Epidemiology and risk factors for bloodstream infections after allogeneic hematopoietic stem cell transplantion

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SUMMARY

A total of 315 patients who underwent allogeneic Hematopoietic Stem Cell Transplantation (HSCT) during a 4-year period were analysed with the aim of collecting information on bloodstream infections (BSI). Eighty-four patients (27%) developed 112 BSI, with a cumulative risk of 20.6% at 30 days and 27.7% at 180 days. Overall, 127 pathogens were isolated, 95 (75%) gram-positive cocci, 27 (21%) gram-negative rods and 5 (4%) fungi. *Enterococcus sp.* accounted for 46 of 127 (36%) isolates. In a multivariable analysis only including baseline factors, the type of transplant was the only factor significantly associated with the risk of BSI and the risk was higher for patients receiving transplant from mismatched or unrelated donors.
In a case-control study aimed at evaluating the predictive role of additional factors during transplant, the risk appeared to be higher in patients with a positive CMV antigenemia (p=0.03; OR of 4.82; 95% CI, 1.21-19.17), long duration of severe granulocytopenia (p=0.015; OR 7.53; 95% CI, 1.92 – 29.58) and lower platelet count (p<0.001; OR 0.14; 95% CI, 0.05 – 0.40). By day 180 post-transplant, 87 (28%) out of 314 patients had died. The cumulative risk of death was significantly higher among patients with BSI than among other patients.

KEY WORDS: Bacteremia, neutropenia, transplantation, HSCT, BMT, infection

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INTRODUCTION

Infectious complications continue to represent a significant challenge in patients undergoing HSCT (Ninin *et al.,* 2001; Walter *and* Bowden, 1995; Mark, 2004).
In particular microbiologically documented bloodstream infections (BSI) remain an important cause of morbidity and mortality in hematopoietic stem cell transplant (HSCT) recipients, particularly in the pre-engraftment phase (Collin *et al.,* 2001). Important changes occurred in the etiology of these infections during the last decades (Collin *et al.,* 2001; Viscoli *et al.,* 2002; Paganini *et al.,* 2003). The aims of this study were:

1) to describe epidemiology, clinical presentation and outcome of BSI in HSCT;
2) to understand factors associated with the risk of developing BSI in the first 180 days after transplant;
3) to understand the role of BSI on mortality.

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PATIENTS AND METHODS

All patients who underwent allogenic HSCT between July 1st, 1998 and June 30th, 2002 at the Department of Hematology and HSCT Unit at San Martino Hospital in Genoa, Italy were analyzed. Patients were cared for in HEPA filtered positive air pressure rooms until engraftment, and then, if needed, were transferred to a regular ward with HEPA filtered rooms. All patients had an indwelling central venous catheter of Hickman-Broviac type and received ciprofloxacin and fluconazole prophylaxis until engraftment and until day 100, respectively. Both types of prophylaxis were discontinued in case of need for antibacterial or antifungal therapy, respectively, according to the IDSA guidelines.
At the onset of fever (>37°C) or in the presence of any clinical symptom compatible with an infection, 3 sets of blood cultures were drawn and an empirical antibiotic therapy was started, usually with a 3rd generation cephalosporin and an aminoglycoside. In case of persistent fever an empirical antifungal therapy was also started after 3 to 5 days of antibacterial therapy, usually with standard amphotericin B. At least one of the initial 3 sets of blood culture was taken in duplicate both from a peripheral vein and from the central catheter. Blood cultures were then repeated at least daily throughout the febrile/infectious episode. Blood cultures were processed with an automatic system (Bactec 9000, BB), which did not change over the study period. For the management of CMV infection, a pre-emptive approach was used, based on the weekly detection of the viral reactivation (expression of pp65 protein) in peripheral leukocytes (CMV antigenemia) (Zaia, 2002).

DEFINITIONS

A BSI was defined as the isolation of a bacterial or fungal pathogen from at least one blood culture. For coagulase negative staphylococci, corynebacteria other than *Corynebacterium jeikeium*, and commom skin contaminants at least two sets of positive blood cultures were required. All episodes of BSI were then sub-classified as single-agent (gram positive, gram negative or fungal) or polymicrobial. Polymicrobial BSI were those in which two or more pathogens were isolated in a single blood culture or in at least two separate blood cultures obtained 96h apart. For patients who had more than one BSI during the study period, the first episode was defined as the primary one, while subsequent episodes were numbered sequentially (second, third, etc.) and defined as secondary episodes. Fever was defined as an axillary temperature >37ºC. For each day, the highest temperature was recorded. Neutropenia and severe neutropenia were defined as an absolute granulocyte count <500 or <100 cells/mm^3, respectively. Septic shock was defined as the development of hypotension (systolic blood pressure lower than 90 mmHg) in the presence of at least one of the following conditions: cutaneous hypoperfusion, tachycardia, polypnea, cyanosis, mental status changes, or oliguria (<0.5 ml/kg/h urinary output). Positive CMV antigenemia was defined as the detection of at least 1 polymorphonuclear leukocyte expressing the CMV pp65 protein. Mucositis and GvHD were graded according to previously defined criteria (8, 9). BSI was considered as the primary cause of death if the patient died within one week after the last positive blood culture and no other cause (including the underlying disease, rejection, severe GvHD, other infections and hemorrhage) was identified. Conversely, BSI was considered an associated cause of death when another cause was also present (as a relapsing underlying disease, rejection, severe GvHD). Death was considered to be due to another cause when BSI was cleared at time of death (as indicated by the lack of infection-related symptoms or positive cultures).

