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Bacterial Blood Stream Infections Negatively Impact on Outcome of Patients Treated with Allogeneic Stem Cell Transplantation: 6 Years Single-Centre Experience

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Abstract. ***Background:*** **Blood stream infections (BSIs) represent a major complication of allo-SCT and are a major cause of morbidity and mortality during and after bone marrow aplasia.**
Objectives: **The objective of this study was to describe the incidence and outcome of BSIs in a cohort of patients submitted to allo-SCT, in order to track changes of the epidemiology and bacteria resistance.**
Methods: **We retrospectively analyzed the microbiological data of 162 patients allotransplanted in Brescia University Hospital, over a period of 6 years.**
Results: **Eighty patients experienced a BSIs for a total of 119 isolates. In 77 cases (65%) a Gram positive bacteria was isolated, being coagulase negative *Staphilococci* the most frequent species (77% of the cases). In 42 cases (35%) a Gram negative bacteria was isolated (E. *coli* 57% and P. *aeruginosa* 24%). Fluoroquinolones resistance was frequent (90% for S. *epidermidis*, 92% for E. *coli*, 90% for P. *aeruginosa*). Methycillin resistance of S. *epidermidis* was 100%, 76% of E. *coli* were ESBL positive and among P. *aeruginosa* resistance to carbapenems was 40%. The 2 years overall survival of patients with BSIs *vs* patients without BSIs was 46% vs 60% (HR1,48, p=0,07). P. *areuginosa* and E. *coli* were the species with the highest mortality (50% and 33%, respectively).**
Conclusions: **These data confirm that BSIs, mainly sustained by Gram positive bacteria, are frequent in allotransplanted patients (50% of the cases) and may influence the outcome of allotransplanted patients, being antibiotics resistance highly frequent among these bacteria.**

Keywords: Bone Marrow Transplantation, Bacterial Infections, Antimicrobical Resistance.

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Introduction. Allogeneic stem cell transplantation (allo-SCT) is widely considered a curative option for many hematological malignancies, particularly for acute leukemias. Morbidity and mortality of allo-SCT are mainly influenced by relapse and transplant-related factors (e.g. conditioning toxicity), infections and immunological events, such as graft versus host disease (GVHD).[1-13] Blood stream infections (BSIs) represent the most frequent infective event in allotransplanted patients, and their incidence may vary from 20 to 70%.[1-12] Prolonged neutropenia, gastro-intestinal mucosal damage and extensive use of central venous catheters (CVC) are the major risk factors for BSIs.[3,5,7,9]

Usually BSIs occur during the pre-engraftment phase, but they can occur in later phases too.[5,10] Prophylactic antimicrobical therapy is conventionally used during agranulocytosis, as well as an empirical use of antibiotics in case of suspected BSI, despite it is well known that multidrug resistant bacteria may emerge.[14-17] At present, a selective gut decontamination, with the aim to reduce the translocation of intestine Gram negative bacteria from the gut to periheral blood, is conventionally based on fuoroquinolones, but this policy, during the last decade, induced the emergence of Gram negative fluoroquinolones resistant bacteria and an increase in Gram positive infections.[12,15]

In the event of a suspected bacterial infection, the common clinical practice in patients submitted to allo-SCT is to identify the specie involved in the infection as soon as possible, and to use a targeted antibiotic therapy, based on the antibiograms. However, it should be considered that only 30-40% of the febrile episodes in patients submitted to allo-SCT can be defined as BSIs. In fact, more than 50% of blood cultures (BCs) during fever do not give rise to any bacteria, and, as a consequence, the antimicrobical therapy is often empirical and, thus, the surveillance of infections in a bone marrow transplant Unit is mandatory, in order to correctly drive the use of empirical therapy.

The aim of this retrospective study was to describe the incidence and outcome of BSIs in a cohort of 162 patients submitted to allo-SCT, over a period of 6 years of transplant activity and to compare these data with the ones reported in the litterature, in order to track changes of the epidemiology and bacteria resistance.

Patients and Methods. This retrospective analysis was pointed out on the BSIs occured from January 2010 to December 2015 in the Bone Marrow Transplant Unit of Brescia University Hospital in Italy. Data were analysed from the computerized database including all the informations of patients submitted to allo-SCT. At the same time, clinical charts of the patients who experienced a BSIs were reviewed. All the BSIs occuring during the patient hospitalization were recorded.

Definitions: BCs were obtained from peripheral blood (PB) and CVC at fever onset (defined as body temperature of at least 38°C) or whenever in suspicion of infection. BSIs were defined as isolation of bacterial or fungal pathogen from at least 1 blood culture, with the exception of bacteria commonly considered skin contaminants (e.g. coagulase negative *Staphylococci* or *Corynebacteria*), for whom at least two positivity were requested. The CVC related infection was defined when a positive CVC-BC preceeded by two hours the positivity of a PB-BC. CVC contamination was defined by the presence of a positive CVC-BC and a negative PB-BC. For the porpuse of this study, CVC contaminations were included in the analysis and considered as bacteremia, considering that the clinical management of these cases is not different with respect to the clinical management of any other BSI. Subsequent positivity of a blood culture after at least 7 days following first positivity was considered as a separate BSI, if blood culture negativity was defined, in the meanwhile. Gram negative bacteria were considered extended spectrum beta-lactamase (ESBL) producers according to the published laboratory tests.[18,19] Resistance to at least 3 antibiotics among ceftazidime or cefepime, piperacillin/tazobactam, ciprofloxacin, gentamicin, imipenem or meropenem were the basis for multi-drug resistance definition (MDR). For the definition of resistance we referred to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) 2016.[20] Briefly, the Minimal Inhibitory Concentrations (MIC) considered for antibiotics resistance were the followings: ceftazidime or cefepime > 8 mg/L for P. *aeruginosa* and > 4 mg/L for enterobacteria, respectively; piperacillin/tazobactam > 16 mg/L, cirpofloxacin > 1 mg/L, gentamicin > 4 mg/L and

carbapenems > 8 mg/L both for P. *aeruginosa* and for enetrobacteria.

