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Engraftment Syndrome after Nonmyeloablative Allogeneic Hematopoietic Stem Cell Transplantation: Incidence and Effects on Survival

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ABSTRACT

Engraftment syndrome (ES) encompasses a constellation of symptoms that occur during neutrophil recovery after both autologous and allogeneic hematopoietic stem cell transplantation (HCT). Although it is well characterized after conventional myeloablative procedures, limited data exist on this complication after nonmyeloablative allogeneic HCT. The clinical manifestations, incidence, and risk factors associated with ES were investigated in a consecutive series of patients undergoing cyclophosphamide/fludarabine-based nonmyeloablative allogeneic HCT from a related HLA-compatible donor. Fifteen (10%) of 149 patients (median age, 53 years; range, 27-66 years) developed ES; the onset of symptoms occurred at a median of 10 days (range, 3-14 days), and they consisted of fever (100%), cough (53%), diffuse pulmonary infiltrates (100%), rash (13%), and room air hypoxia (87%). ES was more likely to develop in patients who received empiric amphotericin formulations after transplant conditioning (Fisher exact test; $P = .007$). In a multivariate analysis, older patient age, female sex, and treatment with amphotericin were predictors for the development of ES. Intravenous methylprednisolone led to the rapid resolution of ES; however, transplant-related mortality was significantly higher (cumulative incidence, 49% versus 16%; $P = .0005$), and median survival was significantly shorter (168 versus 418 days; $P = .005$) in patients with ES compared with non-ES patients. In conclusion, ES occurs commonly after cyclophosphamide/fludarabine-based nonmyeloablative transplantation and responds rapidly to corticosteroid treatment, but it is associated with a higher risk of nonrelapse mortality and with shorter overall survival.

KEY WORDS

Nonmyeloablative allogeneic hematopoietic stem cell transplantation • Survival • Engraftment syndrome • Neutrophil recovery

INTRODUCTION

Myeloablative allogeneic hematopoietic cell transplantation (HCT) can cure a variety of advanced hematologic malignancies. Unfortunately, toxicities associated with high-dose conditioning regimens contribute substantially to transplant-related morbidity and mortality and limit conventional HCT to relatively younger and healthier patients. Recently, investigators have demonstrated that graft-versus-tumor effects after nonmyeloablative HCT can cure chemotherapy-resistant hematologic malignancies. It is important to note that reductions in the intensity of the preparative regimen reduce conditioning-related toxicities, thus allowing this transplantation approach to be applied to older patients or to those with medical comorbidities. Although randomized trials have yet to be performed, transplant-related mortality (TRM) seems to be reduced with nonmyeloablative transplantations compared with historical controls treated with conventional myeloablative HCT [1].

Engraftment syndrome (ES) involves a constellation of signs and symptoms that occur during neutrophil recovery after both autologous and allogeneic HCT. Differences in the intensity and types of agents used in myeloablative conditioning regimens and the lack of a standard definition likely account for the high degree of variability in the reported incidence (5%-59%) of this complication [2-16]. Although the clinical findings associated with ES vary among reports, they consistently include skin rash, fever, weight gain, and the development of noncardiogenic pulmonary edema associated with respiratory distress and hypoxia not attributable to any known causes such as infection, pulmonary embolism, or hemorrhage [7-12,16-19]. The pathophysiology of ES is poorly understood; proinflammatory cytokines such as interleukin 1, tumor necrosis factor α, and interferon γ, released as a consequence of tissue injury from dose-intensive conditioning or from recovering neutrophils during engraftment, have been hypothesized to play a role [19].

Although several conditioning-related toxicities occur less often after reduced-intensity conditioning (eg, severe mucositis, veno-occlusive disease, and idiopathic pneumonia syndrome), relatively few data exist on the incidence and severity of ES after nonmyeloablative transplantation [20]. Reducing the intensity of transplantation conditioning results in less tissue damage and decreases inflammatory cytokine release compared with myeloablative transplantation [21]. On the basis of the proposed pathophysiology of this syndrome, we hypothesized that ES would occur infrequently with nonmyeloablative transplantations. In contrast, we observed a relatively high incidence of this complication in patients undergoing nonmyeloablative HCT after cyclophosphamide/fludarabine-based conditioning. The clinical manifestations, risk factors, and outcomes in patients who developed ES after nonmyeloablative HCT are described herein.

MATERIALS AND METHODS

One hundred forty-nine consecutive patients underwent a cyclophosphamide/fludarabine-based allogeneic HCT on National Heart, Lung and Blood Institute Institutional Review Board–approved protocols investigating nonmyeloablative transplantation in nonmalignant hematologic disorders (severe aplastic anemia, paroxysmal nocturnal hemoglobinuria, and pure red cell aplasia; n = 15), hematologic malignancies (n = 38), and metastatic solid tumors (n = 96) [22]. Patients were required to have a pretransplantation chest radiograph to exclude an active pulmonary infection, a pretransplantation echocardiogram to exclude cardiac failure, and baseline pulmonary function testing to ensure adequate lung function (ie, diffusing capacity for carbon monoxide [corrected for hemoglobin] ≥65%). The preparative regimen consisted of intravenous cyclophosphamide (60 mg/kg/d) on days −7 and −6, followed by intravenous fludarabine (25 mg/m^2/d) on days −5, −4, −3, −2, and −1. An unmanipulated granulocyte colony-stimulating factor–mobilized allograft from a related 6/6 or 5/6 HLA antigen–matched donor was infused on day 0; the target CD34$^+$ cell dose was 5×10^6 cells per kilogram of recipient weight. Seventeen patients who received an allograft from a 5/6 HLA antigen–matched donor or who had a history of significant red blood cell or platelet transfusions had antithymocyte globulin added to the conditioning regimen (40 mg/kg/d on days −5 to −2). All patients received standard doses of oral fluconazole and acyclovir as fungal and viral prophylaxis. Cyclosporine for graft-versus-host disease (GVHD) prophylaxis was given either alone (n = 66) or combined with either mycophenolate mofetil (n = 78) or low-dose methotrexate (5 mg/m^2 on days +1, +3, and +6; n = 5). None of the patients received granulocyte colony-stimulating factor after infusion of the allograft. Blood was obtained for weekly cytomegalovirus (CMV) antigenemia testing from engraftment until posttransplantation day +100. Patients who developed CMV antigenemia received preemptive therapy with ganciclovir or foscarnet at standard induction doses to prevent CMV disease.

