Biol Blood Marrow Transplant 19 (2013) 1190–1196

Risk Factors for Invasive Fungal Disease after Allogeneic Hematopoietic Stem Cell Transplantation: A Single Center Experience

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Article history:
Received 4 April 2013
Accepted 29 May 2013

Key Words:
Invasive fungal disease
Allogeneic hematopoietic stem cell transplantation
$CD34^+$ cell

A B S T R A C T

Invasive fungal disease (IFD) is a major cause of morbidity and mortality after hematopoietic stem cell transplantation (HCT). We performed a retrospective review of 271 adults with a hematologic malignancy undergoing allogeneic HCT to determine the incidence of and risk factors for IFD and to examine the impact of IFD on nonrelapse mortality and overall survival. We defined IFD using standard criteria and selected proven and probable cases for analysis. Diagnoses in the study group included acute leukemia (42%), non-Hodgkin lymphoma (24%), myelodysplastic syndrome (15%), chronic lymphocytic leukemia (5%), and other hematologic disorders (14%). Conditioning included reduced-intensity (64%) and myeloablative (36%) regimens. Donor sources were HLA-matched sibling (60%), matched unrelated (20%), haploidentical (12%), and cord blood (8%). A total of 51 episodes of IFD were observed in 42 subjects (15%). *Aspergillus* spp (47%) was the most frequent causative organism, followed by *Candida* spp (43%). The majority of IFD cases (67%) were reported after day +100 post-HCT. In multivariate analysis, haploidentical donor transplantation (hazard ratio [HR], 3.82; 95% confidence interval [CI], 1.49-9.77; $P = .005$) and grade II-IV acute graft-versus-host disease (HR, 2.55; 95% CI, 1.07-6.10; $P = .03$) were risk factors for the development of IFD. Conversely, higher infused $CD34^+$ cell dose was associated with a lower risk of IFD (HR, 0.80; 95% CI, 0.68-0.94; $P = .006$, per 1×10^6 cells/kg increase in $CD34^+$ cell infusion). IFD-related mortality was 33.3%. Nonrelapse mortality was significantly higher in patients who developed IFD compared with those without IFD ($P < .001$, log-rank test). Patients with IFD had lower overall survival (5.8 months versus 76.1 months; $P < .001$, log-rank test). Further studies exploring strategies to increase the infused cell dose and determine adequate prophylaxis, especially against aspergillus, beyond day +100 are needed.

INTRODUCTION

Invasive fungal disease (IFD) is a major cause of morbidity and mortality after hematopoietic stem cell transplantation (HCT) [1-9]. The reported incidence of IFD in allogeneic HCT recipients has ranged from 6% to 33% in previous studies [2-11]. Martino et al. [4] reported an incidence of 14% in 395 recipients of allogeneic HCT from HLA-matched sibling donors. A much smaller study by Hagen et al. [5], involving 31 patients receiving nonmyeloablative allogeneic HCT, found a 32% incidence of IFD.

Although routine administration of systemic antifungal prophylaxis in the HCT setting significantly reduces both the incidence of proven IFD and IFD-related mortality, IFD remains a potentially lethal complication after HCT [12]. Since the 1990s, after the use of fluconazole for prophylaxis in HCT became a routine, the incidence of invasive candidiasis has decreased, to its current range of 1.1%-5% [2-4,11,13,14]. Non-*albicans* candidal species are more frequently observed than *C albicans*, and *Aspergillus* species have emerged as the most frequently encountered fungal pathogen after allogeneic HCT [3,5,9,12,15]. The Transplant-Associated Infection Surveillance Network (TRANSNET) database, currently the most comprehensive multicenter epidemiologic surveillance study of invasive fungal infections in transplantation, reports a 1-year survival of 33% for invasive candidiasis after HCT [11]. The multicenter Prospective Antifungal Therapy (PATH) Alliance registry also reports high mortality in this patient population, with a 12-week mortality of 49% after the diagnosis of invasive candidiasis [16]. Despite continuing advances in treatment and supportive care, invasive aspergillosis after HCT also carries a grave prognosis, with reported mortality of 35%-67% [16-18]. The 1-year overall survival for patients developing invasive aspergillosis after HCT was 25% in the TRANSNET study [11].

