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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*

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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

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* 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).

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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.

All tests were 2-tailed and a P value $< .05$ was considered statistically significant. All analyses were performed by using Stata (StataCorp. Stata Statistical Software, Release 11.0 College Station, TX).

RESULTS

During the study period, 198 patients underwent a total of 217 allogeneic HSCT, with 8.1% (n = 16) receiving more than 1 HSCT, and their total follow-up time was 104,117 person-days at risk. Table 1 summarizes the demographic data and the general characteristics of the performed transplantations. For 20 transplantations (9.2%), aGVHD was not evaluable because of absence of engraftment, it was absent or mild (grade 0 to I) in 44.7% of cases, and it was severe (grade II to IV) in 100 (46.1%).

Table 1
Characteristics of Evaluable Patients Undergoing Allogeneic HSCT

Characteristic	Value
Patient characteristics	
No. patients	198
No. transplantations	217
Gender, n (%)	
Male	120 (60.6)
Female	78 (39.4)
Underlying disease, n (%)	
Nonmalignant	59 (29.8)
Malignant	139 (70.2)
Number of HSCT procedures, n (%)	
1	182 (91.9)
2	13 (6.6)
3	3 (1.5)
Number of infectious episodes, n (%)	
0 (none)	109 (55.0)
1	55 (27.8)
2	23 (11.6)
3	11 (5.6)
Immunosuppression days, median (IQR)	385.5 (187-698)
Transplantation characteristics	
No. of allogeneic transplantations	217
Age at HSCT, median (IQR), years	8.4 (3.4-12.1)
Type of donor, n (%)	
Matched related donor	59 (27.2)
Alternative donor	158 (72.8)
Source of stem cells, n (%)	
Bone marrow	167 (77.0)
Cord blood	25 (11.5)
Peripheral blood stem cells	25 (11.5)
Type of conditioning regimen, n (%)	
Nonmyeloablative	54 (24.9)
Myeloablative	163 (75.1)
Grade of acute GVHD, n (%)	
Absent/mild	97 (44.7)
Grade 0	55 (56.7)
Grade I	42 (43.3)
Severe	100 (46.1)
Grade II	55 (55.0)
Grade III	25 (25.0)
Grade IV	20 (20.0)
Not evaluable (not engraftment)	20 (9.2)
Refractory acute GVHD	38 (17.5)
Days to infection, median (IQR); min-max [n]	
Bacteremia	19 (6-167); 1-961 [103]
Grade 0-I	12 (5-131); 1-443 [85]
Grade II-IV	149 (107-310); 15-961 [16]
Not evaluable	7 (4-11), 4-11 [2]
Invasive fungal disease	67 (8-127); 1-487 [31]
Grade 0-I	15 (7-127); 3-250 [13]
Grade II-IV	108 (91-136); 43-487 [12]
Not evaluable	9 (7-15); 1-42 [6]

HSCT indicates hemopoietic stem cell transplantation; IQR, interquartile range; GVHD, graft-versus-host disease.

At least 1 infectious episode was diagnosed in 89 (44.9%) patients, and 34 (38.2%) experienced more than 1 episode, for a total of 134 infections: 97 single-agent bacteremias (72.4%), 6 mixed infection (4.5%), and 31 IFD (23.1%). Gram-positives were isolated as a single pathogen in 53 bacteremic episodes, whereas single-agent Gram-negative bacteremia was diagnosed in 44. In the 6 mixed infections, etiologies were represented only by bacteria (6 Gram-negatives and 6 Gram-positives), and no case of mixed bacterial-fungal infection was documented. As a consequence, there were a total of 103 bacteremic episodes. As for IFD, isolated fungemia without deep organ localization was diagnosed in 6 (19.4%) cases, a proven-probable IFD with deep organ involvement in 17 (54.8%), and possible IFD was considered in 8 (25.8%) episodes. Table 1 also reports on time distribution since HSCT of the infectious complications by grade of aGVHD.

Table 2 reports on analysis of risk factors associated with the development of a severe infectious episode. Only the type of donor was significantly associated with HR of infection, both in univariate ($P = .0022$) and multivariable ($P = .0052$) analysis. In more detail, children receiving AD HSCT had a 94% (univariate) and 91% (multivariable) increased risk of infections compared with those receiving MRD transplantation.

The risk factors associated with the development of bacteremias and IFD considered separately were analyzed both in univariate and multivariable analyses. As for the risk of bacteremia, univariate analysis showed a significant association of the HR only with the type of HSCT donor ($P = .0147$), with higher risk after AD HSCT (HR, 1.81; 95% CI, 1.31 to 2.02). This result was confirmed in the multivariable model (Table 3, Panel A), where the type of donor resulted in the only variable significantly associated with the development of bacteremia. Interestingly, the effect of the grade of aGVHD was not statistically significant, but it is noteworthy that the HR of bacteremia was almost 3 times lower (HR, .32) in patients not evaluable for acute GVHD (engraftment failure or early death) as compared with those with absent/mild disease.