Diagnosis and Management of ES

We defined ES by using the diagnostic criteria most commonly reported from prior publications [2-15,19], which included the development of 2 or more of the following symptoms within 96 hours of the start of neutrophil recovery (absolute neutrophil count [ANC] >100): (1) fever (temperature >38.5°C) without an identifiable infectious cause; (2) weight gain ≥2.5% over the pretransplantation baseline weight; (3) erythematous rash not attributable to a medication; and (4) hypoxia, pulmonary infiltrates, or both not attributable to infection, thromboembolism, pulmonary hemorrhage, fluid overload, or cardiac disease. An infectious etiology of fever, hypoxia, and respiratory distress was ruled out in all ES cases by blood, urine, and sputum cultures; CMV antigenemia testing; and bronchoalveolar lavage. Bronchoalveolar lavage samples were sent for cytopathology (to rule out viral cytopathic changes) and cultured for bacterial, fungal, and common respiratory viral pathogens (shell vial cultures for CMV, parainfluenza 1-3, influenza A and B, respiratory syncytial virus, and adenovirus). Because of the low diagnostic yield and risk of procedure-associated morbidity, open lung biopsies on patients developing ES were not performed. After a diagnosis of ES, patients were treated with intravenous methylprednisolone 1 to 3 mg/kg/d. Once symp-

toms improved, corticosteroids were typically tapered over 2 to 3 weeks.

Statistical Methods

An analysis of factors associated with the development of ES was performed. Pretransplantation variables analyzed included patient age and sex, underlying diagnosis, previous treatment (including chemotherapy and prior HCT), smoking history, history of thoracic radiation, and baseline pulmonary function tests. Transplant-related variables included donor sex, ABO blood group compatibility, $CD34^+$ and $CD3^+$ cell dose, GVHD prophylactic regimen, development and grade of acute GVHD, and use of amphotericin formulations.

Comparisons between patients with ES and those without were made by using the Wilcoxon test for continuous variables and either a χ^2 test or Fisher exact test for categorical variables [23]. Survival was estimated by the Kaplan-Meier method, and differences were assessed by using both the Gehan-Wilcoxon test and the log-rank test [23,24]. Because ES developed by day 14, all time-to-event comparisons were made from day 14 after transplantation, although summary statistics (eg, medians) are reported from the time of transplantation. This eliminated 2 patients who did not have ES from the analysis because of early death. Time to grades II to IV and III to IV acute GVHD and TRM were estimated by using cumulative incidence methods; death was considered a competing risk [25]. Comparisons between estimates of cumulative incidence functions were made by using the χ^2 test proposed by Gray [26]. Logistic regression was performed to model the probability of ES based on variables occurring before the development of ES [23]. Both ordinary logistic regression and penalized likelihood logistic regression, which reduces the bias in coefficient estimates due to the small number of events, were used [24,27]. The 2 sets of results were very similar, and the penalized logistic regression results (with coefficients closer to 0) are reported.

RESULTS

One hundred forty-nine consecutive patients received a nonmyeloablative allogeneic HCT from an HLA-identical (n = 144) or single HLA antigen–mismatched (n = 5) related donor. Fifteen patients (10%; median age, 53 years; range, 27-66 years) developed ES (Table 1); two thirds with this complication had a solid tumor as an underlying diagnosis (6 renal cell carcinoma, 2 melanoma, 1 pancreatic cancer, and 1 hepatocellular carcinoma), and the remainder had a diagnosis of either a hematologic malignancy (1 chronic lymphocytic leukemia, 1 chronic myelogenous leukemia, and 2 myelodysplastic syndromes) or a

Table 1. *Characteristics and Outcome in Patients Developing Engraftment Syndrome (ES)*

