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Incidence of Human Cytomegalovirus Infection in Patients with Refractory Solid Tumors Receiving Nonmyeloablative Allogeneic Stem Cell Transplants versus Recipients of Standard SCT for Hematologic Malignancies

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ABSTRACT

Human cytomegalovirus (HCMV) infection is the most frequent infectious complication after conventional allogeneic stem cell transplantation (alloSCT). From December 1998 to December 2002, we prospectively monitored HCMV reactivation in 59 patients affected by solid tumors and undergoing nonmyeloablative alloSCT (NST). Patients were allografted from HLA-identical sibling donors after fludarabine/cyclophosphamide-based conditioning regimens. Seventeen (28.8%) of 59 patients presented with HCMV antigenemia, and 14 received ganciclovir, with successful HCMV clearance in all cases. No patient developed HCMV viremia or disease. The median time to HCMV reactivation was 54 days (range, 16-245 days) after NST. These patients were compared with a cohort of hematologic patients who were treated with conventional myeloablative alloSCT. Matching criteria included HCMV risk group, stem cell source, donor type, and age. In the myeloablative group, HCMV active infection was observed in 47 (85.4%) of 55 patients at a median time of 30 days (range, 13-64 days) after alloSCT, and HCMV infection occurred more frequently ($P < .001$) and earlier ($P = .001$) than in NST patients. Patients affected with solid tumors undergoing NST had a reduced and delayed incidence of HCMV active infection.

KEY WORDS

Human cytomegalovirus infection • Nonmyeloablative allogeneic stem cell transplantation • Refractory solid tumor

INTRODUCTION

Whereas the potential antitumor effect mediated by donor lymphocytes has been established in many hematologic malignancies, [1] its efficacy in inducing clinically meaningful responses in solid tumors has been largely unexplored, despite evidence of its potential benefit in experimental animal models. [2-4] Only in recent years has the investigational application of nonmyeloablative allogeneic stem cell transplantation (NST) in patients with refractory nonhematologic cancers proved that a graft-versus-tumor effect can be generated in patients with metastatic renal cell cancer and possibly other solid tumors. [5-8]

Nonmyeloablative regimens have a lower treatment-related mortality than conventional myeloablative allogeneic regimens. [9] However, infectious complications remain a consistent cause of morbidity and mortality, and human cytomegalovirus (HCMV)

is the most common viral infection in the early posttransplantation period. [10] To evaluate the incidence and outcome of viral infections, we prospectively monitored active HCMV infection in 59 patients affected by refractory solid tumors and receiving NST in our institutions, and we compared these results with those of 55 matched hematologic patients treated with conventional myeloablative allogeneic stem cell transplantation (alloSCT).

PATIENTS AND METHODS

Patients

From December 1998 to December 2002, 59 consecutive patients, with a minimum follow-up of 100 days, underwent NST from an HLA-identical sibling at Istituto Scientifico HS Raffaele and Ospedale Niguarda Ca' Granda, both in Milan, or at Fondazione Maugeri in Pavia (all in Italy). These 3 institutions share a phase II clinical trial of NST in refractory solid tumors and followed a similar surveillance and prevention protocol for infections.

To compare the rate of HCMV infection of these patients with that of patients treated with conventional alloSCT, we analyzed the incidence of HCMV infection in a comparable cohort of patients affected by hematologic malignancies treated and monitored for HCMV infection during the same time period (December 1998 through December 2002). Fifty-five patients who met the criteria for HCMV risk group, stem cell source, donor type (HLA-identical sibling), age, and duration of follow-up were identified among 72 consecutive patients and were analyzed for HCMV infection. Patient characteristics relevant to the study are reported in Table 1.

