14. Narayanasami U, Kanteti R, Morelli J, et al. Randomized trial of filgrastim versus chemotherapy plus filgrastim mobilization of hematopoietic progenitor cells for rescue in autologous transplantation. *Blood*. 2001;98:2059-2064.
15. Jantunen E, Putkonen M, Nousiainen T, et al. Low-dose or intermediate-dose cyclophosphamide plus granulocyte colony-stimulating factor for progenitor cell mobilisation in patients with multiple myeloma. *Bone Marrow Transplant*. 2003;31:347-351.
16. Wood WA, Whitley J, Moore D, et al. Chemomobilization with etoposide is highly effective in patients with multiple myeloma and overcomes the effects of age and prior therapy. *Biol Blood Marrow Transplant*. 2011;17:141-146.
17. To LB, Haylock DN, Simmons PJ, et al. The biology and clinical uses of blood stem cells. *Blood*. 1997;89:2233-2258.
18. Sezer O, Possinger K, Metzner N, et al. Optimal CD34+ dose in autologous peripheral blood stem cell transplantation. *J Clin Oncol*. 2000;18:3319-3320.
19. Bensinger W, Appelbaum F, Rowley S, et al. Factors that influence collection and engraftment of autologous peripheral-blood stem cells. *J Clin Oncol*. 1995;13:2547-2555.
20. Weaver CH, Hazelton B, Birch R, et al. An analysis of engraftment kinetics as a function of CD34 content of peripheral blood progenitor cell collections in 692 patients after the administration of myeloablative chemotherapy. *Blood*. 1995;86:3961-3969.
21. Glaspy JA, Shpall EJ, LeMaistre CF, et al. Peripheral blood progenitor cell mobilization using stem cell factor in combination with filgrastim in breast cancer patients. *Blood*. 1997;90:2939-2951.
22. Dugan MJ, Maziarz RT, Bensinger WI, et al. Safety and preliminary efficacy of plerixafor (Mozobil) in combination with chemotherapy and G-CSF: an open-label, multicenter, exploratory trial in patients with multiple myeloma and non-Hodgkin's lymphoma undergoing stem cell mobilization. *Bone Marrow Transplant*. 2010;45:39-47.
23. Attolico I, Pavone V, Ostuni A, et al. Plerixafor added to chemotherapy plus G-CSF is safe and allows adequate PBSC collection in predicted poor mobilizer patients with multiple myeloma or lymphoma. *Biol Blood Marrow Transplant*. 2012;18:241-249.
24. D'Addio A, Curti A, Worel N, et al. The addition of plerixafor is safe and allows adequate PBSC collection in multiple myeloma and lymphoma patients poor mobilizers after chemotherapy and G-CSF. *Bone Marrow Transplant*. 2011;46:356-363.
25. Costa LJ, Abbas J, Hogan KR, et al. Growth Factor plus preemptive ('just-in-time') plerixafor successfully mobilizes hematopoietic stem cells in multiple myeloma patients despite prior lenalidomide exposure. *Bone Marrow Transplant*. 2012;47:1403-1408.

Role of Acute Graft-Versus-Host Disease in the Risk of Bacteremia and Invasive Fungal Disease after Allogeneic Hemopoietic Stem Cell Transplantation in Children. Results from a Single-Center Observational Study

Elio Castagnola [1,*], Francesca Bagnasco [2], Roberto Bandettini [3], Ilaria Caviglia [1], Giuseppe Morreale [4], Edoardo Lanino [4], Stefano Giardino [4], Cristina Moroni [1], Riccardo Haupt [2], Maura Faraci [4]

[1] *Infectious Disease Unit, Istituto Giannina Gaslini, Genoa, Italy*
[2] *Epidemiology, Biostatistics and Committees Unit, Istituto Giannina Gaslini, Genoa, Italy*
[3] *Laboratory Analysis, Istituto Giannina Gaslini, Genoa, Italy*
[4] *HSCT Unit - Department of Haematology-Oncology, Istituto Giannina Gaslini, Genoa, Italy*

Article history:
Received 2 December 2013
Accepted 24 March 2014

Key Words:
Acute graft-versus-host disease
Bacteremia
Invasive fungal disease
Allogeneic hemopoietic stem cell transplantation
Children