Patient No.	Age (y)/ Sex	Disease	Onset of ES Symptoms (Days after HCT)	ES Symptoms	Steroids Given to Treat ES	ES Response	Survival (Days after Transplantation)	TRM	Cause of Death	Interval from ES to Death (d)
1	64/M	RCC	3	F, P	Yes	CR symptoms	223	Yes	Culture-negative sepsis	220
2	51/M	RCC	12	F, R, P	Yes	CR symptoms	62	Yes	Culture-negative sepsis	50
3	30/M	PNH	13	F, P	Yes	CR symptoms	1232+	No	N/A	N/A: alive
4	27/M	MEL	9	F, C, P	No	N/A*	15	No	Disease progression	6
5	49/F	MEL	9	F, C, P	Yes	CR symptoms	42	Yes	Culture-negative sepsis	33
6	55/F	MDS	11	F, C, P	Yes	CR symptoms	71	No	Disease progression	60
7	66/M	CLL	9	F, C, P	Yes	CR symptoms	42	Yes	Bacterial pneumonia	33
8	65/M	MDS	10	F, P	Yes	CR symptoms	1225+	No	N/A	N/A: alive
9	53/M	RCC	7	F, P	Yes	CR symptoms	168	Yes	Bacterial pneumonia	161
10	47/M	RCC	10	F, C, P	Yes	CR symptoms	200	No	Disease progression	190
11	55/M	RCC	10	F, P	Yes	CR symptoms	612+	No	N/A	N/A: alive
12	51/F	RCC	11	F, C, P	Yes	CR symptoms	164+	No	N/A	N/A: alive
13	65/F	Pancreatic	14	F, R, P	Yes	CR symptoms	67	Yes	CMV colitis	53
14	51/F	HCC	7	F, C, P	Yes	CR symptoms	136	Yes	GVHD: invasive aspergillosis	129
15	57/F	CML	10	F, C, P	Yes	CR symptoms	1658+	No	N/A	N/A: alive

RCC indicates renal cell carcinoma; PNH, paroxysmal nocturnal hemoglobinuria; MEL, metastatic melanoma; MDS, myelodysplastic syndrome; CLL, chronic lymphocytic leukemia; Pancreatic, pancreatic cancer; HCC, hepatocellular cancer; CML, chronic myelogenous leukemia; F, fever; R, rash; C, cough; P, pulmonary infiltrates; CR, complete resolution; N/A, not applicable.

*Patient 4 died from rapid disease progression before therapy for ES could be initiated.

Table 2. *Univariate Comparisons of Patients with and without Engraftment Syndrome (ES)*

Variable	ES (n = 15)	No ES (n = 134)	*P* Value
Median age, y (range)	53 (27-66)	47 (14-71)	.026
Male	9/15 (60%)	100/134 (67%)	.23
Tobacco use	4/15 (27%)	43/134 (32%)	.78
Diagnosis			.82
Solid tumor	10/15 (67%)	82/134 (64%)	
Hematologic malignancy, PNH, or SAA	5/15 (33%)	52/134 (36%)	
Median FEV_1/FVC	1.0	1.041	.34
Median $DLCO_c$ (% predicted)	92.6	92.2	.76
Median TLC (% predicted)	88.2	91.9	.61
Median number of prior therapies (range)	1 (0-6)	2 (0-7)	.17
Prior chest radiation	5/15 (33%)	39/134 (29%)	.77
Prior fludarabine	0/15 (0%)	9/134 (7%)	.60
GVHD prophylaxis			.32
CSA	8/15 (53%)	58/134 (43%)	
CSA + MMF	6/15 (40%)	72/134 (54%)	
CSA + MTX	1/15 (7%)	4/134 (3%)	
Median CD34 dose, 10^6/kg (range)	8.20 (5.10-21.06)	7.39 (1.91-30.0)	.34
Median CD3 dose, 10^7/kg (range)	3.62 (1.10-7.10)	3.52 (0.88-16.0)	.69
Median number of days to ANC ≥ 100 (range)	10 (6-13)	9 (4-16)	.20
Median number of days to ANC ≥ 500 (range)	11 (7-15)	11 (6-21)	.39
Amphotericin use before engraftment*	7/15 (47%)	20/134 (15%)	.007

PNH indicates paroxysmal nocturnal hemoglobinuria; SAA, severe aplastic anemia; FEV_1/FVC, forced expiratory volume at 1 second/forced vital capacity; $DLCO_c$, diffusion capacity for carbon monoxide (corrected for hemoglobin); TLC, total lung capacity; CSA, cyclosporine; MMF, mycophenolate mofetil; MTX, methotrexate.

*Three received amphotericin deoxycholate, and 4 received lipid formulations of amphotericin.

bone marrow failure syndrome (1 paroxysmal nocturnal hemoglobinuria). The onset of ES symptoms occurred at a median of 10 days (range, 3-14 days); the median ANC at the onset of symptoms was 376 cells per microliter (range, 6-4675 cells per microliter). Patients presented with fever (100%), room air hypoxia (87%; mean room air oxygen saturation, 89%; range, 78%-93%), diffuse pulmonary infiltrates (100%; observed by chest radiograph or computerized tomography scan), cough (53%), wheezing (26%), and weight gain (53%; defined as an increase of body weight >2.5% above admission baseline); only a minority (13%) had an associated skin rash.