Transplantation procedures: The patients included in this analysis were transplanted during the 6-years period with either reduced intensity conditioning regimen (RIC) or myeloablative conditioning regimen (MAC), depending on age, disease type and comorbidity. All the RIC regimens were fludarabine and thiotepa or busulfan-based, whereas the MAC regimens were busulfan or total-body irradiation-based. GVHD prophylaxis consisted on cyclosporine and methotrexate, with the addition of anti-thymocyte globulin (ATG) in cases of matched unrelated donors (MUD). In the haploidentical setting, GVHD prophylaxis consisted on cyclosporine, mycophenolate and post-transplant cyclofosfamide.[21] HLA matching was based on molecular four digits typing of a minimum of 4 loci (A, B, C, DRB1). Peripheral blood was used as the preferred stem cells source. Acute and chronic GVHD were graded as previously published.[22,23] All the patients had a Groshong CVC at the time of conditioning regimen intiation. At hospital admittance, mucosal swabs (nasal, mouth, axillary, rectal and genital) were performed in all the patients, with the objective to detect bacterial colonization. In case of positive coltures, antibiograms were included in the laboratory report, with focus on vancomycin resistant *Enterococci* (VRE), bacteria ESBL-producing or carbapenemase-producing enterobacteriacae. Engraftment was defined as an absolute count of neutrophils greater than 500/mmc for at least three consecutive days.

Porphylaxis and management of infections: All the patients received standard antimicrobical prophylaxis with levofloxacine, acyclovir and fluconazole from day 0. Levofloxacine was discontinued at the engraftment. Patients with a previous history of possible or probable invasive fungal infection (IFI) received secondary anti-fungal prophylaxis with liposomal B-amphotericine. Pneumocystis *carinii* prophylaxis with trimethoprim sulphametoxazole was started at the time of neutrophil engraftment. Pre-emptive therapy for CMV with gancyclovir was started in the event of CMV-DNA positivity as detected by quantitative real-time PCR, in at least two examinations within 1 week.

As previosuly described, the diagnostic work up at fever onset, or in case of any symptom of infection, consisted on blood cultures collection, collection of culture samples from any site with suspect infection and chest X-ray. Then, floroquinolone prophylaxis was stopped and broad spectrum i.v. antibiotics were started. Empirical antimicrobical therapy consisted on ceftazidime or piperacillin + tazobactam with or without the addition of glycopeptide (e.g. teicoplanine) depending on the clinical conditions of the patient (e.g. hypotension, gastrointestinal mucositis). In case of fever persistance after 72 hours of broad spectrum antibiotics with persistent negativity of blood cultures, the diagnostic work-up for an IFI was started. This consisted on a high-resolution CT scan of the lung, blood samples for galactomannan dosage and broncoscopy with bronco-alveolar lavage and tissue specimen whenever clinically possible. In case of possible IFI according to the published criteria,[24] empirical therapy was started, using liposomal B-amphotericine or caspofungin.

Statistical analysis: For the purpose of descriptive analysis, continuous variables were summarized as median and range, categorical as frequencies and percentages. Differences between groups were analyzed with Chi-square and Mann-Whitney U tests for categorical and continuous variables, respectively. Survival analysis was performed according to Kaplan-Meier method and log-rank test was used to evaluate differences between subgroups. All tests were 2-sided and p values below 0.05 were considered statistically significant. Analyses were carried out with EZR software version 1.33.[25]

Results.

Patients' characteristics: From 2010 to 2015, 162 patients with hematological malignancies were submitted to allo-SCT. Among these, 80 patients (49%) experienced a BSI, for a total of 119 isolates. Eighty-two (51%) out of 162 patients had no BSIs. Patient demografics are reported in **Table 1**. Briefly, the median age was 48 years (17-68), approximately two thirds of the patients were transplanted for acute leukemias (AL) (93/162, 57%) and 70/162 (43%) patients were transplanted in 1st complete remission (CR). Seventy-one (44%) out of 162 patients received a MAC, 122 (76%) were transplanted using peripheral blood

Table 1. Clinical and biological characteristics of the 80 allotransplanted patients with BSI vs the 82 allotransplanted patients without BSI.

Variables	Patients submitted to allo-SCT (n=162)	
	N	**%**
Sex		
- M	103	64
- F	59	36
Age (Median – range)	48 (17-68)	-
Disease		
- AL/MDS	93	57
- HL	4	9
- NHL	14	9
- MM	19	12
- Other	22	13
Disease phase		
- 1st CR	70	43
Patients with pre-SCT colonization		
- ESBL producer enterobacteria	6	4
- Carbapenem-resistant enterobacteria	7	4
- MDR P. *aeruginosa*	0	0
- VRE	0	0
Conditioning		
- MAC	71	44
- RIC	91	56
SC source		
- BM	33	20
- PB	122	76
- CB	7	4
Donor		
- Sibling	58	36
- MUD	86	53
- Haplo	11	7
- CB	7	1
GVHD prophylaxis		
- CyA + MTX + ATG	86	53
- CyA + MTX	58	36
- CyA + MMF + ATG	7	4
- CyA + MMF + other	11	7
aGVHD ≥ 2	56	35
cGVHD (extensive)	19	12
Follow up (months; median – range)	14 (0 - 68)	-