Data collection and statistical analysis

Data were collected from the computerized bone marrow transplant registry database, and from chart review when necessary. Two statistical analyses were performed. First, the univariate effect of variables available at the time of transplant (baseline variables) on the risk of developing a first BSI was evaluated in the overall patient population by means of the Kaplan-Meier cumulative incidence curves. The statistical significance of differences was assessed with the log-rank test. In the estimation of cumulative incidences, the follow-up time of patients who died before the occurrence of BSI was censored at the time of

death. Baseline variables included year of HSCT, underlying disease, disease status at the time of transplantation, type of transplantation, ABO compatibility, sex, age, total body irradiation, number of infused cells, CMV baseline serological status, and type of stem cell used (BM vs PBSC). Then, a multivariable Cox model was fitted to the data, with time to occurrence of the first BSI as dependent variable and, as independent variables, all baseline factors. Second, in order to assess the effect of both baseline and time-dependent variables, a nested case control study was performed. Case patients were those patients who developed at least one BSI. For each case, 2 controls were randomly selected among the cohort who had no BSI during the study period. Case and controls were matched by the day of development of the BSI in case patients. Variables analysed in the case-control study included the above listed baseline factors and additional factors available both at the time of match (granulocyte and platelet counts) and in the 15 days preceding the episode (type of immunosuppressive therapy, use of thymoglobulins, occurrence and duration of neutropenia, presence of severe mucositis, highest degree of GvHD, presence of gastrointestinal symptoms, positive CMV antigenemia and administration of antimicrobial agents). The combined effect of post-transplant and baseline variables was assessed by means of a multivariate logistic regression model with the case-control status as the dependent variable. In all multivariate analyses the final model was obtained by means of a step-down procedure based on the likelihood ratio test, starting from the full model with all the covariates. All p values are 2-sided. All analyses were performed using SPSS (Chicago, Illinois). A p value less than 0.05 was considered significant.

RESULTS

A total of 315 patients underwent a first allogeneic HSCT during the study period. One patient died from congestive heart failure on the day of the transplant and was excluded from the analysis. The baseline patient's characteristics are shown in table 1. Briefly, almost all patients had a malignant hematological disorder and, of them, 115 (37%) had an acute leukemia. The underly-

TABLE 1 - *Characteristic of 314 SCT recipients who underwent HSCT.*

Characteristic	*Value (%)*
Male sex	176 (56.1)
Age at transplantation (mean)	37 (range: 9-66)
Year of HSCT	
1998	42 (13.4)
1999	69 (22)
2000	72 (22.9)
2001	83 (26.4)
2002	48 (15.3)
Underlying disease	
Aplastic Anemia	17 (5.4)
Acute Myeloid Leukemia	59 (18.8)
Acute Lymphoblastic Leukemia	56 (17.8)
Myelodisplastic Syndrome	34 (10.8)
Lymphoproliferative Syndrome	31 (9.9)
Myeloproliferative Syndrome	117 (37.3)
Disease status at time of transplantation	
1st remission	132 (42)
2nd or 3rd remission	90 (28.7)
Relapse	72 (22.9)
Disease not neoplastic	20 (6.4)
Type of transplantation	
Identical twins	2 (0.6)
HLA identical	172 (54.8)
Matched unrelated donor	100 (31.9)
Mismatched	40 (12.7)
Type of stem cell used	
Bone marrow	297 (94.6)
Peripheral Blood Stem Cells	17 (5.4)
ABO Compatibility	
Major mismatch	43 (13.7)
Matched	194 (61.8)
Minor/Major mismatch	77 (24.5)
Patient who received TBI	156 (49.7)
Prophylaxis GVHD	
Cyclosporin + methotrexate	308 (98.1)
CMV serological status (D/R)[1]	
D–/R–	23 (7.3)
D+/R–	19 (6.1)
D–/R+	64 (20.4)
D+/R+	205 (65.3)

[1]CMV serostatus was not available for 3 patient-donor pairs.

ing disease was not in remission in about 23% of the patients, and about 28% of them were transplanted in second or third remission. A total of 172 (55%) patients received an HLA-identical HSCT, 2 (1%) received HSCT from an identical twin and 140 (44%) from alternative donors, of whom 100 (32%) were matched unrelated donors and 40 (13%) mismatched donors. Conditioning regimens included cyclosphosphamide and TBI in 156 patients (50%), thiotepa and cyclosporin in 123 patients (39%), and other regimens in 35 patients (11%). About 50% of the patients received TBI as part of the conditioning regimen. Interestingly, the large majority of patients (269 of 314-86%) were seropositive for CMV before transplantation, reflecting the high CMV seropositivity rate in Mediterranean populations.