Table 1. *Characteristics of Patients*

Variable	NST	AlloSCT
No. of patients	59	55
Sex (female/male)	34/25 (57/43)	22/33 (40/60)
Median age at transplantation, y (range)	42 (17-62)	44 (18-55)
HLA-identical sibling donors	59 (100)	55 (100)
Solid tumors		
RCC	23 (39)	NA
MBC	14 (24)	NA
OC	7 (12)	NA
Sarcoma	7 (12)	NA
Melanoma	2 (3)	NA
Others	6 (10)	NA
Hematologic disease		
ALL	NA	8 (15)
AML	NA	19 (35)
CML	NA	18 (32)
MM	NA	5 (9)
MDS	NA	2 (4)
Others	NA	3 (5)
Prior CT	42 (71)	30 (54)
Prior ASCT	17 (29)	2 (3.6)
HCMV risk group		
Low (D^-/R^-)	2 (3)	3 (5)
Intermediate (D^+/R^-)	1 (2)	5 (9)
High (D^-/R^+; D^+/R^+)	56 (95)	47 (86)
NST conditioning		
Flu/Cy	25 (42)	NA
Flu/Cy/TT	34 (58)	NA
AlloSCT conditioning		
Bu/Cy	NA	30 (55)
Bu/Mel	NA	4 (7)
TBI/Cy	NA	14 (25)
Others	NA	7 (13)
RBC/Plt transfusion	25/59 (42)	55/55 (100)
Median overall survival, d (range)	330 (21-1377)	910 (50-2350)
Nonrelapse mortality (at 1 y)	10 (17)	10 (18)

RCC indicates renal cell carcinoma; MBC, metastatic breast cancer; OC, ovarian cancer; ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; CML, chronic myeloid leukemia; MM, multiple myeloma; MDS, myelodysplastic syndrome; CT, chemotherapy; ASCT, autologous stem cell transplantation; Flu/Cy, fludarabine/cyclophosphamide; TT, thiotepa; Bu, busulfan; Mel, melphalan; TBI, total body irradiation; RBC, red blood cell; Plt, platelet; NA, not applicable; D, donor; R, recipient.
Data are No. patients (%) unless otherwise noted.

Conditioning Regimen, Hematopoietic Cell Transplantation, and Posttransplantation Immunosuppression

In 34 patients, the NST conditioning regimen consisted of intravenous thiotepa 5 to 10 mg/kg body weight on day −6, cyclophosphamide 30 mg/kg/d on day −4 and day −3, and fludarabine 30 mg/m^2/d on day −4 to day −3 before transplantation. Twenty-five subjects received cyclophosphamide 30 mg/kg/d on day −5 and day −4 and fludarabine 30 mg/m^2/d daily from day −5 to day −2 before transplantation.

Granulocyte colony-stimulating factor–mobilized peripheral blood stem cells were transplanted without further manipulation on day 0. Patients received a median of 4.88×10^6 CD34$^+$ hematopoietic cells per kilogram (range, 1.5-14) and 2.2×10^8 CD3$^+$ T cells per kilogram (range, 0.8-3.9). From day +7 until neutrophil recovery, granulocyte colony-stimulating factor was administered at 5 μg/kg/d. The transfusion requirement was reported in 25 (42%) of 59 patients.

Cyclosporin A (Cs-A), used to prevent both graft rejection and graft-versus-host disease (GVHD), was started 7 days before hematopoietic cell transplantation (HCT) as an intravenous infusion at 3 mg/kg/d. Subsequently, patients received Cs-A orally at 6 mg/kg/d in 2 single doses. Methotrexate was administrated as part of GVHD prophylaxis at 10 mg/m^2 intravenously on day 1 and 8 mg/m^2 on days 3 and 6 after HCT. After transplantation, Cs-A was decreased by 25% every week starting from day +70 or +90 (depending on disease status) and was discontinued if severe GVHD had not developed.

In patients with disease progression at day +30 or

+60, but not before day +45, after transplantation, Cs-A was withheld without tapering. Tapering schedules were modified at the discretion of the attending physician if active GVHD was present.

Patients who underwent conventional alloSCT received different types of conditioning regimens, most commonly cyclophosphamide (60 mg/kg/d for 2 consecutive days) followed by busulfan (4 mg/kg/d for 4 consecutive days) or by total body irradiation (TBI; 12 Gy). All patients received red blood cell and/or platelet support. No patient received antilymphocyte antibody. GVHD prophylaxis was the same as reported for NST patients.

Anti-infectious Prophylaxis: Supportive Care

For both NST and alloSCT, antimicrobial therapy followed institutional protocols that consisted of itraconazole or fluconazole for antifungal prophylaxis and ciprofloxacin for antibacterial prophylaxis. Acyclovir was administered at 800 mg/m^2 every 8 hours until 90 days after transplantation. No ganciclovir prophylaxis was administered in either group. The HCMV serologic status was determined before transplantation in all patients and their donors. Platelet transfusions were given on a prophylactic basis when the platelet count was $<10 \times 10^9$/L or in the presence of bleeding episodes, whereas red blood cell units were transfused in patients with hemoglobin <8 g/dL. All blood products were filtered and irradiated before transfusion.