Studies performed in the early 21st century have investigated factors for the development of IFD in HCT recipients. Risk factors associated with IFD include older age, indwelling catheter uses, chemotherapy-induced severe mucositis, conditioning regimen, gastointestinal tract colonization, prolonged neutropenia, graft source, cytomegalovirus (CMV) infection, and graft-versus-host disease (GVHD) and chronic

Financial disclosure: See Acknowledgments on page 1195.

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1083-8791/$ – see front matter © 2013 American Society for Blood and Marrow Transplantation.
http://dx.doi.org/10.1016/j.bbmt.2013.05.018

steroid use for its treatment [3-5,9,19,20]. Recent data are limited regarding the risk factors for IFD in adult patients undergoing allogeneic HCT after both myeloablative and reduced-intensity conditioning [21,22]. None of the aforementioned studies explored the relationship between the cell dose infused and development of IFD.

We undertook the present study to explore the incidence of IFD after allogeneic HCT in a single center, determine the risk factors for development of IFD, study the association of cell dose with IFD, and analyze the impact of IFD on nonrelapse mortality (NRM) and overall survival (OS).

PATIENTS AND METHODS

To study the incidence, risk factors, and outcomes in patients who developed IFD, we retrospectively analyzed the medical records of 300 consecutive adult patients (age ≥18 years) with a hematologic malignancy undergoing allogeneic HCT at Massachusetts General Hospital between 2000 and 2010. Patients who had undergone previous allogeneic HCT or combined bone marrow/kidney transplantation were excluded from our analysis, but patients who had undergone previous autologous HCT were included. Only the first allogeneic HCT was analyzed for those who underwent multiple transplantations. A total of 271 patients were included in the final analysis.

All patients received antifungal prophylaxis from the day before HCT up to at least day +100; 90% received fluconazole 400 mg/day. Data are limited on antifungal prophylaxis before HCT. The majority of patients with leukemia received fluconazole 200 mg during induction chemotherapy, whereas most lymphoma patients did not receive routine antifungal prophylaxis during preconditioning chemotherapy. During transplantation, routine antiinfective prophylaxis was also provided by a fluoroquinolone and acyclovir, and *Pneumocystis jiroveci* prophylaxis was provided by trimethoprim-sulfamethoxazole or another agent. Patients were screened weekly for CMV, and ganciclovir therapy was started on the detection of CMV antigenemia. All patients were housed in rooms with high-efficiency particulate air filtration. Myeloablative conditioning consisted of either high-dose total body irradiation–based regimens in combination with cyclophosphamide or high-dose busulfan–based regimen, combined with cyclophosphamide. Reduced-intensity conditioning regimens were fludarabine-based, including fludarabine and busulfan in most cases [23]. For double cord blood HCT, the conditioning regimen consisted of fludarabine, melphalan, and antithymocyte globulin (ATG). GVHD prophylaxis was provided by tacrolimus or cyclosporine plus methotrexate with or without ATG, sirolimus and tacrolimus, or cyclosporine and mycophenylate mofetil. This study was undertaken on approval from the Partners Institutional Review Board.

Definitions of IFD

We defined IFD using the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group Consensus Group definitions [24] and included proven and probable cases for analysis. Proven IFD was defined as evidence of fungal elements in a sterile tissue specimen by microscopy and/or a positive culture obtained either from peripheral blood or from needle aspirate or biopsy from normally sterile tissues. Probable IFD was defined as isolation of fungal elements in a nonsterile tissue (bronchoalveolar lavage fluid or sputum) or positive Galactomannan antigen or β-D-glucan test in patients with clinical and radiologic features suggestive of IFD. We did not include patients with possible IFD, defined as those with clinical manifestations suggestive of IFD but lacking mycologic evidence to support the diagnosis, in our analysis.