During the 180 days following HSCT, 84 (27%) patients developed at least one episode of BSI. The incidence of BSI was particularly high during the first month following HSCT and decreased thereafter. Indeed, the cumulative risk of having a first episode was 20.6% (SE=2.3%) at 30 days, versus only 8.9% (SE=1.9%) from day 30 to day 180, with a total cumulative risk of 27.7% (SE=2.6%) from 0 to 180 days (Figure 1). Out of 84 patients with BSI, 62 (73.8%) developed only one episode, 17 (20.2%) two episodes, and 5 (6.0%) three or four episodes, with a total of 112 episodes.

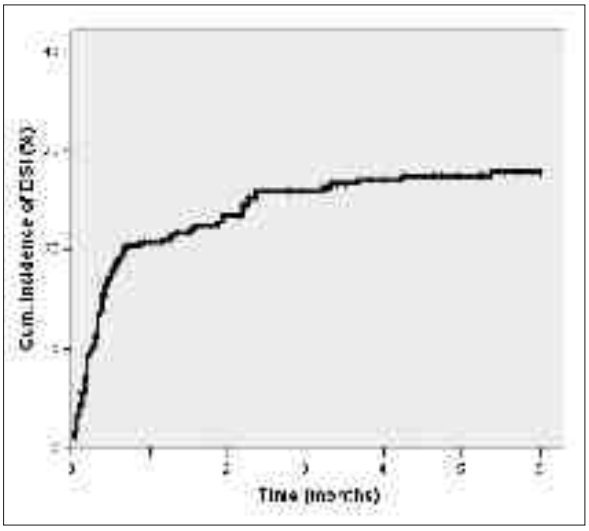

FIGURE 1 - *Cumulative incidence of BSI among 314 recipients of allogeneic HSCT, until day 180 after transplantation.*

Of these 112 episodes, 76 (68%) occurred during the first month after transplant. Although the difference was not statistically significant, the incidence rate of primary episodes of BSI in the overall population was 1.54/100 patients-week (95%CI 1.23-1.91), while the incidence rate of secondary episodes among the 84 individuals with a primary episode was 2.19 (95% CI 1.45-3.16) (RR=1.42, 95% CI=0.90-2.22, p=0.11).

Clinical presentation of primary episodes of BSI

At the time of bacteremia (day of the first positive blood culture), 70 of 84 (83%) patients had no detectable source of infection. Sites of infection in the remaining 14 patients were lungs (11 patients) and skin and soft tissues (3 patients). The highest temperature during the 24 hours preceding the BSI varied from 36.3ºC to 40.5ºC, with a median temperature of 38.2ºC. A total of 18 patients (21%) had only low-grade fever (<38ºC). Of these, 13 were receiving corticosteroids. Septic shock was found to complicate the BSI in 6 (8%) patients, of which 3 had polymicrobial infections. Five of these 6 patients died. The median absolute white blood cell count on the day of the first positive blood culture was 250 cells/mm^3 (20 - 22000). A total of 60 (71%) patients were neutropenic and 46 (55%) were severely neutropenic. All patients were receiving antibiotics for prophylaxis or therapy. In details, 36 (42.8%) patients were on prophylaxis (30 patients with ciprofloxacin and 6 with trimethoprim/sulfamethoxazole) and 48 (57.2%) on empiric therapy, usually for a previous episode of unexplained fever.

Pathogens isolated in primary and secondary episodes of BSI

As shown in table 2, of the 112 episodes of BSI, 80 (71%) were due to gram-positive cocci, 14 (12%) to gram-negative rods, and 3 (3%) to fungal organisms, while the remaining 15 episodes (13%) were polymicrobial. A total of 127 pathogens were isolated, of which 95 (75%) were gram-positive cocci, 27 (21%) gram-negative rods and 5 (4%) fungi. *Enterococcus sp.* was the most frequently isolated pathogen, accounting for 46 of 127 (36%) isolates, followed by coagulase-negative staphylococci (29 of 127-23%), viridans streptococci (12 of 127-9%) and *Pseudomonas* sp (10 of 127-8%). Five of 46 (11%) enterococcal isolates were resistant to vancomycin. As compared