Virologic Follow-up and Preemptive HCMV Therapy

In both NST and alloSCT patients, monitoring of HCMV infection by antigenemia and viremia determination started when patients reached a leukocyte count of $>0.5 \times 10^9$/L. Ethylenediaminetetraacetic acid–anticoagulated blood samples were collected weekly until day +100 in all patients and monthly thereafter until 1 year after transplantation in 48% of NST patients and in 72% of alloSCT patients. Antigenemia was quantified under a fluorescence microscope by counting the number of pp65-positive peripheral blood leukocytes/2×10^5 peripheral blood leukocytes (PBLs) examined on cytospin preparations stained with a pool of 3 pp65-specific monoclonal antibodies, according to a previously reported [11] and recently standardized [12] procedure. Viremia was quantified by inoculating 2×10^5 PBLs onto human embryonic lung fibroblast cell cultures by the shell vial technique and then, 16 to 24 hours after inoculation, by counting the number of fibroblast nuclei positive for the HCMV immediate-early antigen p72. [13]

During the first 100 days after transplantation, all patients (from both the NST and the alloSCT group) with HCMV antigenemia at any level, confirmed at 2 consecutive controls, or with HCMV viremia received ganciclovir therapy (5 mg/kg intravenously twice daily) until 2 consecutive negative controls. After day +100, preemptive therapy was given in the presence of at least 4 positive cells per 2×10^5 PBLs per slide or HCMV viremia.

Definitions

HCMV active infection was defined as HCMV presence in the blood, as shown by positive antigenemia confirmed on 2 consecutive tests, in the absence of clinical manifestations or organ function abnormalities. Self-limiting infection was defined in case of antigenemia positive on the first test and not confirmed at the subsequent control. HCMV infection was defined as early if it occurred within 100 days after transplantation or late when it occurred thereafter. Diagnosis of HCMV disease required documentation of HCMV infection in tissue samples by virus isolation, histopathology, or immunohistochemistry, along with organ function abnormalities. [14]

The HCMV risk groups were defined on the basis of previous results from myeloablative transplantations: [15] low risk (donor and recipient serologically negative), intermediate risk (donor serologically positive and recipient negative), and high risk (recipient positive and donor either negative or positive). The day of onset of active infection was defined as the day when the diagnostic test was found positive or (if applicable) on the basis of signs and symptoms of organ involvement. Diagnosis and clinical grading of acute and chronic GVHD were performed according to established criteria. [16]

Statistical Analysis

Cumulative incidence curves were produced for HCMV infection up to 365 days after transplantation; comparison between the 2 curves was made with use of the log-rank test. Failures from other causes (death) were classified as censored cases. Differences between time to HCMV infection and duration of treatment were determined by the Mann-Whitney *U* test. Univariate and multivariate logistic regression models were used to analyze the influence of selected variables (occurrence of acute GVHD, conditioning regimen [with or without thiotepa for NST and with or without TBI for alloSCT], age, and underlying disease) on the risk of HCMV infection; variables for the multivariate models were selected with backward stepwise elimination, and significance exceeding .05 was the criterion for removal from the models.

RESULTS

Incidence and Treatment of HCMV Infection

Characteristics of HCMV infection in the 2 patient populations are reported in Table 2. After a

Table 2. *Characteristics of HCMV Infection in the 2 Patient Populations*

Variable	Type of Transplantation		P Value
	NST	AlloSCT	
Incidence of HCMV infection (%)	17/59 (28.8)	47/55 (85.4)	<.001
Time to HCMV infection, median (range) days after transplantation	54 (16-245)	30 (13-64)	.001
Peak antigenemia level, median (range)*	2 (1-39)	4 (1-55)	NS
Peak viremia level, median (range)†	2 (1-10)	4 (1-20)	NS
Duration of HCMV treatment, d, median (range)	9 (7-21)	20 (6-53)	<.001
Incidence of HCMV recurrent infection (%)	0	30 (54.5)	<.001
Incidence of HCMV disease	0	0	NS

NS indicates not significant.

*pp65-positive/2 $\times$ 10^5 PBLs.