List of abbreviations: AL: acute leukemia; MDS: myelodisplastic syndrome; HL: Hodgkin Lymphoma; NHL: Non Hodgkin Lymphoma; MM: Multiple Myeloma; CR: Complete Remission; VRE: vancomycin-resistantant enterobacteria; MAC: Myeloablative Conditioning; RIC: Reduced Intensity Conditioning; BM: Bone Marrow; PB: Peripheral Blood; CB: Cord Blood; MUD: Matched Unrelated Donor; Haplo: Haploidentical Transplant; CyA: Cyclosporine A; MTX: Methotrexate; ATG: Anti-Thymocyte Globulin; MMF: Mophetil Mycophenolate; GVHD: Graft Versus Host Disease; NS: non significant.

stem cells (PBSC) and 86 (53%) received a transplant from a MUD. During patients' follow up the incidence of aGVHD grade ≥ 2 was 35% (56/162 cases) and the incidence of extensive cGVHD was 12% (19/162 cases). The incidence of possible/probable IFI was 35% (57/162 cases) and the incidence of CMV reactivation was 59% (95/162 cases). The median follow 14 months (range 0-68). No significant differences were observed comparing the clinical and biological characteristics of patients with BSIs (BSI-pos) and patients without BSIs (BSI-neg) (data not shown). As a consequence we were not able to identify any factor possibly correlated with the risk of developing a BSI.

Bloodstream infections: One-hundred nineteen isolates were obtained from the blood samples of 80 patients with BSI (BSI-pos). The median time of positivity of BCs was 19 days from transplant (range day -4 to day +921). In 42/80 patients (52%) the positive blood culture was detected

before day +19 from allo-SCT. Half (n° 59) of the positive BCs derived from PB samples and half (n° 60) could be considered CVC-related. Thirty-five out of 119 (29%) positive BCs were considered as contamination as previously defined. Therefore, 84/119 (71%) of the BSIs could be considered real bacteremia according to microbiological criteria. In 27/84 cases (24%) the BSI could be defined CVC-related, according to the above reported criteria.

Considering epidemiology, 77/119 (65%) and 42/119 (35%) BCs were positive for Gram positive and Gram negative bacteria, respectively. Data on the different species distribution is reported in **Table 2**. Polimicrobical BSIs were found in 11/119 (9%). In one patient the BCs were positive for a Gram positive bacteria (S. *heamolyticus*) toghether with a Candida parapsilosis.

Antibiotics resistance is reported in **Table 2**. S. *epidermidis* was resistant to methycillin in all the cases and to fluoroquinolones in 90% of the cases. Sixty-seven percent of E. *coli* were ESBL producers and 92% were resistant to fluoroquinolones. Moreover, 40% and 90% of P. *aeruginosa* were resitant to carbapenems and fluoroquinolones, respectively. No carbapenemase-producing K. *pneumoniae* (KPC) was isolated in our series.

When we analyzed the clinical and transplant characteristics of the patients who experienced a Gram positive or a Gram negative BSI, we found that: patients with Gram negative BSIs were more frequently affected by acute leukemia/myelodisplastic syndrome (83% vs 54%, p=0,006) and were more frequently transplanted from cord blood (14% vs 3%, p=0,04). On the other hand, patients with Gram positive BSIs were more frequently allotransplanted using a RIC regimen (69% vs 51%, p=0,004).

Gram positive / Gram negative ratio was 1 and 3,2 considering early BSIs (before day +19) *vs* late BSIs (after day +19) (p= 0,004). This difference was related to the reduction of Gram negative bacteremia after recovery from agranulocytosis (p=0,001). When we separately analyzed the clinical and biological characteristics and the different species distribution according to the time of positivity of the blood culture (before or after day +19 from allo-SCT), we were not able to identify any significant difference (data not shown). With the aim to identify factors correlated with the time of development of BSIs (during or after aplasia), we analyzed the clinical and biological variables of patients with positive BCs, according to the time of BC positivity [before engraftement (n=47) and after engraftment (n=33)]. We found that BSIs in patients transplanted from a sibling donor were more frequent after engraftement (45% vs 23%; p=0,05) and that patients who developed a BSI during aplasia had a higher incidence of IFI (47% vs 24%; p=0,04).

Forty-four (55%) out of 80 patients had an organ involvement together with the BSI. This was the lung in 57% of the cases (25/44 cases), and the gut in 14% (6/44 cases). Within the 6 years of the observation time, we found a homogeneous Gram positive / Gram negative ratio in all the years, with the exception of 2012 and 2013, where we observed a reduction in the number of positive BCs (11 in 2012 and 13 in 2013) and a reduction in Gram positive / Gram negative ratio (1,2 in 2012 and 0,6 in 2013). No statistical significant differences were observed comparing the number of positive BCs and the Gram positive / Gram negative ratio in the single years (data not shown).

Pre-transplant microbiological-history: Twenty-nine out of 80 (36%) patients with BSIs experienced other microbiological isolates during the treatment of the hematological disease before allo-SCT, for a total of 55 isolates. In 12/29 cases (34%) the specie responsible of the BSI before and after the transplant treatment phase was the same, and the antibiograms were comparable. In 27 (49%) and 28 (51%) out of 55 BSIs a Gram positive and Gram negative bacteria was isolated, respectively. Among Gram positive and Gram negative bacteria, coagulase negative Staphylococci (14/27, 52%) and E. *coli* (22/28, 76%) were mostly represented.