TABLE 2 - *Pathogens isolated in primary and secondary episodes of BSI .*

Pathogen isolated	*First episode*	*Secondary episodes*	*Total (%)*
Gram positive	**78 (83%)**	**17 (51.5%)**	**95 (74.8)**
Enterococcus sp.	36	10	46 (36.2)
Coagulase-negative Staphylococci	24	5	29 (22.8)
S. viridans	12	0	12 (9.4)
S. aureus	3	1	4 (3.1)
Corynebacterium sp.	2	1	3 (2.4)
S. pyogenes	1	0	1
Gram negative	**13 (14%)**	**14 (42.4%)**	**27 (21.3)**
Pseudomonas sp.	5	5	10 (7.9)
E. coli	3	2	5 (3.9)
Enterobacter sp.	2	2	4 (3.1)
Klebsiella sp.	1	1	2 (1.6)
Acinetobacter sp.	0	1	1
Bacteroides sp.	1	0	1
Citrobacter sp.	0	1	1
Serratia sp.	0	1	1
S. maltophilia	1	0	1
Other	0	1	1
Fungi	**3 (3%)**	**2 (6.1%)**	**5 (3.9)**
Candida albicans	2	2	4 (3.1)
Fusarium sp	1	0	1
Total	**94 (74%)**	**33 (26%)**	**127**

to primary episodes, the pattern of pathogens isolated in secondary episodes was significantly different, with a relative increase in gram-negative rods and fungal organisms (p<0,001).

Risk factors for BSI

As shown in table 3, the univariate analysis identified the type of transplantation and the number of stem cell infused as the only baseline factors significantly associated with the risk of developing a BSI. The risk was higher in mismatched and unrelated transplants and in patients receiving a number of cells lower than $3.5x10^8$/Kg. In the multivariate analysis only the type of transplant remained in the final model, with an OR of 1.92 (95% CI 1.24-2.96 - p=0.003). The risk was higher in patients receiving transplant from mismatched or unrelated donors.

Table 4 reports the results of the nested case-control study, in which 84 patients with BSI were compared with 168 control patients. The univariate analysis identified a baseline variable (type of transplantation) and 4 post-transplant variables (thrombocyte and granulocyte counts at time of match, duration of severe neutropenia within 15 days before the day of match and administration of thymoglobulins) as significantly associated with the risk of developing a BSI. In the multivariate analysis only the platelet count at time of BSI and the duration of neutropenia before the day of match were retained in the final model. The risk of BSI was inversely related to platelet count at time of match (p<0.001; OR 0.14; 95% CI, 0.05-0.40) and directly related to number of days of severe granulocytopenia (p=0.015; OR 7.53; 95% CI, 1.92-29.58). Due to the high collinearity between duration of severe neutropenia within the 15 days preceding the BSI and the absolute granulocyte count on the day of BSI, the latter variable could not be included in multivariable models. The development of a positive CMV antigenemia, which was not significant in the univariate model (P=0.17), became significant in the multivariate one. Indeed, a positive CMV antigenemia in the 15 days prior to the day of match was associated in an increase in the risk of BSI with an OR of 4.82 (p=0.03; 95% CI, 1.21-19.17).

TABLE 3 - *Univariable and multivariable analysis of baseline factors associated with the risk of developing primary episodes of BSI.*

Baseline host variables	*No BSI (n = 230)*	*BSI (n = 84)*	*Univariate P value*	*Multivariate P value*
Type of transplantation			**0.003**	**0.003[1]**
HLA identical and identical twins donors	139 (79.9%)	35 (20.1%)		
Matched unrelated and mismatched donors	91 (65.0%)	49 (35.0%)		
Underlying disease			**0.39**	**0.24[2]**
Aplastic Anemia	13 (76.5%)	4 (23.5%)		
Acute Myeloid Leukemia	41 (69.5%)	18 (30.5%)		
Acute Lymphoblastic Leukemia	39 (69.6%)	17 (30.4%)		
Myelodisplastic Syndromes	22 (64.7%)	12 (35.3%)		
Linfoproliferative Syndromes	24 (77.4%)	7 (22.6%)		
Myeloproliferative Syndromes	91 (77.8%)	26 (22.2%)		
Status at time of transplantation			**0.49**	**0.84[2]**
Disease not neoplastic	16 (80.0%)	4 (20.0%)		
1st remission	101 (76.5%)	31 (23.5%)		
2nd or 3rd remission	64 (71.1%)	26 (28.9%)		
Relapse	49 (68.1%)	23 (31.9%)		
Recipient's Sex			**0.42**	**0.76[2]**
Male	132 (75.0%)	44 (25.0%)		
Female	98 (71.0%)	40 (29.0%)		
Recipient's Age (years)			**0.59**	**0.77[2]**
<30	66 (70.2%)	28 (29.8%)		
30-50	134 (75.7%)	43 (24.3%)		
>50	30 (69.8%)	13 (30.2%)		
Year of HSCT			**0.48**	**0.22[2]**
1998	31 (73.8%)	11 (26.2%)		
1999	45 (65.2%)	24 (34.8%)		
2000	57 (79.2%)	15 (20.8%)		
2001	61 (73.5%)	22 (26.5%)		
2002	36 (75.0%)	12 (25.0%)		
ABO Compatibility			**0.54**	**0.61[2]**
Major mismatch	29 (67.4%)	14 (32.6%)		
Matched	145 (74.7%)	49 (25.3%)		
Minor/Major mismatch	56 (72.7%)	21 (27.3%)		
Total body irradiation			**0.54**	**0.74[2]**
No	114 (72.2%)	44 (27.8%)		
Yes	116 (74.4%)	40 (25.6%)		
Number infused cells (x 10^8/Kg)			**0.03**	**0.11[2]**
<3.5 cells	53 (62.4%)	32 (37.6%)		
3.5-5.1 cells	90 (78.3%)	25 (21.7%)		
>5.1 cells	87 (76.3%)	27 (23.7%)		
CMV serological status (D/R)[3]			**1.00**	
-/-	17 (73.9%)	6 (26.1%)		
+/-	14 (73.7%)	5 (26.3%)		
-/+	47 (73.4%)	17 (26.6%)		
+/+	150 (73.2%)	55 (26.8%)		
Type of stem cell used			**0.63**	**0.47[2]**
Bone marrow	218 (73.4%)	79 (26.6%)		
PBSC	12 (70.6%)	5 (29.4%)		