Univariate comparisons of patient characteristics and risk factors for the development of ES are shown in Table 2. Patients with ES were significantly older (median, 53 years) than patients without ES (median, 47 years; $P = .026$). ES was significantly more likely to develop in patients who received empiric amphotericin formulations after transplantation (between post-transplantation days 0 and 14; Fisher exact test; $P = .007$). Twenty-seven (18%) of 149 patients were receiving amphotericin formulations before the development of ES. Five patients (1 ES patient and 4 non-ES patients) with a history of an invasive fungal infection treated successfully before transplantation received amphotericin preparations instead of fluconazole as fungal prophylaxis. The remaining 22 patients (6 ES and 16 non-ES) received amphotericin as empiric treatment (ie, no infectious pathogen identified) for neutropenic fever that persisted longer than 72 hours after the initiation of empiric antibiotic therapy. There was no significant difference between groups in the type of amphotericin formulation used (lipid versus nonlipid formulation) or the indication for antifungal coverage (prophylaxis versus treatment). No significant difference was observed between groups in prior tobacco use, prior therapy with nucleoside analogues, number of prior chemotherapy regimens, prior chest radiation therapy, baseline pulmonary function (forced expiratory volume in 1 second/forced vital capacity, diffusing capacity for carbon monoxide [corrected for hemoglobin], and total lung capacity), tumor histology, $CD34^+$ and $CD3^+$ transplant doses, GVHD prophylactic regimen (cyclosporine alone, cyclosporine plus mycophenolate mofetil, or cyclosporine plus methotrexate), or change in baseline weight to weight during engraftment (ANC ≥ 500). None of the patients who developed ES had a pretransplantation history of pulmonary edema, pneumonia, or noninfectious pneumonitis syndrome. The median time from transplantation to an ANC of ≥100 cells per microliter and an ANC of ≥500 cells per microliter was 9 and 10 days ($P = .20$) and 11 and 11 days ($P = .39$) for ES and non-ES patients, respectively. ES was not associated with graft rejection. Donor myeloid and T-cell engraftment profiles were similar between groups (data not shown).

Pulmonary and other symptoms related to ES improved rapidly after the initiation of corticosteroid therapy; 14 (93%) of 15 patients were treated with intravenous methylprednisolone (1-3 mg/kg/d), whereas 1 patient with rapidly growing metastatic melanoma died from disease progression without re-

ceiving steroid therapy. Steroid therapy was initiated 24, 48, 72, 96, and 120 hours after the onset of ES symptoms in 8 (57%) of 14, 3 (21%) of 14, 1 (7%) of 14, 1 (7%) of 14, and 1 (7%) of 14 patients, respectively. The median time from the initiation of steroid therapy to symptomatic pulmonary improvement was 1 day (range, 1-4 days), and symptoms related to ES resolved completely in all patients within 2 weeks of corticosteroid therapy initiation. After an improvement in symptoms associated with ES, the steroid dose was initially decreased by 50% in most patients, followed by a 15% to 20% reduction in steroid dose every 4 to 6 days thereafter. Although no patients required mechanical ventilation for respiratory failure, most received supplemental oxygen via nasal canula or face mask for room air hypoxia (ie, oxygen saturation <90%).

Although corticosteroid therapy resulted in rapid improvement of symptoms, TRM was significantly higher (Figure 1) in ES patients compared with non-ES patients (49% versus 16%, respectively; P = .005). Culture-negative sepsis (n = 3) or uncontrolled systemic infection (n = 3) was the cause of death in 6 of 7 patients who died from TRM (Table 1). Among the 7 patients with ES who died from TRM, the median time from the onset of ES symptoms to death was 57 days (range, 6-220 days). ES was also associated with reduced early survival (Figure 2); median survival was significantly shorter in ES patients (168 days) compared with non-ES patients (418 days; Gehan-Wilcoxon test; P = .005). At day 100, 53% of patients with ES were alive, compared with 91% without ES. The causes of death among ES patients included GVHD (n = 1), systemic infection or culture-negative sepsis (n = 6), and progressive disease (n = 3); 5 ES patients were alive at last follow-up (Table 1). The time to acute GVHD grades II to IV, acute GVHD grades III and IV, and death from GVHD did not differ significantly for ES patients compared with non-ES patients (Figure 3). In logistic regression, patient age, sex, prior amphotericin treatment, disease, $CD3^+$ cell dose, $CD34^+$ cell dose, and their logarithms were considered as prognostic factors. Older patient age (P = .04), amphotericin use before day 14 after transplantation (P = .0029), and female sex (P = .02) were found to be predictors for the development of ES (Figure 4). Because the number of cases of ES was small in our series, these prognostic factors should be confirmed by others.

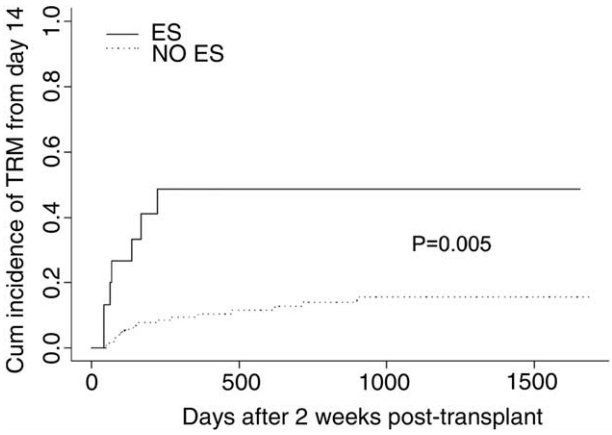

Figure 1. Cumulative incidence of treatment-related mortality (TRM) from day 14 after transplantation. A significantly higher percentage of patients who developed engraftment syndrome (49%) died from TRM compared with non–engraftment syndrome patients (16%; P = .0005).