A B S T R A C T

Data on epidemiology of severe infectious complications, ie, bacteremia or invasive fungal disease (IFD), in children with acute graft-versus-host disease (aGVHD) after allogeneic hemopoietic stem cell transplantation (HSCT) are scarce. In a retrospective, single-center study, we analyzed the risk (hazard ratio [HR]) and the rate (episodes/1000 patients days at risk) of bacteremias and IFD in children receiving allogeneic HSCT, according to the type of donor (matched related [MRD] or alternative [AD]) and presence and grade of aGVHD. From 2000 to 2009, 198 children receiving 217 allogeneic HSCT developed 134 severe infectious episodes (103 bacteremias and 31 IFD). The type of donor (AD versus MRD) was the most important risk factor for the severe infections ($P = .0052$). In separate multivariable analysis for bacteremia and IFD, children receiving an AD HSCT had increased HR and rate of bacteremia compared with those receiving a MRD transplantation ($P = .0171$ and $P = .0001$, respectively), whereas the HR and the rate of IFD were significantly influenced by the grade of aGVHD ($P = .0002$ and $P < .0001$, respectively). Finally, infectious episodes occurred late after HSCT, especially in presence of severe aGVHD, and bacteremias were 3 to 6 times more frequent than IFD. These data may be important to design management strategies of infections in pediatric allogeneic HSCT.

INTRODUCTION

Bacteremia and invasive fungal diseases (IFD) represent severe complications for patients receiving allogeneic hemopoietic stem cell transplantation (HSCT) [1-4]. These infections are more frequent in subjects receiving HSCT from an alternative donor (AD) than from a matched related donor (MRD) [1]. During a prospective survey of adverse events occurring in patients with steroid-resistant acute graft-versus-host disease (aGVHD), we observed that the

Financial disclosure: See Acknowledgments on page 1072.

* Correspondence and reprint requests: Elio Castagnola, Infectious Diseases Unit, Istituto Giannina Gaslini, Largo G.Gaslini, 5 16147, Genoa, Italy.
E-mail address: eliocastagnola@ospedale-gaslini.ge.it (E. Castagnola).

1083-8791/$ – see front matter © 2014 American Society for Blood and Marrow Transplantation.
http://dx.doi.org/10.1016/j.bbmt.2014.03.026

incidences of bacteremia and IFD were much higher than previously reported [5]. The major criticism to that study was that incidence was compared with that observed in a "general" population of pediatric allogeneic HSCT recipients, as no data were available for the subgroup of children with aGVHD.

The aim of the present study was to analyze the role of aGVHD in the risk of severe infectious complications (bacteremia and IFD) in pediatric allogeneic HSCT recipients.

PATIENTS AND METHODS

Clinical records of children or adolescents with cancer or other hematological disorders who received allogeneic HSCT at the Hematopoietic Stem Cell Unit of the Istituto Giannina Gaslini in Italy between January 2000 and December 2009 were reviewed for the occurrence of aGVHD and development of bacteremia or IFD. The period at risk for developing aGVHD or any infectious episode was defined as the interval between the day of transplantation and that of discontinuation of any immunosuppressive treatment, which could have been due to its elective end, relapse, or death, whichever occurred first. If a subsequent transplantation was performed, another treatment period was calculated using the same criteria as stated above, starting from the date of the subsequent HSCT. Follow-up information was censored at June 30, 2011.

For each eligible patient, data on demographics, underlying disease, date and type of transplantation(s), development of aGVHD (date of onset and end, maximum grade, and refractoriness to steroids), and updated follow-up status were already available in an institutional database. In a separate database, information (ie, etiology, localization, and date of diagnosis) had also been prospectively collected on any infectious episode. Bacteremia and IFD were classified as previously described [1], but for IFD, the revised version of the European Organization for Research and Treatment of Cancer/Mycosis Study Group criteria was adopted [6].

For the purpose of this study, the underlying disease was categorized as malignant (including leukemias, lymphomas, hemophagocytic lymphohistiocytosis, and solid tumors) and nonmalignant (including severe aplastic anemia, Fanconi anemia, immunodeficiency, and inborn errors). Recipients of transplants from an HLA-geno/phenotypically identical donor or from a single-locus–mismatched related donor were categorized as receiving a MRD HSCT, whereas recipients of transplants from an unrelated source (adult volunteer or cord blood) or from a related donor with more than 1 HLA mismatch were classified as receiving an AD HSCT. The source of stem cells was categorized as bone marrow, peripheral blood stem cells, or umbilical cord blood. The conditioning regimen was defined as myeloablative (MA) or nonmyeloablative. According to our previous definitions [7], aGVHD was grouped into 3 categories: (1) not evaluable, in case of primary graft failure or rejection and in case of death before engraftment; (2) absent or mild in case of grades 0 to I; and (3) severe in case of grades II to IV. Acute GVHD was further defined as refractory to first-line therapy when clinical signs (cutaneous, intestinal, or hepatic) worsened or remained stable 5 to 7 days after starting of standard methylprednisolone therapy.