Regarding the risk of IFD, univariate analysis showed a significant association with the grade of aGVHD ($P = .0002$) that resulted higher in presence of severe aGVHD (HR, 3.26; 95% CI, 1.58 to 6.71) and it was highest when aGVHD was not evaluable (HR, 8.01; 95% CI, 3.59 to 17.88). Also, the presence of refractory aGVHD ($P = .0150$), with a 3.03 HR (95% CI, 1.60 to 5.77), was associated with the risk of IFD in univariate analysis. However, the multivariable analysis (Table 3, Panel B) showed that the HR was significantly associated only with the grade of aGVHD ($P = .0002$). Compared with children with absent or mild aGVHD, the risk of IFD was more than 3 times higher in children with severe aGVHD, but it was almost 9 times higher when GVHD was not evaluable. The association with refractory aGVHD was no longer present in the multivariable model. Results of all multivariable models (overall severe infections, bacteremia, IFD) were confirmed also in the stepwise backward selection procedure (data not shown).

The rates of bacteremias and IFD, according to HSCT donor type and grade of aGVHD, were also analyzed. In univariate analysis, the type of donor was significantly associated with the rates of both bacteremia ($P = .0002$) and IFD ($P = .0068$), whereas the grade of aGVHD was significantly associated with the rate of IFD ($P < .0001$) but not with

Table 2
Univariate and Multivariable Analysis of Risk Factors Associated with the Development of Infectious Episodes

Risk Factor	Infectious Episodes (n = 134)			
	Univariate Analysis		Multivariable Analysis	
	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
Gender		.0843		.1291
Male	*ref.*		*ref.*	
Female	.73 (.50-1.05)		.76 (.53-1.08)	
Underlying disease		.4754		.2743
Nonmalignant	*ref.*		*ref.*	
Malignant	.88 (.63-1.22)		.79 (.54-1.15)	
Age at HSCT	.99 (.97-1.03)	.9206	1.01 (.98-1.04)	.6616
Type of donor		.0022		.0052
Matched related	*ref.*		*ref.*	
Alternative	1.94 (1.19-3.18)		1.91 (1.19-3.05)	
Source of stem cells		.4973		.6809
Bone marrow	*ref.*		*ref.*	
Cord blood	1.28 (.84-1.96)		1.04 (.64-1.68)	
Peripheral blood	.84 (.46-1.53)		.77 (.40-1.47)	
Type of conditioning regimen		.3347		.4638
Nonmyeloablative	*ref*		*ref.*	
Myeloablative	1.23 (.78-1.93)		1.21 (.73-1.99)	
Grade of acute GVHD		.6517		.9136
Absent/mild	*ref.*		*ref.*	
Severe	1.02 (.69-1.50)		1.01 (.68-1.49)	
Not evaluable	1.43 (.80-2.57)		1.18 (.65-2.12)	
Refractory acute GVHD		.3920		-
No	*ref.*		-	
Yes	1.24 (.85-1.82)			

GVHD indicates graft-versus-host disease; HR, (subdistribution) hazard ratio; HSCT, hemopoietic stem cell transplantation.

that of bacteremia ($P = .553$). Table 4 reports the results of multivariable analysis for the rate of bacteremia and IFD. The rate of bacteremia was still significantly associated with the type of donor ($P = .0001$), with an incidence rate ratio that was more than 2 times higher in AD recipients compared with those receiving HSCT from a MRD (Table 4, Panel A), whereas that of IFD was significantly associated only with aGVHD grade ($P < .0001$) (Table 4, Panel B). In this case, compared with those with absent/mild aGVHD, children experiencing severe aGVHD had almost 4 times increased risk of IFD, whereas those without engraftment had > 24 times increased risk of IFD.

DISCUSSION

Severe infections represent an important complication after allogeneic HSCT. Studies, mostly on allogeneic HSCT in adults, have shown that aGVHD, steroids administration, and secondary neutropenia are associated with the development

Table 3
Multivariable Analysis of Risk Factors Associated with the Development of Bacteremia and Invasive Fungal Disease

Risk Factor	Panel A: Bacteremia		Panel B: Invasive Fungal Disease	
	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
Gender		.1615		.5940
Male	*ref.*		*ref.*	
Female	.74 (.51-1.10)		.81 (.43-1.55)	
Underlying disease		.1011		.4012
Nonmalignant	*ref.*		*ref*	
Malignant	.67 (.45-.99)		1.51 (.71-3.21)	
Age at HSCT	1.01 (.98-1.05)	.5211	.99 (.94-1.05)	.8702
Type of donor		.0171		.1445
Matched related	*ref.*		*ref*	
Alternative	1.85 (1.15-2.98)		2.13 (.84-5.38)	
Source of stem cells		.1132		.0987
Bone marrow	*ref.*		*ref*	
Cord blood	1.32 (.80-2.18)		.23 (.03-1.86)	
Peripheral blood	.48 (.21-1.10)		1.86 (.68-5.07)	
Type of conditioning regimen		.4873		.9419
Nonmyeloablative	*ref.*		*ref.*	
Myeloablative	1.23 (.75-2.04)		1.04 (.42-2.55)	
Grade of acute GVHD		.0660		.0002
Absent/mild	*ref.*		*ref.*	
Severe	.66 (.40-1.09)		3.21 (1.62-6.36)	
Not evaluable	.32 (.09-1.17)		8.71 (4.22-17.99)	
Refractory acute GVHD				
No	-		-	
Yes				