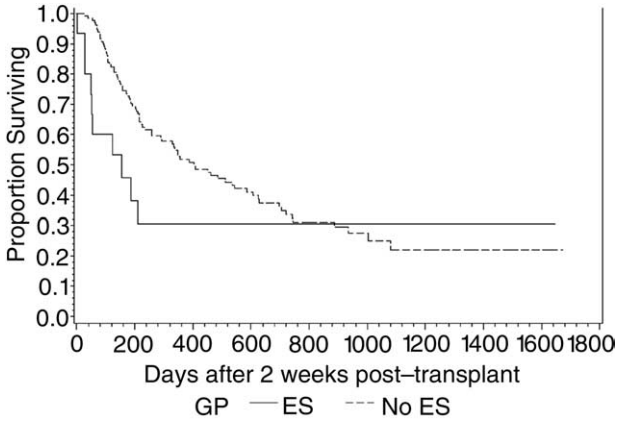

Figure 2. Survival from 14 days after transplantation. The median survival for engraftment syndrome (ES) patients was significantly shorter (168 days) compared with non-ES patients (418 days; P = .005; Gehan-Wilcoxon test). GP indicates group.

DISCUSSION

Reducing the intensity of transplantation conditioning has been shown to decrease a variety of complications associated with allogeneic HCT. As a consequence, nonmyeloablative transplantation approaches are increasingly being used to treat older or debilitated patients in whom a high risk of regimen-related mortality precludes conventional HCT. Although several studies have reported a reduction in a variety of transplant-related morbidities, surprisingly few data exist on ES and other pulmonary complications associated with this newer transplantation strategy [8]. Because tissue damage from myeloablative conditioning is thought to play a critical role in the development of complications such as diffuse alveolar hemorrhage, ES, or idiopathic pneumonia syndrome, one might hypothesize that these pulmonary complications would occur infrequently after nonmyeloablative conditioning. Indeed, a recent retrospective analysis from the Fred Hutchinson Cancer Center reported a lower incidence of idiopathic pneumonia syndrome (defined as noninfectious interstitial pneumonitis occurring up to 120 days after transplantation) after low-dose total

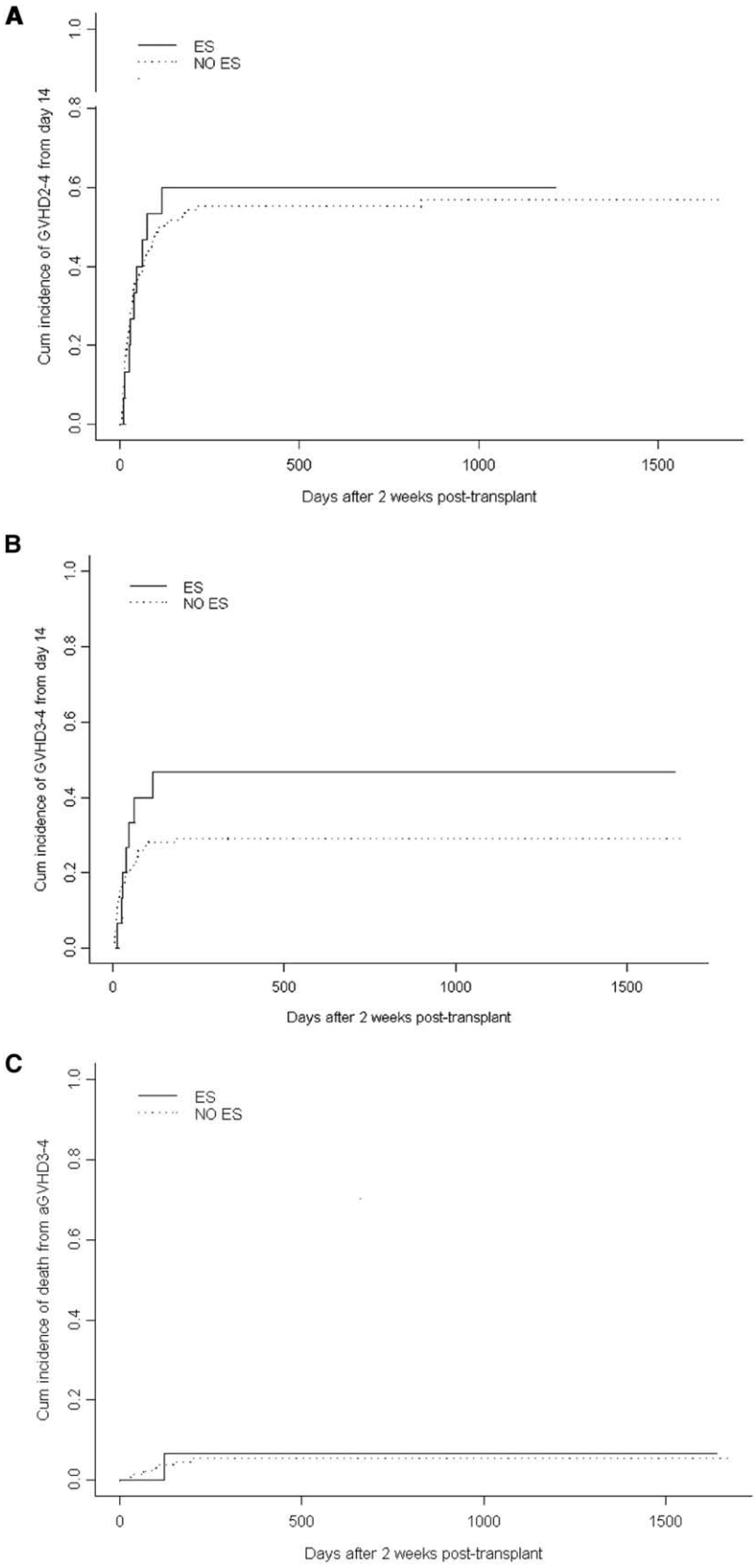

Figure 3. A, Cumulative incidence of grades II to IV acute graft-versus-host disease (AGVHD). B, Cumulative incidence of grades III and IV AGVHD. C, Cumulative incidence of death from AGVHD. The differences between the engraftment syndrome (ES) patients and non-ES patients were not significantly different.

$$P(ES) = e^{L} / (1 + e^{L}), \text{ where}$$

$$L = -3.77153 + 0.05205\ AGE + 1.50824\ SEX + 1.91087\ AMP,$$

Where AGE is age transplant, SEX = 1 if female, 0 if male,
and AMP = 1 if the patient was treated with Amphotericin B before
or within 14 days of transplant and 0 if not.

