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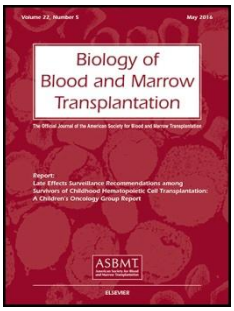

Title: Clinical Impact of Pre-Transplant Multidrug-Resistant Gram-Negative Colonization in Autologous and Allogeneic Hematopoietic Stem Cell Transplantation

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MANUSCRIPT TITLE:

Clinical impact of pre-transplant multidrug-resistant Gram-negative colonization in autologous and allogeneic hematopoietic stem cell transplantation.

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Running title: Pre-transplant multidrug-resistant Gram-negative colonization in HSCT

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Highlights

- Mortality due to multidrug-resistant Gram - strains (MDR-GNB) is high
- Pre-transplant MDR-GNB carriers of are often excluded from HSCT
- A pre-transplant MDR-GNB colonization did not impact on clinical outcomes
- Early targeted therapy in carriers may improve OS and reduce mortality

Abstract

Multidrug-resistant Gram-negative bacteria (MDR-GNB) are an emerging cause of morbidity and mortality after hematopoietic stem cell transplantation (HSCT). Three-hundred-forty-eight consecutive patients transplanted at our hospital from July 2012 to January 2016 were screened for a pre-transplant MDR-GNB colonization and evaluated for clinical outcomes. A pre-transplant MDR-GNB colonization was found in 16.9% of allo-HSCT and in 9.6% of auto-HSCT recipients. Both in auto- and in allo-HSCT, carriers of a MDR-GNB showed no significant differences in overall survival (OS), transplant-related mortality (TRM) nor infection-related mortality (IRM) compared to non-carriers. OS at two years for carriers compared to non-carriers was 85% vs 81% (P=0.262) in auto-HSCT and 50% vs 43% (P=0.091) in allo-HSCT.TRM at two years was 14% vs 5%; (P=0.405) in auto-HSCT and 31% vs 25% (P=0.301) in allo-HSCT. IRM at two years was 14% vs 2% (P=0.142) in auto-HSCT and 23% vs 14% (P=0.304) in allo-HSCT. In multivariate analysis, only grade III-IV acute graft-versus-host disease (GVHD) was an independent factor for reduced OS (P<0.001) and increased TRM (P<0.001) and IRM (P<0.001). During the first year after transplant, we collected 73 GNB bloodstream infectious episodes (BSI) in 54 patients, 42.4% of which sustained by a MDR-GNB. Rectal swabs positivity associated with the pathogen causing subsequent MDR-GNB BSI in 13/31 (41.9%). Overall, OS at four months from MDR-GNB BSI onset was of 67.9%, with a 14-day attributed mortality of

12.9%, not being significantly different between carriers and non-carriers (P=0.207). We conclude that in this extended single-center experience, a pre-transplant MDR-GNB colonization did not significantly influence OS, TRM and IRM both in auto- and allo-HSCT settings and that MDR-GNB attributed mortality can be controlled in carriers when an early pre-emptive antimicrobial therapy is started in case of neutropenic fever.

Introduction

Infections caused by multidrug-resistant Gram-negative bacteria (MDR-GNB) are a public health challenge worldwide[1-4]. An increasing trend in MDR-GNB infections in hematological malignancies and in hematopoietic stem cell transplantation (HSCT) has been recently reported [2,5-7]. The emergence of MDR-GNB is a potential limiting factor in HSCT, facing important clinical issues such as the eligibility to transplant for pre-transplant carriers of a MDR-GNB, the risk of local infection outbreaks and the unacceptably high related mortality. Moreover, the susceptibility pattern to antibiotics and global bacterial epidemiology are changing, as well as the transplanted population and the availability of new drugs[1, 5, 8-11].