†pp72-positive human embryonic lung fibroblast (HELF)/2 $\times$ 10^5 PBLs inoculated.

median follow-up of 196 days (range, 102-390 days), HCMV active infection occurred in 17 (28.8%) of 59 NST patients, all of whom were allocated to the HCMV high-risk group. The median time to HCMV active infection was 54 days (range, 16-245 days), with 4 cases of late infection, and the median peak number of positive cells was 2 per 2 $\times$ 10^5 PBLs per slide (range, 1-39). Five (8%) of 59 patients developed HCMV viremia after a median time of 28 days (range, 13-58 days), with a median peak number of 2 (range, 1-10) p72-positive human embryonic lung fibroblast (HELF) per 2 $\times$ 10^5 PBLs inoculated. Overall, 14 (82.3%) of 17 patients received ganciclovir preemptive treatment, and in all cases active infection was successfully cleared after a median of 9 days of therapy (range, 7-21 days). No patient had recurrent infection after ganciclovir treatment. Three patients had self-limiting HCMV active infection, with only 1 positive test, not confirmed at the subsequent control, and they therefore did not receive antiviral treatment. No patient with HCMV disease was observed.

When looking at the matched cohort of hematologic patients treated with alloSCT, we observed an HCMV infection incidence rate of 85.4% (47/55), with 2 cases of self-limiting infection. On the whole, 44 cases of HCMV infection occurred in the high-risk group (recipient positive), whereas 1 case was observed in the low-risk group (donor and recipient both negative) and 2 cases were observed in the intermediate-risk group (donor positive, recipient negative). Active HCMV infection occurred earlier (30 days; range, 13-64 days; $P = .001$), with no case of late infection. The median antigenemia peak level was not different from that of NST patients (4; range, 1-55 pp65-positive PBLs), whereas 17 (30.1%) of 55 patients developed viremia (median peak level, 4; range, 1-20 p72-positive HELF) at a median time of 50 days (range, 27-150 days) after transplantation. In addition, 45 of 47 patients received ganciclovir therapy, with virus clearance in all cases, after a longer median treatment duration (20 days; range, 6-53 days; $P <$ .001). After the first course of ganciclovir treatment, 30 patients (54.5%) had recurrent infection (6 had self-limiting and 24 had treatment-requiring infections). Preemptive therapy was effective in preventing overt HCMV disease in all patients. As shown in Figure 1, the rate of HCMV infection was significantly higher compared with that observed in patients with solid tumors after NST (85.4% versus 28.8%; $P < .001$; hazard ratio, 8.1; 95% confidence interval, 5.5-17.1).

GVHD and HCMV Infection

GVHD data were available for all patients. In NST patients, the distribution according to the degree of acute GVHD expression was as follows: grade 0, 25 patients (42.5%); grade I, 14 patients (23.7%); grade II, 11 patients (18.6%); grade III, 6 patients (10%); and grade IV, 3 patients (5%). The acute GVHD was usually treated with prednisolone, reinstitution of Cs-A, or both. The median duration of Cs-A administration was 106 days (range, 57-510 days), with no significant difference with respect to alloSCT patients.

In logistic regression analysis, the risk of HCMV

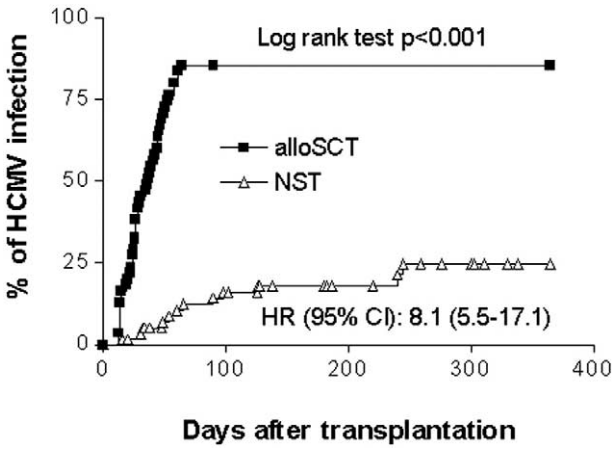

Figure 1. Cumulative incidence of HCMV infection in patients receiving NST for solid tumors as compared with patients receiving alloSCT for hematologic malignancies. HR indicates hazard ratio; CI, confidence interval.

infection was significantly associated with the occurrence of acute GVHD ($P = .01$; 95% confidence interval, 1.96-135). Conversely, no significant differences in the risk of HCMV infection were observed between the 2 nonmyeloablative regimens administered (with or without thiotepa) or according to the underlying disease and age (≥50 and <50 years).