Figure 4. Logistic model to predict probability of ES, P(ES).

body irradiation–based conditioning than after myeloablative transplantation [20].

This is the first published analysis to investigate the incidence, risk factors, and outcome associated with the development of ES in patients undergoing nonmyeloablative allogeneic HCT. Although this cyclophosphamide/fludarabine-based regimen was generally well tolerated and spared patients from conditioning-related toxicities such as mucositis (0/149) and hepatic veno-occlusive disease (1/149), 10% developed ES that presented as acute onset hypoxia, fever, and noninfectious pulmonary infiltrates coincident with neutrophil recovery.

As observed by others [20], symptoms related to ES (including fever, respiratory distress, and hypoxia) improved quickly with the use of corticosteroid therapy. Nevertheless, patients with this complication had a significantly higher risk of TRM and a significantly lower overall survival. A reduction in survival and an increased incidence of TRM in patients who developed ES after myeloablative conditioning has previously been reported [5,12]. A recent multivariate analysis of children undergoing autologous transplantation for malignant diseases revealed ES to be the major contributor to TRM [28]. In our series, death from TRM occurred primarily as a consequence of systemic infection or culture-negative sepsis, in most cases after all symptoms related to ES had resolved and corticosteroid therapy had been discontinued. The immunosuppressive effects of corticosteroid therapy may have contributed to the ES cohort having an increase in infection-related mortality. Alternatively, patients who developed ES may have altered immune function before the initiation of steroid therapy that might predispose to the development of opportunistic infections.

Older patient age is an independent risk factor for TRM, even with the use of nonmyeloablative conditioning [29]. The older age of ES patients may in part account for their increased incidence of TRM. However, it is possible that patients who developed ES have biologic differences that predispose to other morbidities associated with allogeneic transplantation. Specific genetic polymorphisms have previously been shown to protect against or predispose to the development of acute GVHD [30-33]. Although further study in this area is needed, it is possible that polymorphisms in genes that encode proinflammatory cytokines might also predispose to other transplant-associated morbidities, such as ES.

Several factors have been associated with the development of ES after dose-intensive conditioning [2,3,5,7,10,11,13,15-17]. Our observation that older patient age, female sex, and amphotericin use are strongly associated with the development of ES after nonmyeloablative conditioning is consistent with similar findings in patients undergoing myeloablative HCT [4,7,10,13].

Life-threatening noninfectious pulmonary injury has previously been reported with amphotericin use [34-36]. Pulmonary endothelial damage, increased neutrophil aggregation, and increased tumor necrosis factor α production could potentially mediate amphotericin-induced pulmonary injury [37-40]. The early and brisk autologous neutrophil recovery that occurred with this nonmyeloablative regimen might have amplified amphotericin-induced lung injury by mechanisms analogous to those that cause pulmonary toxicity when granulocyte transfusions are combined with amphotericin preparations. Furthermore, amphotericin deoxycholate has been shown to increase levels of proinflammatory cytokine gene and protein expression in immune cells [41]. Whether the incorporation of newer, non–amphotericin-based antifungal agents (eg, voriconazole and caspofungin) as empiric therapy for febrile neutropenia will decrease the incidence of ES is currently being investigated. Finally, it is important to consider that amphotericin use may have served as a surrogate marker for those who were more ill, perhaps accounting at least in part for the reduced survival observed in the ES cohort.

In contrast to studies with myeloablative transplantations, we found no relationship between the type of underlying malignancy, GVHD prophylactic regimen, prior chest radiation, $CD34^{+}$ cell dose, or early neutrophil engraftment on the development of ES. Fludarabine has been reported to cause steroid-responsive pulmonary toxicity in a small percentage of patients who have received prior treatment with this agent [42]. In this series, all patients were treated with

fludarabine as part of the nonmyeloablative preparative regimen; however, because none who developed ES had received prior therapy with fludarabine (Table 2), it is less likely this agent played a direct role in this complication.

Several reports have implicated early-onset GVHD as being associated with the development of ES after myeloablative conditioning [2,15,16]. Acute GVHD is a time-dependent variable; in our series, ES occurred early (median, 10 days) and always preceded the development of acute GVHD. Therefore, in our multivariate analysis of factors associated with development of ES, we were able to consider only factors that preceded ES onset. We found that the incidence of acute GVHD and the cumulative incidence of death from acute GVHD did not differ between ES and non-ES patients, although the power to detect such a difference was not high. It is important to note that graft rejection has also been reported to be associated with a high incidence of ES after an antithymocyte globulin/thymic irradiation–based nonmyeloablative transplantation approach [8]. This is in contrast to our cyclophosphamide/fludarabine-based regimen, in which sustained donor engraftment occurred in all patients who developed ES.