In a recent large prospective Italian multicenter study performed on behalf of Gruppo Italiano Trapianto Midollo Osseo (GITMO) in the HSCT population, the overall survival (OS) at 4 months in patients developing pre-engraftment *Pseudomonas aeruginosa* and carbapenem-resistant (CR) *Klebsiella pneumoniae* (CR-Kp) bloodstream infectious episodes (BSI) was 25% and 40%, respectively, with pre-transplant carriers having a probability of approximately 30% of development a subsequent colonizing-pathogen related infection[12]. According to some recent studies, patients developing an

MDR-GNB infection after allo-HSCT have a poor outcome, so that their eligibility to allogeneic procedure is generally carefully evaluated for the risk-to-benefit ratio[13].

The aim of this study was to analyze the impact of a pre-transplant MDR-GNB colonization on overall survival (OS), transplant-related mortality (TRM) and infection-related mortality (IRM) in both autologous and allogeneic HSCT population and to analyze the epidemiology of Gram-negative BSI in MDR-GNB carriers and non-carrier patients.

Patients and methods

Study population

We analyzed data collected from 348 consecutive adult patients undergoing autologous (94/348, 27%) and allogeneic transplant (254/348, 72.9%), hospitalized in the Hematology and Bone Marrow Transplant Unit at IRCCS San Raffaele Scientific Institute Milan, from July 2012 to January 2016 (43 months). Patients undergoing a second or subsequent transplant during the study period were excluded. The median follow-up for survivors was of 602 days (range 4-1375). Gram-negative BSI episodes occurring during the first year after transplant were also recorded.

Microbiological definitions

MDR-GNB colonization was defined as the isolation of the microorganism from rectal swabs, a non-sterile body site, in absence of clinical signs or symptoms of active infection. A GNB BSI was defined as the isolation of the microorganism from one or more blood cultures; a recurrent GBN BSI was defined as the isolation of the microorganism in a patient who previously experienced a GNB BSI and cleared it with documented negative

blood cultures after the first episode. Multi-drug resistance was defined as acquired non-susceptibility to at least 1 agent in 3 or more antimicrobial categories[14].

Microbiological surveillance and transplant procedures

All candidate patients to a first HSCT were screened with a rectal swab before transplant to detect ESBL-producers and carbapenem-resistant Enterobacteriaceae and non-fermenting GNB. Cultures were performed with MacConkey agar plates. Selective chromogenic media plates were not routinely used, so our screening policy did not cover vancomycin-resistant *Enterococcus faecium* (VRE) colonization.

Rectal swabs were then regularly repeated with weekly frequency until discharge. Patients were isolated in single rooms with high-efficiency particulate air filtration from the beginning of conditioning. When MDR-GNB colonization was detected, infection-control strategies were adopted as previously described[15]. Transplants were conducted according to local procedures and treatment protocols. All patients received primary antibacterial prophylaxis (usually with levofloxacin) until engraftment. In case of fever, standard blood cultures were performed with at least two sets of aerobic and anaerobic bottles collected from central lines and peripheral blood during the acute febrile episode.

Empirical antibiotic therapy was usually administered intravenously following our institutional antimicrobial guidelines. At the early onset of neutropenic fever in MDR-GNB carriers, the de-escalation approach was preferred, starting promptly a combination therapy based on the results of the antimicrobial susceptibility test of the rectal swab isolate. In all cases of GNB positivity on blood cultures patients were treated accordingly as most appropriate.

Statistical methods

Continuous variables were summarized according to median and range. Categorical variables were summarized according to their frequency distributions. Comparison of medians of continuous variables between groups was made according to Mann-Whitney U test. Comparison of proportions of categorical variables between groups was made according to Fischer exact test. The probability of OS was estimated using the Kaplan-Meyer estimator. Cumulative incidences (CI) were estimated for graft-versus-host disease (GVHD), TRM and IRM to accommodate competing risks. Relapse or progression of the original disease were competing risks for TRM, death without relapse was a competing risk for relapse, death from any cause was a competing risk for engraftment.