In alloSCT patients, acute GVHD distribution was as follows: grade 0, 27 patients (49.1%); grade I, 17 patients (30.9%); grade II, 7 patients (12.7%); grade III, 2 patients (3.6%); and grade IV, 2 patients (3.6%). No significant correlation with HCMV infection was observed in alloSCT patients for acute GVHD, conditioning regimen (with or without TBI), underlying disease, or age (≥50 and <50 years).

DISCUSSION

Whether the incidence of HCMV infection is reduced after NST as compared with conventional alloSCT has been addressed so far only in patients with hematologic diseases. Martino et al. [17] analyzed 71 nonmyeloablative and 123 myeloablative patients receiving allogeneic HCT from HLA identical siblings and observed a significant reduction in HCMV infection in the nonmyeloablative setting (21% versus 43% in the HCMV high-risk group; $P = .03$).

In a matched control study of 56 NST and 112 conventional alloSCT patients, Junghanss et al. [18] observed that the incidence of HCMV infection was significantly reduced during the first 100 days in nonmyeloablated as compared with myeloablated patients (53% versus 78% in the HCMV high-risk group; $P = .01$) but that the overall 1-year incidence was similar; they concluded that the 2 groups of patients should receive the same surveillance. A greater risk of delayed HCMV infection in NST patients was also shown in that study. Other authors, focusing on HCMV and NST in hematologic patients, reported a rate of HCMV infection that varied from 42% to 65%. [19-22] These results, not always concordant, differ in regard to the NST regimen administered and suggest that modulation of immunosuppression (with low doses of TBI, Campath-1H, or T-cell depletion) plays a major role in determining the risk of HCMV infection.

The incidence and outcome of HCMV infection after NST for solid tumors are largely unknown, because small series of patients have been reported so far. In our study, 59 patients, which represent the largest cohort reported so far, were prospectively evaluated. Only approximately 30% of patients had an active HCMV infection, and none had clinically overt disease. The observed rate of active HCMV infection is lower than that reported previously in this setting. [6,23,24] In addition, the general trend was toward a delayed appearance and a shorter treatment duration of HCMV infection.

Because conventional alloSCT represents the standard model to which the NST approach is compared, we investigated the incidence of HCMV infection in a cohort of matched hematologic patients undergoing alloSCT, and we observed a lower and delayed rate and a shorter treatment duration of HCMV infection in NST patients. Even though this analysis was conducted in 2 different cohorts of patients (hematologic versus solid tumors), it is worth noting the strength of these data, which may have clinical implications if they are confirmed in a prospective trial.

As expected, the risk of HCMV reactivation was associated with acute GVHD in NST patients; conversely, this correlation was not observed in alloSCT patients. Although this lack of correlation was probably a bias due to the limited number of patients analyzed, another hypothesis might be the more intensive corticosteroid treatment (methylprednisolone 2-4 mg/kg/d in divided doses) administered in some cases of alloSCT patients suspected of having early acute GVHD (grade 0). In our experience, the different follow-up in the 2 groups of patients (330 versus 910 days) allowed an effective comparison for the HCMV infection during the first 100 days and in the first year after transplantation, but it rendered the risk of HCMV incidence/recurrence after the first year not evaluable.

One possible explanation for such a difference in HCMV infection rates might be that our NST patients had not previously received intensive chemotherapy, and the conditioning regimens per se bear a low rate of infection and toxicity. In addition, it has been shown [25,26] that NST is associated with a more rapid immune reconstitution as compared with conventional alloSCT. This could entail a potentially faster development of an effective immune response against pathogens, thus reducing the overall infection risk.

Moreover, the reduced transfusion requirement in NST patients underlines the low risk of exposure to transfusion-transmitted HCMV infection with the NST approach. Finally, an additional hypothesis, which needs confirmation, is that patients harboring solid malignancies would be less prone to HCMV infection as compared with hematologic patients because of the different immunologic impairment related to the underlying disease.

In conclusion, this study documents, regardless of the mechanisms underlying the phenomenon, that patients with solid tumors undergoing NST have a reduced incidence and delayed appearance of active HCMV infection. Thus, in our experience, HCMV infection did not represent a major clinical problem in this setting. Our results need confirmation and, at

present, do not justify a change in conventional HCMV surveillance after NST.

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