[1]OR 1.92 (95% CI 1.24-2.96); [2]Removed from final model; [3]CMV serostatus was not available for 3 patient-donor pairs. Therefore this variable was excluded from the Multivariable analysis.

TABLE 4 - *Nested case-control study for assessing factors associated with BSI, including baseline host variables and post-transplant time dependent variables.*

	Control (n=168)	*Case (n=84)*	*Univariate P value*	*Multivariate P value*	*OR (95% CI)*
BASELINE VARIABLES					
Type of transplantation			**0.007**	**0.50[1]**	
HLA identical and identical twins donors	100 (59.5%)	35 (41.7%)			
Matched unrelated and mismatched donors	68 (40.5%)	49 (58.3%)			
Underlying disease			**0.50**	**0.62[1]**	
Aplastic Anemia	10 (6.0%)	4 (4.8%)			
Acute Myeloid Leukemia	31 (18.5%)	18 (21.4%)			
Acute Lymphoblastic Leukemia	28(16.7%)	17(20.2%)			
Myelodisplastic Syndromes	14 (8.3%)	12 (14.3%)			
Linfoproliferative Syndromes	16 (9.5%)	7 (8.3%)			
Mieliproliferative Syndromes	69 (41.1%)	26 (31.0%)			
Diseases status at time of transplantation			**0.62**	**0.95[1]**	
Disease not neoplastic	10 (6.0%)	4 (4.8%)			
1st remission	73 (43.5%)	31 (36.9%)			
2nd or 3rd remission	50 (29.8%)	26 (31.0%)			
Relapse	35 (20.8%)	23 (27.4%)			
Recipient's Sex			**0.53**	**0.30[1]**	
Male	95 (56.5%)	44 (52.4%)			
Female	73 (43.5%)	40 (47.6%)			
Recipientís Age (years)			**0.60**	**0.44[1]**	
<30	50 (29.8%)	28 (33.3%)			
30-50	97 (57.7%)	43 (51,2%)			
>50	21 (12.5%)	13 (15.5%)			
Year of HSCT			**0.47**	**0.84[1]**	
1998	25 (14.9%)	11 (13.1%)			
1999	34 (20.2%)	24 (28.6%)			
2000	44 (26.2%)	15 (17.9%)			
2001	43 (25.6%)	22 (26.2%)			
2002	22 (13.1%)	12 (14.3%)			
Compatibility ABO			**0.75**	**0.32[1]**	
Major mismatch	22 (13.1%)	14 (16.7%)			
Matched	102 (60.7%)	49 (58.3%)			
Minor/Major mismatch	44 (26.2%)	21 (25.0%)			
Total Body Irradiation			**0.66**	**0.72[1]**	
No	83 (49.4%)	44 (52.4%)			
Yes	85 (50.6%)	40 (47.6%)			
Number infused cells (x 108/Kg)			**0.18**	**0.53[1]**	
<3.5 cells	45 (26.8%)	32 (38.1%)			
3.51-5 cells	61 (36.3%)	25 (29.8%)			
≥5,1 cells	62 (36.9%)	27 (32.1%)			