Thirteen out of 162 (8%) patients of this series were colonized by resistant microorganisms (ESBL producers, carbapenem-resistant enterobacteria, MDR P. *aeruginosa* and VRE). In particular, 5/80 (6%) of patients who developed a BSI were colonized by carbapenem-resitant E. *coli*. All these patients experienced a BSI caused by carbapenem-resistant E. *coli*. Moreover, 8/82 (10%) patients who did not experienced a BSI were colonized by resistant microorganisms (E. *coli* ESBL producers in 5 cases, E. *coli* carbapenem-resistant in 2 cases and K. *pneumonia*

Table 2. Antibiotic resistance of the 119 species isolated from 80 allotransplanted patients.

	N° of BCs (%)	MRSA (%)	Penicillin-R (%)	Quinolones-R (%)
GRAM positive	77/119 (65)	//	//	//
S. *epidermidis*	42/77 (55)	100	100	90
S. *haemolyticus*	9/77 (12)	100	100	100
S. *hominis*	8/77 (10)	75	75	100
S. *aureus*	1/77 (1)	100	0	100
S. *warneri*	1/77 (1)	0	0	0
E. *faecalis*	6/77 (8)	//	33	100
E. *faecium*	3/77 (4)	//	33	100
C. *jeikeium*	3/77 (4)	//	100	100
C. *striatum*	1/77 (1)	//	0	0
C. *urealyticum*	1/77 (1)	//	0	0
B. *firmus*	1/77 (1)	//	0	0
Difteroides	1/77 (1)	//	0	0
	N° of BCs (%)	**ESBL+ (%)**	**Carbapenems-R (%)**	**Quinolones-R (%)**
GRAM negative	42/119 (35)			
E. *coli*	24/42 (57)	67	0	92
P. *aeruginosa*	10/42 (24)	//	40	90
A. *baumanii*	1/42 (2)	//	0	100
E. *cloacae*	1/42 (2)	//	0	0
R. *radiobacter*	1/42 (2)	//	0	0
K. *pneumoniae*	1/42 (2)	0	0	0
S. *liquefaciens*	1/42 (2)	//	0	0
P. *mirabilis*	1/42 (2)	//	0	0
Salmonella *ssp*	1/42 (2)	//	0	0
S. *maltophila*	1/42 (2)	//	0	0

ESBL-producer in 1 case). No MDR P. *aeruginosa* or VRE were isolated.

Outcome: The overall survival of the 80 BSI-pos patients compared to the 82 BSI-neg patients statistical significant differences were observed (data not showed). Thirty out of 47 (64%) and 17/33 (52%) patients with BSIs before and after engraftement died, respectively (p=0,27). The projected 2 years OS for patients with BSI *vs* patients without BSI is 46% *vs* 60% (HR 1.48, 95% CI: 0,96-2,29; p=0,07). Major causes of death in the BSI-pos vs BSI-neg groups were: disease relapse (42% vs 55%; p= 0,37), infections (42% vs 17%; p= 0,02), aGVHD (2% vs 8%; p= 0,36), cGVHD (5% vs 6%; p= 1,00) and toxixity (2% vs 6%; p= 0,30). As reported in **Figure 1B** the transplant related mortality (TRM) among BSI-pos and BSI-neg patients was comparable (p=0,22). is reported in **Figure 1A**. Fourty-seven out of 80 BSI-pos patients (59%) *vs* 35/82 BSI-neg patients (43%) died during follow up (p=0,2). Considering the outcome according to the distribution of BSIs before (n=47) or after (n=33) the engraftement, no

Fifteen out of 80 patients with positive BSI (19%), died because of the bacterial infection. Considering the 162 allotransplanted patients, the BSI related mortality was 9% (15/162 cases). Nine out of these 15 deaths (60%) were related to Gram positive bacteria, leading to a Gram positive related mortality of 12% (9/77 Gram + isolates). Six out of 15 infection-related deaths (40%) were caused by a Gram negative agent, leading to a Gram negative related mortality of 14% (6/42 Gram negative isolates). Interestingly, we observed that among the Gram positive bacteria the mortality rate was 33% for Enterococci (3/9 isolates), 22% for S. *haemolyticus* (2/9 isolates) and 10% for S. *epidermidis* (4/42 isolates).