Finally, in contrast to other nonmyeloablative transplantation strategies, all patients treated with our cyclophosphamide/fludarabine-based regimen developed severe neutropenia (ie, ANC <100 cells per microliter). The observation that ES occurred despite the use of a nonmyeloablative regimen that spared patients from conditioning-associated toxicities such as mucositis and veno-occlusive disease implies that neutrophils and/or soluble factors released during the neutrophil recovery phase play a role equal to, if not greater than, that of conditioning-induced tissue damage in the pathophysiology of ES. Therefore, it is likely that differences in nonmyeloablative regimens that affect the development, depth, and duration of neutropenia will also affect the incidence of this complication. Because ES occurs during neutrophil recovery, one might anticipate that this complication would occur less often with nonmyeloablative regimens that are associated with a lower incidence of neutropenia. In this, as in other analyses, a diagnosis of ES was made on the basis of the development of symptoms (both pulmonary and nonpulmonary) during a relatively brief window of neutrophil recovery (ie, within 96 hours of the neutrophil count recovering to ≥ 100 cells per microliter). Because idiopathic pneumonia syndrome has been defined as acute, noninfectious, diffuse lung injury that occurs after bone marrow transplantation, some overlap in diagnosis may occur. However, unlike ES, idiopathic pneumonia syndrome can occur up to 4 months after transplantation, is not clearly associated with neutrophil recovery, is usually unresponsive to corticosteroid therapy, and typically has a more malignant clinical course characterized by fulminant pulmonary failure and death. In contrast, the rapid improvement in symptoms associated with ES after corticosteroid therapy further suggests a distinct pathophysiology between these processes.

In conclusion, we observed a high incidence of ES in patients undergoing allogeneic HCT after cyclophosphamide/fludarabine-based conditioning. Patients who are older, who are female, or who have received amphotericin-based formulations seem to be at increased risk for this complication. Although corticosteroids rapidly improved symptoms, patients who developed ES after nonmyeloablative transplantation seemed to have an increased risk of TRM and a reduced overall survival.

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REFERENCES

1. Anagnostopoulos A, Giralt S. Critical review on non-myeloablative stem cell transplantation (NST). *Crit Rev Oncol Hematol*. 2002;44:175-190.
2. Cahill RA, Spitzer TR, Mazumder A. Marrow engraftment and clinical manifestations of capillary leak syndrome. *Bone Marrow Transplant*. 1996;18:177-184.
3. Nurnberger W, Willers R, Burdach S, Gobel U. Risk factors for capillary leak syndrome after bone marrow transplantation. *Ann Hematol*. 1997;74:221-224.
4. Capizzi SA, Kumar S, Huneke NE, et al. Peri-engraftment respiratory distress syndrome during autologous hematopoietic stem cell transplantation. *Bone Marrow Transplant*. 2001;27:1299-1303.
5. Madero L, Vicent MG, Sevilla J, et al. Engraftment syndrome in children undergoing autologous peripheral blood progenitor cell transplantation. *Bone Marrow Transplant*. 2002;30:355-358.
6. Oyama Y, Cohen B, Traynor A, et al. Engraftment syndrome: a common cause of rash and fever following autologous hematopoietic stem cell transplantation for multiple sclerosis. *Bone Marrow Transplant*. 2002;29:81-85.
7. Ravoet C, Feremans W, Husson B, et al. Clinical evidence for an engraftment syndrome associated with early and steep neutrophil recovery after autologous blood stem cell transplantation. *Bone Marrow Transplant*. 1996;18:943-947.
8. Colby C, McAfee S, Sackstein R, et al. Engraftment syndrome following non-myeloablative conditioning therapy and HLA-matched bone marrow transplantation for hematologic malignancy [abstract]. *Blood*. 2000;96:520a.
9. Niiya H, Ogasawara T, Kanda Y, et al. Engraftment syndrome (ES) after allogeneic hematopoietic stem cell transplantation (HSCT): a clinically distinct syndrome from acute graft-versus-host disease [abstract]. *Blood* 2000;96:5229a.