segue

continua	*Control (n=168)*	*Case (n=84)*	*Univariate P value*	*Multivariate P value*	*OR (95% CI)*
Type of stem cell used			**0.68**	**0.63[1]**	
Bone marrow	160 (95.2%)	79 (94.0%)			
PBSCs	8 (4.8%)	5 (6.0%)			
CMV serological status (D/R)[2]			**0.98**	**–**	
D–/R–	12 (7.2%)	6 (7.2%)			
D+/R–	11 (6.6%)	5 (6.0%)			
D–/R+	37 (22.3%)	17 (20.5%)			
D+/R+	106 (63.9%)	55 (66.3%)			
POST-TRANSPLANT VARIABLES					
At time of match					**0.001**
Platelet count					
<20000	58 (35.2%)	51 (60.7%)			1 (Ref.)
20001-50000	53 (32.1%)	17 (20.2%)			0.26 (0.17-0.57)
>50000	54 (32.7%)	16 (19%)			0.14 (0.05-0.40)
Granulocyte count[3]				**0.01**	**–**
<100	55 (33.3%)	46 (54.8%)			
101-500	48 (29.1%)	14 (16.7%)			
501-1000	11 (6.7%)	3 (3.6%)			
>1000	51 (30.9%)	21 (25%)			
Within the previous 15 days					
Days of neutropenia			**0.015**	**0.01**	
No neutropenia	86 (51.2%)	36 (42.9%)			1 (Ref.)
1-7 days	65 (38.7%)	28 (33.3%)			1.23 (0.48-3.20)
>7 days	17 (10.1%)	20 (23.8%)			7.53 (1.92-29.58)
CMV Antigenemia			**0.17**	**0.03**	
Negative	155 (92.3%)	73 (86.9%)			1 (Ref.)
Positive	13 (7.7%)	11 (13.1%)			4.82 (1.21-19.17)
Mucositis			**0.19**	**0.18[1]**	
No	127 (75.6%)	57 (67.9%)			
Yes	41 (24.4%)	27 (32.1%)			
GvHD			**0.65**	**0.75[1]**	
No	102(60.7%)	51 (60.7%)			
Grade 1	48 (28.6%)	21 (25%)			
Grade 2-4	18 (10.7%)	12 (14.3%)			
Gastrointestinal symptoms			**0.27**	**0.58[1]**	
No	125 (74.4%)	57 (67.9%)			
Yes	43 (25.6%)	27 (32.1%)			
Administration of thymoglobulin			**0.04**	**0.91[1]**	
No	101 (60.1%)	39 (46.4%)			
Yes	67 (39.9%)	45 (53.6%)			
Administration of antibiotics			**0.72**	**0.94[1]**	
No	76 (45.2%)	36 (42.9%)			
Yes	92 (54.8%)	48 (57.1%)			

segue

continua	*Control (n=168)*	*Case (n=84)*	*Univariate P value*	*Multivariate P value*	*OR (95% CI)*
Administration of antifungals			**0.75**	**0.55[1]**	
No	14 (8.3%)	5 (6%)			
Fluconazol	101 (60.1%)	50 (59.5%)			
Others	53 (31.5%)	29 (34.5%)			
Cyclosporin serum levels			**0.27**	**0.19[1]**	
<130 mg/ml	61 (36.3%)	23 (27.4%)			
130-200 mg/ml	41 (24.4%)	27 (32.1%)			
>200 mg/ml	66 (39.3%)	34 (40.5%)			
Total dose steroids			**0.21**	**0.61[1]**	
No solumedrol	101 (60.1%)	45 (53.6%)			
1-300 mg	30 (17.9%)	12 (14.3%)			
>300 mg	37 (22%)	27 (32.1%)			

[1]Removed from final model; [2]CMV serostatus was not available for 3 patient-donor pairs. Therefore this variable was excluded from multivariable analysis; [3]Due to the high collinearity between the number of days of severe neutropenia and the absolute granulocyte count on the day of BSI, the latter variable could not be included in multivariable models.

Mortality

By day 180 post-transplant, 87 (28%) out of 314 patients had died. Of them, according to our definitions, 10 (11%) and 11 (13%) died with BSI as primary or an associated cause of death, respectively. Among the 11 individuals with BSI as an associated cause of death, the co-morbidities related with death were persistent fever without microbiological documentation (4 patients), rejection, underlying disease and acute GvHD (2 patients each) and hemorrhage (1 patient). The other 66 patients (76%) died from other causes. As shown in figure 2, the cumulative risk of death from any cause was 8.9% at 30 days, 13.4% at 60 days, and 27.7% at 180 days. Out of 87 fatal events, 28 (32.2%) occurred within day 30 and the other 59 between day 30 and 180 after transplantation.

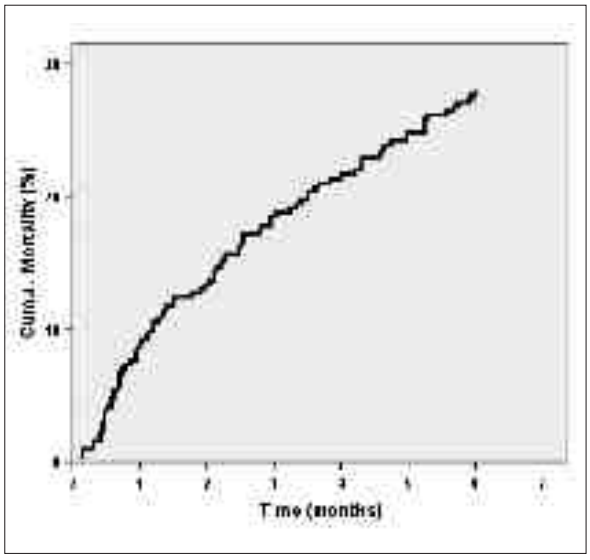

FIGURE 2 - *Cumulative mortality among 314 recipients of allogeneic HSCT, until day 180 after transplantation.*

