno-occlusive disease (n = 2), and hemorrhage (n = 1). The median time to death was 130 days after BMT. Three patients had CMV pneumonitis at the time of death, as well as fungal infection (n = 2), *Pneumocystis carinii* pneumonia (n = 1), or bacterial sepsis (n = 1). Of 13 additional patients with CMV disease, eight had CMV pneumonitis, two had gastrointestinal disease, and three had CMV viral syndrome.

The results of univariate analysis are shown in table 1. Analysis of pretransplantation variables showed that the probability of dying within the first year after BMT was higher for patients with advanced disease (as defined in Methods) pretransplantation and for recipients of allografts from unrelated donors. The pretransplantation CMV serostatus of donors and recipients was not a statistically significant predictor of 1-year mortality, although there was a trend towards increased mortality for seropositive recipients whose donors were seronegative. When all other serological combinations were compared with the seronegative/seronegative combination, mortality was not significantly different (34.3% vs. 21.2%; \( P = .18 \)). Posttransplantation variables that were associated with increased 1-year mortality were a positive CMV antigenemia assay, development of symptomatic CMV disease, and development of severe (grade 3–4) acute GVHD.

Urine, blood, or throat cultures positive for CMV were not associated with a higher probability of death. For the subset of 67 patients who underwent screening bronchoscopy for CMV on day 35, a positive BAL result was associated with an increased 1-year mortality. CMV antigenemia assays were positive a median of 48.5 days after BMT (mean, 46.0 days) for the patients who survived, vs. 40.0 days (mean, 40.8 days) for the patients who died (\( P = \) not significant). Blood cultures for CMV were positive a median of 42.0 days (mean, 44.3 days) after BMT, and there was no significant difference between patients who were alive or dead at 1 year. Three patients had positive BAL fluid specimens, CMV antigenemia, and positive CMV blood cultures, and all of these patients died within the first year after BMT.

Variables that were significant (\( P < .05 \)) in the univariate analysis were included as factors for multivariate analysis (table 2). The development of CMV antigenemia after BMT, the

<table>
<thead>
<tr>
<th>Variable</th>
<th>RR (95% CI)</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unrelated donor</td>
<td>3.20 (1.30–7.92)</td>
<td>.012</td>
</tr>
<tr>
<td>CMV antigenemia</td>
<td>2.74 (1.28–5.86)</td>
<td>.009</td>
</tr>
<tr>
<td>Severe acute GVHD</td>
<td>3.50 (1.50–8.17)</td>
<td>.004</td>
</tr>
<tr>
<td>Advanced underlying disease</td>
<td>1.96 (0.85–4.49)</td>
<td>.11</td>
</tr>
<tr>
<td>Active CMV disease</td>
<td>1.06 (0.40–2.78)</td>
<td>.97</td>
</tr>
</tbody>
</table>

NOTE. CMV = cytomegalovirus; GVHD = graft-versus-host disease.

* Multivariate analysis performed using a stepwise Cox proportional hazards regression model.
development of severe acute GVHD, and the use of unrelated donors remained independent predictors of 1-year mortality. Multivariate analysis showed that the development of active CMV disease and advanced pretransplantation hematologic disease did not contribute significantly to mortality. In a separate multivariate analysis of the 67 patients who underwent screening bronchoscopy on day 35, a positive BAL result was not independently associated with increased mortality ($P = .68; \text{RR} = 1.26; C1 = 0.47–3.77$). CMV antigenemia, unrelated donors, and severe acute GVHD remained independent predictors of mortality for this subgroup.

**Discussion**

Pretransplantation recipient and donor CMV serologies were not correlated with 1-year mortality for our cohort of allogeneic BMT recipients who received ganciclovir prophylaxis based on the results of screening bronchoscopy for CMV on day 35. Despite this finding, there was a trend towards increased mortality among seropositive recipients with seronegative donors. Previous studies have demonstrated that the risk of developing CMV disease is increased for seropositive recipients, regardless of donor status, although this finding was not correlated with 1-year mortality [3]. Similarly, Grob et al. [17] demonstrated that the risk of CMV pneumonitis and fatal CMV disease was significantly higher for CMV-seropositive recipients of marrow from seronegative donors than for recipients of marrow from seropositive donors. This situation contrasts with that in solid organ transplantation, where higher rates of CMV disease and higher 1-year mortality have been reported for seronegative recipients of organs from seropositive donors [5]. Seropositive marrow donors may transfer partial cellular immunity to the recipients, thereby conferring protection against CMV infection, which would account for the difference between bone marrow transplant recipients and solid organ transplant recipients.