Neutrophil engraftment was defined as the first day of an absolute neutrophil count (ANC) >500/μL for 2 consecutive days. Platelet engraftment was defined as the first day of 3 consecutive platelet counts >20 × 10^9/L over a period of at least 7 days, in the absence of platelet transfusion for at least 7 days before this date. Acute GVHD (aGVHD) was graded according to established criteria [25]. The Glucksberg grading system was used to classify the maximum grade of aGVHD [26].

IFD-related mortality was defined as death attributed to a direct consequence of fungal infection (eg, respiratory failure due to fungal pneumonia) or a direct complication of IFD (eg, bleeding or superimposed bacterial infection and sepsis with unresolved fungal infection) in the absence of relapse or progressive disease.

Statistical Analysis

Continuous data are reported as mean ± SD, and time data are reported as median (range or interquartile range [IQR]). Categorical data are reported as frequency (%). NRM was defined as death not caused from disease relapse/progression and was estimated from the day of transplantation to death or last follow-up, with the exception of patients who relapsed/progressed (who were censored at the dates of relapse/progression) [27]. OS was defined as the time from transplantation to death from any cause. Progression-free survival was defined as the time from transplantation to relapse, disease progression, or death from any cause. OS and NRM were calculated using the Kaplan-Meier method. IFD was treated as time-varying explanatory covariate, and time span records were split (into periods before and after IFD; "episode splitting") to account for the effect of IFD on OS and NRM. This methodology allowed us to compare risks in patients with the same survival time with and without IFD [28].

Univariate Cox regression models were used to identify significant moderators (independent covariates) on the occurrence of proven/probable IFD. Time to neutrophil and platelet engraftment was dichotomized at the 75th percentile to define late engraftments and were included in the analysis as independent covariates. Variables with a Mantel-Cox P value <.20 were included in the multivariate analysis. Hazard ratios (HRs) with 95% confidence intervals (CIs) are reported. An HR >1 denotes an unfavorable effect. A P value <.05 was considered significant.

RESULTS

Patient Characteristics

A total of 152 males and 119 females, with a median age of 53 years (IQR, 18-75 years), were included in the final analysis (Table 1). The most common diagnoses were acute myelogenous leukemia (34%), non-Hodgkin lymphoma (24%), myelodysplastic syndrome (15%), acute lymphoblastic leukemia (8%), and chronic lymphocytic leukemia (5%). Forty patients (15%) had undergone previous autologous HCT. Eleven percent of the patients had diabetes, and 6% had a history of IFD before transplantation. The median duration of follow-up was 16.3 months (IQR, 1.0-141.0 months) posttransplantation.

Transplantation Characteristics

Table 2 presents the transplantation characteristics of the study group. The majority of the patients (64%) received reduced-intensity or nonmyeloablative pretransplantation conditioning. The donor source was a fully HLA-matched sibling donor in 60% of patients, a matched unrelated donor in 20%, a haploidentical family member in 12%, and double umbilical cord blood in 8%. All donors and recipients were typed for HLA-A, -B, and -DRB1. Unrelated donors were either 7/8 or 8/8 HLA-A, -B, -C, and -DR allele-level matched. A donor matched for only 3/6, 4/6 or 5/6 alleles was considered haploidentical. The cord blood units were at least a 4/6 HLA match with one another and with the recipient. Using matched-related transplants as reference, matched-unrelated and haploidentical transplants had higher mean $CD34^+ \times 10^6$ cells/kg infused (namely +2.6, 95% CI 1.4-3.8,

Table 1
Patient Characteristics

Characteristic	Value
Male sex, n (%)	152 (56)
Age, years, median (IQR)	53 (18-75)
Diagnosis, n (%)	
Acute myelogenous leukemia	93 (34)
Non-Hodgkin lymphoma	64 (24)
Myelodysplastic syndrome	40 (15)
Acute lymphoblastic leukemia	21 (8)
Chronic lymphocytic leukemia	13 (5)
Myeloproliferative disorder	12 (4)
Hodgkin disease	11 (4)
Multiple myeloma	10 (4)
Other	7 (2)
Previous autologous HCT, n (%)	40 (15)
Previous IFD, n (%)	16 (6)
Diabetes mellitus, n (%)	29 (11)