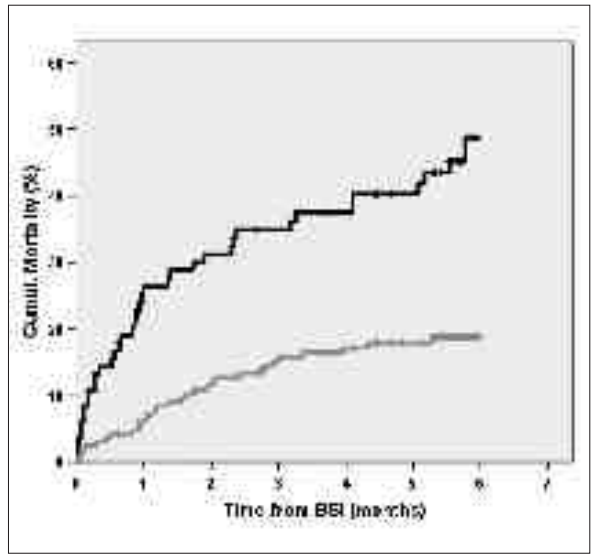

FIGURE 3 - *Cumulative mortality among patients with BSI and control-patients in nested-case control study.*

Among early deaths (within day 30), 12 (43%) were due to or associated with BSI, versus only 9 of 59 (15%) late deaths (after day 30). In the case-control study, the risk of death among BSI cases was then evaluated from the time of occurence of primary BSI and was compared with risk of death among matched controls. The occurrence of BSI was associated with a dramatic increase in overal mortality, with a cumulative risk of death from any cause, among patients with BSI, of 14.3% and 26.3% at 15 and 30 days from the primary episode, respectively (p<0,001) (Figure 3).

DISCUSSION

BSI remain the most frequent infectious complication in HSCT patients, with an incidence rate varying according to definitions, type of transplantation and days of follow-up. For example, Ninin *et al.,* 2001 reported an incidence of bacteremia of about 74% among allogeneic HSCT and 51% among autologous HSCT, while Collin *et al.,* 2001 described 189 patients with bacteremia among 519 HSCT (45% autologous and 55% allogeneic) with an incidence of 36%. We approached the problem with a more sophisticated statistical method and found that the overall risk of BSI (28%) was higher in the early (day 0-30) than in the late post-transplant period. In the population of patients experiencing a first BSI, the risk of developing a second episode was higher than in the overall initial population. The large majority of BSI had no apparent site of infection, and more than 20% of the episodes were associated with only low-grade fever, emphasizing the importance of a high level of clinical suspicion in these patients.

The relatively lower incidence of coagulase-negative staphylococcal infections with respect to other studies (Ninin *et al.*), 2001 might be explained by the conservative definition we used for accepting these episodes as a true BSI (at least 2 positive blood cultures).

On the contrary, the very high rate of enterococcal infections (which did not change over the time of the study) might be related to the characteristics of our patient population (mostly acute leukemia patients transplanted either in relapse or in second or third remission) and might reflect the fact that these patients usually receive large amounts of ciprofloxacin and 3rd generation cephalosporins for either prophylaxis or therapy during remission induction phases. Both these antibiotics have the potential for selecting for enterococcal colonization and overgrowth (Bochud *et al.,* 1994; Kern *et al.,* 1990; Cordonnier *et al.,* 2003). Mucositis, also common in hematological and HSCT patients, has also been found to be an independent predictor of vancomycin-resistant enterococcal bacteremia (Kuehnert *et al.,* 1999).

In the univariate analysis the risk of BSI was higher in mismatched and unrelated transplants and in patients receiving a number of cells lower than $3.5x10^8$/kg. In the multivariate model only the type transplant maintained its independent effect. This is a confirmation that the type of transplant is an important factor in conditioning the infection risk (Mark *et al.,* 2004).

In the case-control study, the type of transplant was still associated with the risk of bacteremia in the univariate model, but disappeared from the multivariate one, in which only neutropenia, trombocytopenia and positive CMV-antigenemia, in the preceding 15 days, remained significantly associated with the occurence of BSI. Neutropenia is a well known risk factor for severe bacterial infections. Less is known about the role of thrombocytopenia and CMV reactivation, although both have already been correlated with the risk of infection in other studies (Viscoli *et al., 1994*; Nichols *et al.,* 2002; Boeckh *and* Nichols, 2004; Hakki *et al.,* 2003). In the case-control study the occurence of a BSI was associated with a dramatic increase in the overall risk of death, particularly in the early post-transplant period.

In conclusion, BSI continues to be a serious problem after HSCT and it is an important primary or associated cause of death, particularly in the early post-transplant period. Continuous surveillance is fundamental to guide antibiotic prophylaxis, to control infections and reduce infection-related mortality.