The increase in 1-year mortality for patients who developed CMV infection, as documented by positive CMV antigenemia assays, was greater than 2.5-fold. This variable remained significant on multivariate analysis and was independent of the development of active CMV disease. It is of interest that the development of active CMV disease was not associated with increased mortality, possibly because of advances in early recognition and treatment that may have resulted in somewhat better outcomes than have been reported in previous studies [18]. Reactivation of CMV infection may represent a marker of overall poor immune reconstitution after BMT, or alternatively, may contribute to further immunosuppression, thereby resulting in increased mortality associated with other causes. This is supported by previously published in vitro data that demonstrated delayed immunologic recovery in bone marrow transplant recipients with CMV infection or disease [19]. Furthermore, CMV infection has been recognized as a risk factor for the development of bacterial and fungal infections [20].

The presence of CMV antigenemia was a better marker of the reactivation of CMV infection than were blood, urine, or throat cultures positive for CMV. None of these latter investigations were predictive of 1-year mortality.

In the subgroup of 67 patients who underwent screening bronchoscopy on day 35, those with BAL specimens positive for CMV had a threefold relative risk of death at 1 year (on univariate analysis) despite the use of prophylactic ganciclovir therapy. Of the 10 patients with positive BAL fluid specimens, two developed active CMV disease, and six died. In a previous study analyzing the clinical utility of screening bronchoscopy on day 35, patients received immunoglobulin both before and during prophylactic treatment with ganciclovir [6]. Since immunoglobulin was not given to this group of patients, except for the treatment of active CMV pneumonitis, it is unknown whether the nonuse of immunoglobulin may have had an effect on outcome.

Our findings are in agreement with the results published by Bacigalupo et al. [13], who also demonstrated a correlation between the development of CMV antigenemia and BMT-related mortality. They were also able to show increased mortality among patients with higher levels of antigenemia. However, in that study, patients with antigenemia were prophylactically treated with ganciclovir or foscarnet. Therefore, the true mortality associated with reactivation of CMV infection may have been confounded by treatment efficacy and/or drug-related adverse effects.

We were blinded to the results of the antigenemia assays and cultures of blood, urine, and throat specimens, and none of the patients received prophylactic therapy based on the result of these assays. Thus, our results provide a better evaluation of the correlation between these assays and 1-year mortality.

Although a trend towards higher mortality among patients with higher CMV antigen levels was observed in our study, this trend was not statistically significant (data not shown). Other investigators have shown that the mortality rate among recipients of marrow from unrelated donors is higher than that among recipients of marrow from related donors [21]. The correlation between acute GVHD and transplantation-related mortality has also been previously documented [10, 12]. The results of the multivariate analysis in our study are in agreement with these findings.

The limitations of our study include the relatively small sample size. For this reason, patients could not be analyzed according to pretransplantation diagnosis or conditioning regimen and were therefore grouped into early or advanced disease categories on the basis of disease status at the time of BMT. In addition, conclusions derived from this cohort of patients must be interpreted on the basis of this small sample size. Although most of the patient data and all the laboratory investigations were gathered and performed prospectively, data about the development of active CMV disease was gathered by retrospective chart review. This methodology may have resulted in potential biases, although whenever possible, retrospective data were gathered without knowledge of the results of CMV laboratory investigations.
The strengths of our study include the fact that consecutive patients were enrolled from a single center. Therefore, protocols used for management of various infectious and noninfectious complications, including the protocol for CMV prophylaxis, were the same for all patients. Furthermore, we analyzed several methods for determining reactivation of CMV infection (cultures of blood, urine, BAL fluid, and throat specimens and assays for CMV antigenemia) to determine which of these best correlated with 1-year mortality.

In conclusion, by using multivariate analysis we have shown that in a cohort of allogeneic bone marrow transplant patients, the development of CMV antigenemia is associated with a higher 1-year mortality, independent of the development of CMV disease. Reactivation of CMV infection may be a marker of poor immune function after BMT or may contribute to further immunosuppression. Although we were unable to show a significant association between pretransplantation donor and recipient CMV serologies and 1-year mortality, there was a trend towards increased mortality among CMV-seropositive recipients with CMV-seronegative donors. Further studies are needed to determine whether prophylactic therapy based on the development of CMV antigenemia will result in prolonged survival for allogeneic bone marrow transplant recipients.

References