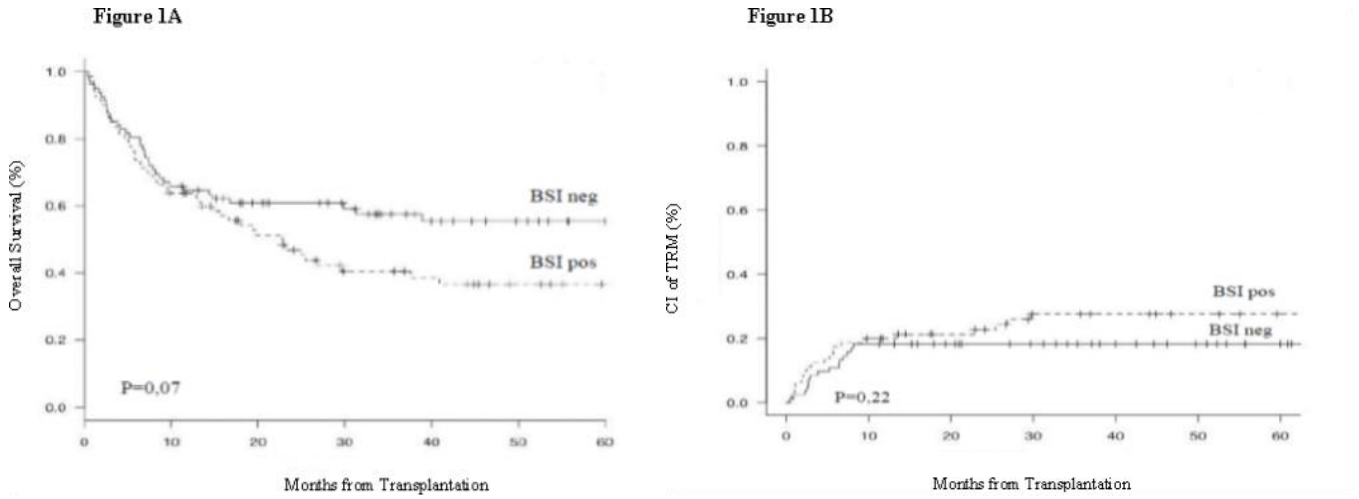

Figure 1A. Overall survival of the 162 allotransplanted patients according to presence or not of a BSI.
Figure 1B. Transplant related mortality of the 162 allotransplanted patients according to presence or not of a BSI. CI= Cumulative Incidence; TRM = Transplant Related Mortality

Considering Gram negative bacteria, the mortality rate was 50% for P. *aeruginosa* (5/10 isolates, in 1 case MDR, in 4 cases multisensible) and 4% for E. *coli* (1/24 isolates, ESBL producer).

Discussion. Infective complications are commonly considered the most relevant event associated with increased morbidity and mortality in allotransplanted patients (1 - 12), and the microbiological surveillance of bacterial isolates in Transplant Units is a mainstay of good clinical practice. In this view, we collected the data on 162 allotransplanted patients, 80 of whom (49%) experienced a BSI, over a period of 6 years of transplant activity. The total number of positive BCs in the period of observation was 119, 84 of whom (71%) could be considered as real bacteremia.

Overall, our data are in line with previous reports, that cover a longer period (10-15 years), showing a predominance of Gram positive over Gram negative bacteria (65% vs 35%), being S. *epidermidis* (55%) and E. *coli* (57%) the predominant species among Gram positive and Gram negative BSIs (**Table 2**).[4,5,6,8,12,16,17] We were not able to identify clinical and transplant variables significantly associated with the development of BSIs, and this is probably related to the relatively low number of patients in each subgroup. Interestingly, patients with a Gram negative BSI were more frequently affected by acute leukemia or myelodisplastic syndrome (p=0,006) and were more frequently transplanted from cord blood (p=0,04). Although the number of patients in each group is relatively small to draw final conclusions, we can speculate that these differences may be caused by the intensive pre-tranplant treatement, by the high prevalence of refractory disease among acute leukemia patients and by the delayed neutrophil recovery observed when a cord blood is used as stem cell source, respectively. On the other hand, a BSI sustained by a Gram positive bacteria was more frequent in patients allotransplanted using a RIC regimen (p=0,04). This may partially reflect the characteristics of these patients (e.g. frail and elderly patients).

One point of interest in the field of bacterial infections is antimicrobical resistance.[14-17] Our data confirm that this problem has now reached the highest level of criticism, as we observed fluoroquinolones resistance both among Gram positive (roughly 100%) and Gram negative (between 90 and 100%) bacteria, toghether with methicillin resistance among Gram positive bacteria (100% of the S. *aureus*, *epidermidis* and *haemolyticus* and 75% of the S. *hominis*) (**Table 2**). This is in line with previously reported series[12,17] and it is strongly associated with the large use of fluoroquinolones for prophylaxis during the aplastic phase, although this practice is commonly suggested by the most recently published guidelines.[26] Considering these data, the debate on the utility of fluoroquinolones prophylaxis is still opened. This policy, indeed, may reduce the mortality of Gram negative bacteria, but other propylaxis are under investigation. Recently, Pohlen and Colleagues, reported on a study comparing ciprofloxacin *versus* colistin prophylaxis in AML patients during

neutropenia.[28] Although this was not a randomized trial, ciprofloxacin prophylaxis was confirmed highly effective in reducing the incidence of infections (69% *vs* 79% for colistin; p=0,07), but was confirmed to be associated with fluoroquinolone resistance, as expected. Moreover, 67% of E. *coli* was ESBL producer and 40% of P. *aeruginosa* was resistant to carbapenems, and this is similar to what previously reported.[12,15] Interestingly, no KPC was isolated. This point is of interest, because currently data from the litterature suggest that carbapenemase-producing enterobacteriacae (namely KPC*)* is an emerging problem in hematological patients, particularly challenging among allotransplanted patients. As reported by Girmenia and Colleagues in a retrospective italian survey, the incidence of KPC infections in allotransplanted patients was 2%, with a high-risk of infections in colonized patients. Moreover, the infection related mortality was 64%.[27] One possible explanation for the absence of KPC in our series may be related to the fact that we did not observe any pre-transplant colonization sustained by this bacteria. Alltogether these data enhance the importance of microbiobiological surveillance with the aim to promptly start patients' isolation and reduce MDR bacteria spreading.

Looking at the species distribution per year in our series, the Gram positive / Gram negative ratio, as well as the isolated species, remained costant, with the exception of 2 years (2012 and 2013), in which an overall reduction in BSIs and a reduction of the Gram positive / Gram negative ratio was observed . Although these differences do not reach the level of significance, they may be partially explained considering the different management of CVC in the years 2012 and 2013, when we had a single nurse dedicated to CVC medications. Considering the high incidence of CVC-related infections (24%), the adoption of clinical-care strategies such as CVC medication under optimal asepsis and by dedicated nurses may be the best way to prevent BSIs.