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REFERENCES

BOCHUD, P., EGGIMAN, P., CALANDRA, T., ET AL. (1994). Bacteremia due to viridans streptococci: a case-control study of predisposing factors. *Clin. Infect. Dis.* **18**: 25-31.

BOECKH, M., NICHOLS, W.G. (2004). The impact of cytomegalovirus serostatus of donor and recipient before hematopoietic stem cell transplantation in the era of antiviral prophylaxis and preemptive therapy. *Blood*. **103** (6): 2003-8.

COLLIN, B.A., LEATHER, H.L., WINGARD, J.R., RAMPHAL, R. (2001). Evolution, incidence, and susceptibility of bacterial bloodstream isolates from 519 bone marrow transplant patients. *Clin. Infect. Dis.* **33** (7): 947-53.

CORDONNIER, C., BUZYN, A., LEVERGER, G., HERBRECHT, R., HUNAULT, M., LECLERCQ, R., ET AL. (2003). Epidemiology and risk factors for gram-positive coccal infections in neutropenia: toward a more targeted antibiotic strategy. *Clin. Infect. Dis.* **36** (2): 149-58.

ELTING, L.S., BODEY, G.P., KEEFE, B.H. (1992). Septicemia and shock syndrome due to viridans streptococci: a case-control study of predisposing factors. *Clin. Infect. Dis.* **14**: 1201-7.

HAKKI, M., RIDDELL, S.R., STOREK, J., CARTER, R.A., STEVENS-AYERS, T., SUDOUR, P., ET AL. (2003). Immune reconstitution to cytomegalovirus after allogeneic hematopoietic stem cell transplantation: impact of host factors, drug therapy, and subclinical reactivation. *Blood*. **102** (8): 3060-7.

KERN, W., KURRLE, E., SCHMEISER, T. (1990). Streptococcal bacteremia in adults with leukemia undergoing aggressive chemotherapy: a review of 55 cases. *Infection*. **18**: 138-45.

KUEHNERT, M.J., JERNIGAN, J.A., PULLEN, A.L., RIMLAND, D., JARVIS, W.R. (1999). Association between mucositis severity and vancomycin-resistant enterococcal bloodstream infection in hospitalized cancer patients. *Infect. Control. Hosp. Epidemiol.* **20**: 660-3.

MARK, K.A. (2004). Stem cell transplant patients. In: Cohen J & Powderly WF (ed.). *Infectious Diseases*. Mosby (Elsevier Limited). 1093-8.

NICHOLS, W.G., COREY, L., GOOLEY, T., DAVIS, C., BOECKH, M. (2002). High risk of death due to bacterial and fungal infection among cytomegalovirus (CMV)-seronegative recipients of stem cell transplants from seropositive donors: evidence for indirect effects of primary CMV infection. *J. Infect. Dis.* **185** (3): 273-82.

NININ, E., MILPIED, N., MOREAU, P., ANDRE-RICHET, B., MORINEAU, N., MAHE, B., ET AL. (2001). Longitudinal study of bacterial, viral, and fungal infections in adult recipients of bone marrow transplants. *Clin. Infect. Dis.* **33** (1): 41-7.

PAGANINI, H., STAFFOLANI, V., ZUBIZARRETA, P., CASIMIR, L., LOPARDO, H., LUPPINO, V. (2003). Viridans streptococci bacteraemia in children with fever and neutropenia: a case-control study of predisposing factors. *Eur. J. Cancer.* **39** (9): 1284-9.

PRZEPIORKA, D., WEISDORF, D., MARTIN, P., ET AL. (1995). Consensus Conference on Acute GVHD Grading. *Bone Marrow Transplant*. **15**: 825-8.

SCHUBERT, M.M., WILLIAMS, B.E., LLOID, M.E., DONALDSON, G., CHAPKO, M.K. (1992). Clinical assessment scale for the rating of oral mucosal changes associated with bone marrow transplantation. Development of an oral mucositis index. *Cancer*. **69**: 2469-77.

VISCOLI, C., on behalf of EORTC IATG. (2002). Management of infection in cancer patients: studies of EORTC International Antimicrobial Therapy Group (IATCG). *Eur. J. Cancer.* **38**: S82-7.

VISCOLI, C., BRUZZI, P., CASTAGNOLA, E., BONI, L., CALANDRA, T., GAYA, H., ET AL. (1994). Factors associated with bacteraemia in febrile, granulocytopenic cancer patients. The International Antimicrobial Therapy Cooperative Group (IATCG) of the European Organization for Research and Treatment of Cancer (EORTC). *Eur. J. Cancer.* **30A** (4): 430-7.

WALTER, E.A., BOWDEN, R.A. (1995). Infection in the bone marrow transplant recipient. *Infect. Dis. Clin. North. Am.* **9**: 823-47.

ZAIA, JA. (2002). Prevention of cytomegalovirus disease in hematopoietic stem cell transplantation. *Clin. Infect. Dis.* **35** (8): 999-1004.