10. Edenfield WJ, Moores LK, Goodwin G, Lee N. An engraftment syndrome in autologous stem cell transplantation related to mononuclear dose. *Bone Marrow Transplant*. 2000;25:405-409.
11. Akasheh M, Eastwood D, Vesole DH. Engraftment syndrome after autologous hematopoietic stem cell transplant supported by granulocyte-colony-stimulating factor (G-CSF) versus granulocyte-macrophage colony-stimulating factor (GM-CSF). *Bone Marrow Transplant*. 2003;31:113-116.
12. Khan SA, Gaa B, Pollock BH, et al. Engraftment syndrome in breast cancer patients after stem cell transplantation is associated with poor long-term survival. *Biol Blood Marrow Transplant*. 2001;7:433-438.
13. Maiolino A, Biasoli I, Lima J, et al. Engraftment syndrome following autologous hematopoietic stem cell transplantation: definition of diagnostic criteria. *Bone Marrow Transplant*. 2003; 31:393-397.
14. Sutkowi L, Pohlman B, Kalaycio M, et al. Clinical correlation of the engraftment syndrome [abstract]. *Blood*. 1999;94:639a.
15. Moreb JS, Kubilis PS, Mullins DL, et al. Increased frequency of autoaggression syndrome associated with autologous stem cell transplantation in breast cancer patients. *Bone Marrow Transplant*. 1997;19:101-106.
16. Lee C-K, Gingrich RD, Hohl RJ, Ajram KA. Engraftment syndrome in autologous bone marrow and peripheral stem cell transplantation. *Bone Marrow Transplant*. 1995;16:175-182.
17. Marks DI, Benjamin K. Engraftment syndrome after autologous peripheral blood stem cell transplantation with high numbers of peripheral blood stem cells followed by granulocyte colony-stimulating factor administration. *Bone Marrow Transplant*. 2000;25:228-229.
18. Marin D, Berrade J, Ferra C, et al. Engraftment syndrome and survival after respiratory failure post-bone marrow transplantation. *Intensive Care Med*. 1998;24:732-735.
19. Spitzer TR. Engraftment syndrome following hematopoietic stem cell transplantation. *Bone Marrow Transplant*. 2001;27: 893-898.
20. Fukuda T, Hackman R, Guthrie K, et al. Risks and outcomes of idiopathic pneumonia syndrome after nonmyeloablative and conventional conditioning regimens for allogeneic hematopoietic stem cell transplantation. *Blood*. 2003; 102:2777-2785.
21. Johansson J-E, Brune M, Ekman T. The gut mucosa barrier is preserved during allogeneic, haemopoietic stem cell transplantation with reduced intensity conditioning. *Bone Marrow Transplant*. 2001;28:737-742.
22. Childs R, Chernoff A, Contentin N, et al. Regression of metastatic renal-cell carcinoma after nonmyeloablative allogeneic peripheral-blood stem-cell transplantation. *N Engl J Med*. 2000; 343:750-758.
23. Altman DG. *Practical Statistics for Medical Research*. Boca Raton, FL: Chapman & Hall/CRC; 1999.
24. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. *J Am Stat Assoc*. 1958;53:457-481.
25. Gooley TA, Leisenring W, Crowley J, Storer BE. Estimation of failure probabilities in the presence of competing risks: new representations of old estimators. *Stat Med*. 1999; 18:695-706.
26. Gray RJ. A class of K-sample tests for comparing the cumulative incidence of a competing risk. *Ann Stat*. 1988;16:1141-1154.
27. Heinze G, Schemper M. A solution to the problem of separation in logistic regression. *Stat Med*. 2002;21:2409-2419.
28. Foncillas M, Diaz M, Sevilla J, et al. Engraftment syndrome emerges as the main cause of transplant-related mortality in pediatric patients receiving autologous peripheral blood progenitor cell transplantation. *J Pediatr Hematol Oncol*. 2004;26: 492-496.
29. Espinoza-Delgado I, Shetty V, Geller N, et al. The impact of age on transplant related mortality following fludarabine and cyclophosphamide-based nonmyeloablative allogeneic hematopoietic cell transplantation [abstract]. *Blood*. 2003;102:2656a.
30. Cavet J, Dickinson AM, Norden J, et al. Interferon-gamma and interleukin-6 gene polymorphisms associate with graft-versus-host disease in HLA-matched sibling bone marrow transplantation. *Blood*. 2001;98:1594-1600.
31. Cavet J, Middleton PG, Segall M, et al. Recipient tumor necrosis factor-alpha and interleukin-10 gene polymorphisms associate with early mortality and acute graft-versus-host disease severity in HLA-matched sibling bone marrow transplants. *Blood*. 1999;94:3941-3946.
32. Takahashi H, Furukawa T, Hashimoto S, et al. Contributions of TNF-alpha and IL-10 gene polymorphisms to graft-versus-host disease following allo-hematopoietic stem cell transplantation. *Bone Marrow Transplant*. 2000;26:1317-1323.
33. Lin M-T, Storer B, Martin PJ, et al. Relation of an interleukin-10 promoter polymorphism to graft-versus-host disease and survival after hematopoietic-cell transplantation. *N Engl J Med*. 2003;349:2201-2210.
34. Wright DG, Robichaud KJ, Pizzo PA, Deisseroth AB. Lethal pulmonary reactions associated with the combined use of amphotericin B and leukocyte transfusions. *N Engl J Med*. 1981; 304:1185-1189.
35. Haber RH, Oddone EZ, Gurbel PA, Stead WW. Acute pulmonary decompensation due to amphotericin B in the absence of granulocyte transfusions. *N Engl J Med*. 1986;315:836.
36. Arning A, Heer-Sonderhoff AH, Wehmeier A, Schneider W. Pulmonary toxicity during infusion of liposomal amphotericin in two patients with acute leukemia. *Eur J Clin Microbiol Infect Dis*. 1995;14:41-43.
37. McDonnell TJ, Chang S-W, Westcott JY, Voelkel NF. Role of oxidants, eicosanoids and neutrophils in amphotericin B lung injury in rats. *J Appl Physiol*. 1988;65:2195-2206.
38. Chia JK, Pollack M. Amphotericin B induces tumor necrosis factor production by murine macrophages. *J Infect Dis*. 1989; 159:113-114.
39. Cutaia M, Bullard SR, Rudio K, Rounds S. Characteristics of amphotericin B-induced endothelial cell injury. *J Lab Clin Med*. 1993;121:244-256.
40. Berliner S, Weinberger M, Ben-Bassat M, et al. Amphotericin B causes aggregation of neutrophils and enhances pulmonary leukostasis. *Am Rev Respir Dis*. 1985;132:602-605.
41. Sau K, Mambula SS, Latz E, et al. The antifungal drug amphotericin B promotes inflammatory cytokine release by a toll-like receptor and CD14-dependent mechanism. *J Biol Chem*. 2003;278:37561-37568.
42. Helman DL, Byrd JC, Ales NC, Shorr AF. Fludarabine-related pulmonary toxicity. *Chest*. 2002;122:785-790.