GVHD indicates graft-versus-host disease; HR, (subdistribution) hazard ratio; HSCT, hemopoietic stem cell transplantation.

Table 4
Multivariable Analysis of Incidence Rates of Bacteremia or Invasive Fungal Disease in Children Undergoing Allogeneic HSCT

Risk Factor	Panel A: Bacteremias				Panel B: Invasive Fungal Disease			
	n/pdr	IR (95% CI)	IRR (95% CI)	*P*	n/pdr	IR (95% CI)	IRR (95% CI)	*P*
Type of donor				.0001				.0746
Matched related	18/35,580	.50 (.31-.86)	*ref.*		4/35,580	.11 (.04-.38)	*ref*	
Alternative	85/68,537	1.24 (.97-1.60)	2.49 (1.42-4.39)		27/68,537	.39 (.26-.60)	2.43 (.85-6.95)	
Grade of acute GVHD				.5536				< .0001
Absent/mild	85/83,959	1.01 (.79-1.31)	*ref.*		13/83,959	.15 (.09-.30)	*ref.*	
Severe	16/18,864	.85 (.48-1.54)	.76 (.39-1.46)		12/18,864	.64 (.34-1.22)	3.74 (1.56-8.97)	
Not evaluable	2/1294	1.55 (.36-12.25)	1.22 (.32-4.69)		6/1294	4.64 (1.91-12.01)	24.01 (8.26-69.83)	

GVHD indicates graft-versus-host disease; IR, incidence rate (episodes/1000 pdr); IRR, incidence rate ratio; pdr, patient-days at risk; HSCT, hematopoietic stem cell transplantation; CI, confidence interval.

of bacteremia or IFD [4,11-15]. To our knowledge, this is the first study that analyzed the role aGVHD, together with other risk factors, on the development of severe infections (bacteremia and IFD) in a pediatric setting. This study confirms that the type of donor (AD versus MRD) is still the most important risk factor for the development of a severe infection in pediatric allogeneic HSCT, as previously described [1]. However, the aim of our study was also to determine if different factors were associated with risk of specific infectious complications, namely bacteremia or IFD. Regarding bacteremias, the type of donor was found to be associated to the risk of this complication, as also shown by Frere et al. in a selected cohort of adults [16], receiving MA HSCT. However, in our study, we did not find any specific role of the type of conditioning regimen. We also observed a lower but not significant HR of bacteremias, in particular, in children with aGVHD not evaluable (ie, engraftment failure or early death). This observation is probably due to the fact that 82.5% of the observed bacteremias occurred in the group of HSCT complicated by grade 0 to I aGVHD that represents only 44.7% of all the study population. Clearly, the proportion of bacteremias in other aGVHD groups was lower, and this could explain the apparent protection from bacteremia in the presence of severe aGVHD, and even in cases when GVHD is not evaluable. The absence of a clinical relevance of this observation is sustained by the wide 95% CI of the hazards that include the value "1" [17], and it is further confirmed by the high rate of bacteremias, just observed in absence of engraftment failure, that is expected in presence of prolonged neutropenia after HSCT [18,19].

Results were different regarding the risk of IFD. In fact, in this case, we confirmed that aGVHD, especially of severe grade, has a pivotal role in the development of IFD, as already reported both in children and adults [15,20-22], as it was the only factor statistically associated with the risk of this complication in the multivariable models also. In fact, in case of severe aGVHD, the HR of IFD was more than 3 times higher than for those with mild or absent aGVHD. Moreover, the risk of IFD was more than 8 times higher in absence of engraftment or early death. Engraftment failure has been described as an important risk factor for the development of IFD [23,24], and the present data quantify this risk, at least in the pediatric population.

This study confirms that both bacteremias and IFD may occur very late during the follow-up of patients receiving allogeneic HSCT [1,25], and it shows that these complications occur later in presence of severe aGVHD. Moreover, in our previous study showing the relationship between incidence of infectious complications and type of HSCT donor [1], we observed that bacteremias were 3 to 6 times more frequent than IFD, and this observation was confirmed by the present results.

In conclusion, our data show that type of HSCT donor is pivotal for the development of severe infectious complications after pediatric allogeneic HSCT. In these settings, severe aGVHD plays an important role in the development of IFD, although it does not represent a significant risk factor for bacteremia. These results must be kept in mind for the development of management strategies.

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