All patients older than 18 years, or the parents or guardians of younger children, had signed a consent form allowing the use of their data for clinical research purposes. The procedures we followed were in accordance with our institution's ethical standards and with the declaration of Helsinki principles.

STANDARD OF CARE

The conditioning regimen was usually MA for patients affected by malignancy or by a congenital disease, whereas for children affected by acquired or congenital aplastic anemia, or with severe comorbidities, the conditioning regimen was usually given at nonmyeloablative doses.

As previously described [7], GVHD prophylaxis varied according to the type of donor and to the diagnosis (malignant versus nonmalignant disease). Patients with malignant disease undergoing hemopoietic stem cell transplant from a matched related donor received cyclosporine (2 mg/kg/day in 2 doses) or tacrolimus (.01 mg/kg/day c.i.) alone, whereas a short course methotrexate (10 mg/m^2 at day +1, 8 mg/m^2 at day +3,+6,+11) was added to the therapy of MRD recipients with a nonmalignant disorder. Rabbit antilymphocyte serum (ATG) (Thymoglobulin, Genzyme, Cambridge, MA) was added to the cyclosporine/short-course methotrexate regimen for patients receiving HSCT from an AD. The dose and timing of ATG varied from 2.5 mg/kg for 2 days to 3.75 mg/kg for 3 days, based on donor-recipient HLA compatibility.

In case of grade > II GVHD, standard methylprednisolone therapy at 2 mg/kg/day was started, and a second-line therapy was considered in case of resistant aGVHD [8]. During the peri- and post-transplantation period, and until discharge from the hospital, patients were admitted in single rooms with air conditioning and high-efficiency particulate air filters. Oral amoxicillin-clavulanate or intravenous ampicillin-sulbactam were administered as antibacterial prophylaxis during the pre-engraftment period, and fluconazole was administered as antifungal prophylaxis up to day 100 after HSCT. Secondary antifungal prophylaxis was administered to all patients with a positive history of IFD before HSCT. All patients received prophylaxis for *Pneumocystis jirovecii* pneumonia starting the second week after HSCT and until the end of immunosuppressive treatment.

STATISTICAL ANALYSIS

Descriptive statistics were performed in terms of absolute frequencies and percentages for qualitative data, and the Pearson's chi-square test or Fisher exact test, if appropriate, were applied to compare proportions. Quantitative data were described in terms of median values and interquartile range values because of their non-normal (Gaussian) distribution.

Analysis was performed considering the overall burden of severe infections. Separate analyses were also performed for bacteremia and IFD. For univariate and multivariable analysis, the counting process approach was applied to take into account that any patient could have received more than 1 HSCT and/or developed more than 1 infection episode [9]. For these reasons, the transplantation-related risk factors (age at HSCT, type of donor, source of stem cell, type of conditioning regimen, and aGVHD occurrence) were considered as time-dependent covariates.

To adjust the analysis for competing risks, relapse or death were the competing risks. Risk factors associated with infections were identified in univariate and multivariable proportional subdistribution hazard regression model according to the method of Fine and Gray [10]. All variables, except refractory aGVHD because it was strictly associated with severe aGVHD, were entered into the multivariable models and then, to test the best-fit model, they were sequentially eliminated in a stepwise backward selection procedure until all remaining variables were statistically significant. The subdistribution hazard ratio (HR) with the 95% confidence interval (CI) was calculated using a robust estimate of variance to incorporate the intraindividual correlation, and the likelihood ratio test was calculated to measure the effect of each predictor. Proportional hazard assumption was tested using scaled Schoenfeld residuals against log of time.

The rates of bacteremia and IFD were calculated as the number of events observed divided by the duration of follow-up (the interval between the day of transplantation and that of discontinuation of any immunosuppressive treatment) and expressed as episodes/1000 person-day at risk and reported with 95% CI. The incidence rate ratio was calculated by a Poisson regression model and the 95% CI was estimated using a robust estimate of variance to incorporate the intraindividual correlation. The likelihood ratio test was calculated to measure the effect of each predictor.