Colonization by multiresistant microorganisms was detected in a small proportion of patients (13/162, 8%), and was mainly sustained by ESBL-producer or carbapenem-resistant enterobacteria (namely E. *coli*). Due to the relatively small number of cases we could not compare the outcome according to colonization. We indeed observed that all the patients colonized by carbapenem-resistant E. *coli* experienced a BSI caused by a similar resistant microorganism.

Moving from epidemiology to outcome, we observed that 15/162 allotransplanted patients (9%) died because of BSIs and this mortality rate is comparable to the one observed in other reports.[7,8,29] P. *aeruginosa* can be still considered the major killer, irrespective of its resistance profile (5 deaths out of 10 cases – 50%, similarly to what reported by Collin et al - in one case only MDR). Enterococci, coagulase negative Staphylococci, and E. *coli* showed a mortality of 33% (3/9 cases), 12% (6/51 cases) and 4% (1/24 cases), respectively. It should be stressed that in 12/15 (80%) of these bacteremia-related deaths other factors, such as GVHD or active disease at transplant, were present at the time of BSI. Moreover, we observed that the long term outcome of patients who experience a BSI was impaired with respect to those who do not experience this complication (2 years OS for patients with BSI *vs* patients without BSI: 46% vs 60%; p=0,07). Although the TRM of the two groups is comparable (**Figure 1B**), the difference in mortality among patients with BSI and those without BSI is more evident after at least 12 months from transplant. This may be partially related to the fact that bacteremia are present in the late phase of the transplant too and usually affects extremely frail patients, such as those with GVHD and chronic steroid treatment. In such critically hill patients, a bacteremia may rapidly and negatively influence the outcome.

In conclusion, BSIs continue to be a significant event in allotransplanted patients, with Gram positive bacteria being the species at highest incidence and P. *aeruginosa* being the specie with the highest mortality. Routinely use of fluoroquinolone prophylaxis and prompt empirical antimicrobical therapy significantly reduces the mortality related to bacterial infections, but emergence of quinolones resistance in the great majority of Gram positive and negative species remain an unsolved issue. Even though further studies on prophylaxis in allotransplanted patients during neutropenia are warranted, we have no data to change our policy, considering the outcome of the reported cases of BSIs. A modern approach to the problem of BSIs is probably the investigation of the patients' microbioma before allo-SCT. Some data from the literature reported that intestinal domination, defined as occupation of at least 30 %

of the microbiota by a single bacterial taxon, is associated with BSI in patients undergoing allo-SCT, that the gut microbiota can identify high-risk patients before allo-SCT and that manipulation of the gut microbiota for prevention of BSIs in high-risk patients may be a useful direction for future research.[30,31,32] Thus, in the near future, we will probably need to include this analysis in the baseline work up and in the follow up of patients addressed to allo-SCT.

Authors' contributions: MM, BR and DR designed the study. All the Authors collected the data. MM, BR, GR, CC, NP and DR analyzed the results. All the Authors gave their final approval to the Manuscript.

References:

1. Williamson EC, Millar MR, Steward CG, Cornish JM, Foot AB, Oakhill A, Pamphilon DH, Reeves B, Caul EO, Warnock DW, Marks DI. Infections in adults undergoing unrelated donor bone marrow transplantation. Br J Haematol 1999; 104:560-8. https://doi.org/10.1046/j.1365-2141.1999.01229.x PMid:10086795
2. Ninin E, Milpied N, Moreau P, André-Richet B, Morineau N, Mahé B, Vigier M, Imbert BM, Morin O, Harousseau JL, Richet H. Longitudinal study of bacterial, viral, and fungal infections in adult recipients of bone marrow transplants. Clin Infect Dis 2001; 33:41-7. https://doi.org/10.1086/320871 PMid:11389493
3. Marena C, Zecca M, Carenini ML, Bruschi A, Bassi ML, Olivieri P, Azzaretti S, Locatelli F. Incidence of, and risk factors for, nosocomial infections among hematopoietic stem cell transplantation recipients, with impact on procedure-related mortality. Infect Control Hosp Epidemiol 2001; 22:510-7. https://doi.org/10.1086/501942 PMid:11700879
4. Poutsiaka DD, Price LL, Ucuzian A, Chan GW, Miller KB, Snydman DR. Blood stream infection after hematopoietic stem cell transplantation is associated with increased mortality. Bone Marrow Transplant 2007 ;40:63-70. https://doi.org/10.1038/sj.bmt.1705690 PMid:17468772
5. Almyroudis NG, Fuller A, Jakubowski A, Sepkowitz K, Jaffe D, Small TN, Kiehn TE, Pamer E, Papanicolaou GA. Pre- and post-engraftment bloodstream infection rates and associated mortality in allogeneic hematopoietic stem cell transplant recipients. Transpl Infect Dis 2005 ;7:11-7. https://doi.org/10.1111/j.1399-3062.2005.00088.x PMid:15984943
6. Collin BA, Leather HL, Wingard JR, Ramphal R. Evolution, incidence, and susceptibility of bacterial bloodstream isolates from 519 bone marrow transplant patients. Clin Infect Dis 2001 ;33:947-53. https://doi.org/10.1086/322604 PMid:11528564
7. Cappellano P, Viscoli C, Bruzzi P, Van Lint MT, Pereira CA, Bacigalupo A. Epidemiology and risk factors for bloodstream infections after allogeneic hematopoietic stem cell transplantion. New Microbiol 2007;30:89-99. PMid:17619251
8. Ortega M, Rovira M, Almela M, Marco F, de la Bellacasa JP, Martínez JA, Carreras E, Mensa J. Bacterial and fungal bloodstream isolates from 796 hematopoietic stem cell transplant recipients between 1991 and 2000. Ann Hematol 2005 ;84:40-6. https://doi.org/10.1007/s00277-004-0909-0 PMid:15480665
9. Lukenbill J, Rybicki L, Sekeres MA, Zaman MO, Copelan A, Haddad H, Fraser T, DiGiorgio MJ, Hanna R, Duong H, Hill B, Kalaycio M, Sobecks R, Bolwell B, Copelan E. Defining incidence, risk factors, and impact on survival of central line-associated blood stream infections following hematopoietic cell transplantation in acute myeloid leukemia and myelodysplastic syndrome. Biol Blood Marrow Transplant 2013; 19:720-4. https://doi.org/10.1016/j.bbmt.2013.01.022 PMid:23380342
10. Shigematsu A, Yamamoto S, Sugita J, Kondo T, Onozawa M, Kahata K, Endo T, Shiratori S, Ota S, Yamaguchi K, Wakasa K, Takahata M, Goto H, Ito S, Takemura R, Tanaka J, Hashino S, Nishio M, Koike T, Asaka M, Imamura M. Increased risk of bacterial infection after engraftment in patients treated with allogeneic bone marrow transplantation following reduced-intensity conditioning regimen. Transpl Infect Dis 2010 ;12:412-20. https://doi.org/10.1111/j.1399-3062.2010.00560.x PMid:20738830
11. Liu CY, Lai YC, Huang LJ, Yang YW, Chen TL, Hsiao LT, Liu JH, Gau JP, Chen PM, Tzeng CH, Chiou TJ. Impact of bloodstream infections on outcome and the influence of prophylactic oral antibiotic regimens in allogeneic hematopoietic SCT recipients. Bone Marrow Transplant 2011 ;46:1231-9. https://doi.org/10.1038/bmt.2010.286 PMid:21113186
12. Busca A, Cavecchia I, Locatelli F, D'Ardia S, De Rosa FG, Marmont F, Ciccone G, Baldi I, Serra R, Gaido E, Falda M. Blood stream infections after allogeneic stem cell transplantation: a single-center experience with the use of levofloxacin prophylaxis. Transpl Infect Dis 2012 ;14:40-8. https://doi.org/10.1111/j.1399-3062.2011.00650.x PMid:21599817
13. Ferrara JL, Levine JE, Reddy P, Holler E. Graft-versus-host disease. Lancet 2009; 373:1550-61. https://doi.org/10.1016/S0140-6736(09)60237-3
14. El-Mahallawy H, Samir I, Abdel Fattah R, Kadry D, El-Kholy A. Source, pattern and antibiotic resistance of blood stream infections in hematopoietic stem cell transplant recipients. J Egypt Natl Canc Inst 2014; 26:73-7. https://doi.org/10.1016/j.jnci.2013.12.001 PMid:24841157
15. Therriault BL, Wilson JW, Barreto JN, Estes LL. Characterization of bacterial infections in allogeneic hematopoietic stem cell transplant recipients who received prophylactic levofloxacin with either penicillin or doxycycline. Mayo Clin Proc 2010; 85:711-8. https://doi.org/10.4065/mcp.2010.0006 PMid:20675508 PMCid:PMC2912731
16. Oliveira AL, de Souza M, Carvalho-Dias VM, Ruiz MA, Silla L, Tanaka PY, Simões BP, Trabasso P, Seber A, Lotfi CJ, Zanichelli MA, Araujo VR, Godoy C, Maiolino A, Urakawa P, Cunha CA, de Souza CA, Pasquini R, Nucci M. Epidemiology of bacteremia and factors associated with multi-drug-resistant gram-negative bacteremia in hematopoietic stem cell transplant recipients. Bone Marrow Transplant 2007; 39:775-81. https://doi.org/10.1038/sj.bmt.1705677 PMid:17438585
17. Mikulska M, Del Bono V, Raiola AM, Bruno B, Gualandi F, Occhini D, di Grazia C, Frassoni F, Bacigalupo A, Viscoli C. Blood stream infections in allogeneic hematopoietic stem cell transplant recipients: reemergence of Gram-negative rods and increasing antibiotic resistance. Biol Blood Marrow Transplant 2009; 15:47-53. https://doi.org/10.1016/j.bbmt.2008.10.024 PMid:19135942
18. Rupp ME, Fey PD. Extended spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae: considerations for diagnosis, prevention and drug treatment. Drugs 2003; 63:353-65. https://doi.org/10.2165/00003495-200363040-00002
19. Harada S, Ishii Y, Yamaguchi K. Extended-spectrum beta-lactamases: implications for the clinical laboratory and therapy. Korean J Lab Med 2008; 28:401-12. https://doi.org/10.3343/kjlm.2008.28.6.401 PMid:19127103
20. The European Committee on Antimicrobial Susceptibility Testing. (EUCAST). http://www.eucast.org/
21. Slade M, Fakhri B, Savani BN, Romee R. Halfway there: the past, present and future of haploidentical transplantation. Bone Marrow Transplant 2016; e-pub ahead of print Jul 25; doi: 10.1038/bmt.2016.190. https://doi.org/10.1038/bmt.2016.190
22. Rowlings PA, Przepiorka D, Klein JP, Gale RP, Passweg JR, Henslee-Downey PJ, Cahn JY, Calderwood S, Gratwohl A, Socié G, Abecasis MM, Sobocinski KA, Zhang MJ, Horowitz MM. IBMTR Severity Index for grading acute graft-versus-host disease: retrospective comparison with Glucksberg grade. Br J Haematol 1997; 97:855-64. https://doi.org/10.1046/j.1365-2141.1997.1112925.x PMid:9217189

23. Filipovich AH, Weisdorf D, Pavletic S, Socie G, Wingard JR, Lee SJ, Martin P, Chien J, Przepiorka D, Couriel D, Cowen EW, Dinndorf P, Farrell A, Hartzman R, Henslee-Downey J, Jacobsohn D, McDonald G, Mittleman B, Rizzo JD, Robinson M, Schubert M, Schultz K, Shulman H, Turner M, Vogelsang G, Flowers ME. National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: I. Diagnosis and staging working group report. Biol Blood Marrow Transplant 2005; 11:945-56.https://doi.org/10.1016/j.bbmt.2005.09.004 PMid:16338616
24. Pagano L, Caira M, Nosari A, Van Lint MT, Candoni A, Offidani M, Aloisi T, Irrera G, Bonini A, Picardi M, Caramatti C, Invernizzi R, Mattei D, Melillo L, de Waure C, Reddiconto G, Fianchi L, Valentini CG, Girmenia C, Leone G, Aversa F. Fungal infections in recipients of hematopoietic stem cell transplants: results of the SEIFEM B-2004 study--Sorveglianza Epidemiologica Infezioni Fungine Nelle Emopatie Maligne. Clin Infect Dis 2007; 45:1161-70. https://doi.org/10.1086/522189 PMid:17918077
25. Kanda Y. Investigation of the freely available easy-to-use software 'EZR' for medical statistics. Bone Marrow Transplant 2013; 48:452-8. https://doi.org/10.1038/bmt.2012.244 PMid:23208313 PMCid:PMC3590441
26. Bucaneve G, Micozzi A, Menichetti F, Martino P, Dionisi MS, Martinelli G, Allione B, D'Antonio D, Buelli M, Nosari AM, Cilloni D, Zuffa E, Cantaffa R, Specchia G, Amadori S, Fabbiano F, Deliliers GL, Lauria F, Foà R, Del Favero A; Gruppo Italiano Malattie Ematologiche dell'Adulto (GIMEMA) Infection Program. Levofloxacin to prevent bacterial infection in patients with cancer and neutropenia. N Engl J Med 2005; 353:977-87. https://doi.org/10.1056/NEJMoa044097 PMid:16148283
27. Girmenia C, Rossolini GM, Piciocchi A, Bertaina A, Pisapia G, Pastore D, Sica S, Severino A, Cudillo L, Ciceri F, Scimè R, Lombardini L, Viscoli C, Rambaldi A; Gruppo Italiano Trapianto Midollo Osseo (GITMO); Gruppo Italiano Trapianto Midollo Osseo GITMO. Infections by carbapenem-resistant Klebsiella pneumoniae in SCT recipients: a nationwide retrospective survey from Italy. Bone Marrow Transplant 2015;50:282-8. https://doi.org/10.1038/bmt.2014.231 PMid:25310302
28. Pohlen M, Marx J, Mellmann A, Becker K, Mesters RM, Mikesch JH, Schliemann C, Lenz G, Müller-Tidow C, Büchner T, Krug U, Stelljes M, Karch H, Peters G, Gerth HU, Görlich D, Berdel WE. Ciprofloxacin versus colistin prophylaxis during neutropenia in acute myeloid leukemia: two parallel patient cohorts treated in a single center. Haematologica 2016; 101:1208-1215. https://doi.org/10.3324/haematol.2016.147934 PMid:27470601 PMCid:PMC5046650
29. Orasch C, Weisser M, Mertz D, Conen A, Heim D, Christen S, Gratwohl A, Battegay M, Widmer A, Flückiger U. Comparison of infectious complications during induction/consolidation chemotherapy versus allogeneic hematopoietic stem cell transplantation. Bone Marrow Transplant 2010; 45:521-6. https://doi.org/10.1038/bmt.2009.187 PMid:19668238
30. Taur Y, Xavier JB, Lipuma L, Ubeda C, Goldberg J, Gobourne A, Lee YJ, Dubin KA, Socci ND, Viale A, Perales MA, Jenq RR, van den Brink MR, Pamer EG. Intestinal domination and the risk of bacteremia in patients undergoing allogeneic hematopoietic stem cell transplantation. Clin Infect Dis 2012; 55:905-14. https://doi.org/10.1093/cid/cis580 PMid:22718773 PMCid:PMC3657523
31. Montassier E, Al-Ghalith GA, Ward T, Corvec S, Gastinne T, Potel G, Moreau P, de la Cochetiere MF, Batard E, Knights D. Pretreatment gut microbiome predicts chemotherapy-related bloodstream infection. Genome Med 2016; 8:49. https://doi.org/10.1186/s13073-016-0301-4 PMid:27121964 PMCid:PMC4848771
32. Laterza L., Rizzatti G., Gaetani E., Chiusolo P., Gasbarrini A. The gut microbiota and immune system relationship in human graft-versus-host disease. Mediterr J Hematol Infect Dis 2016, 8(1): e2016025, DOI: http://dx.doi.org/10.4084/MJHID.2016.025https://doi.org/10.4084/mjhid.2016.025