Recurrence of original disease, GVHD and death from any other cause were competing risks for IRM. Both relapse or progression and death from any causes were competing risks for GVHD. Univariate comparisons of survival curves were made using the log-rank test, while the Gray's test was used for univariate comparisons of CI functions. The association between time to death and colonization status and other relevant variables was evaluated in a multivariate analysis using Cox's proportional hazards regression analysis. Potential covariates included in the model were: pre-transplant gut colonization, patient age (continuous variable), type of diagnosis (myeloid vs lymphoid origin), refined disease risk index (DRI) at transplantation, donor type, Sorror Comorbidity index score, GVHD prophylaxis backbone, development of grade III-IV acute GVHD (time dependent variable). Affirmation of the proportional hazard assumption was met for all variables. All tests were two-sided. The type I error rate was fixed at 0.05 for determination of factors associated with time to event. Statistical analyses were performed with the SPSS (SPSS Inc./IBM, Armonk, NY, USA) and R (R Development Core Team, Vienna) software packages.

Results

Patients' characteristics are summarized in **Table 1.** We found no significant differences between carriers and non-carriers regarding age, sex, Sorror HCT Comorbidity Index, CMV host/donor serostatus matching distribution, disease type, disease status at transplant or donor type as reported in **Table 2**.

OS, TRM and IRM in auto-HSCT population

Only nine out of 94 auto-HSCT recipients (9.6%) were found to be colonized by a MDR-GNB before transplant. Distribution of MDR-GNB genus and species and resistance patterns in pre-transplant carriers of auto-HSCT and allo-HSCT recipients are summarized in **Table 3**. The 2-yrs OS in auto-HSCT recipients was 82%, overall. We did not find any significant differences in 2-yrs OS (85% vs 81%; P=0.262), 2-yrs TRM (14% vs 5%; P=0.405) and 2-yrs IRM (14% vs 2%; P=0.142) comparing MDR-GNB carriers to non-carriers (**Figure 1**). Multivariate analysis was not performed in this setting due to the low numbers of patients.

OS, TRM, IRM and GVHD in allo-HSCT population

Forty-three out of 254 (16.9%) patients were found to be colonized by a MDR-GNB before allo-HSCT. The 2-yrs OS after allo-HSCT was 49%. We did not find any significant difference in 2-yrs OS when pre-transplant MDR-GNB carriers to non-carriers were compared (50% vs 43%; P=0.091). Interestingly, in multivariate analysis, only the time-dependent development of acute GVHD (aGVHD) of grade ≥ III (P<0.0001) and a refined disease-risk index (DRI) high or very high (P<0.0001) were significantly associated with a reduced OS. In particular, a pre-transplant MDR-GNB colonization was not associated with a reduced OS (HR=1.417; P=0.129). Transplant-related mortality at two years was 25% overall, and comparable between pre-transplant MDR-GNB carriers and non-carriers (31%

vs 25%; P=0.301). In multivariate analysis, only the development of aGVHD of grade ≥III (HR=7.933; P<0.001) and age > 53 years (HR=1.029; P=0.005) were independent risk factors for increased TRM. As for OS, also for TRM a pre-transplant colonization status was not significantly associated with an increased mortality (HR=1.428; P=0.268). Infection-related mortality at two years was 16% in the allo-HSCT population. A pre-transplant MDR-GNB colonization was not associated with a significant increase of IRM, being of 23% in carriers and 14% in non-carriers (P=0.304) (**Figure 2**). Multivariate analysis for IRM confirmed again that only the development of aGVHD of grade ≥III (HR=18.690; P<0.0001) and the presence of a myeloid-lineage disease (HR=1.450; P=0.034), were the independent risk factors for an increased IRM. A pre-transplant MDR-GNB colonization status was not significantly associated with an increased IRM (HR=1.690; P=0.215). Incidence of aGVHD of grade ≥ III by day 100 was 13% in the entire allo-HSCT population, with no significant differences according to the pre-transplant colonization status, being 21% in carriers and 12% in non-carriers, respectively (P=0.152). Moreover, multivariate analysis showed that a pre-transplant MDR-GNB colonization did not influence the likelihood of developing severe aGVHD (HR=1.948; P=0.069). Results of multivariate analysis are summarized in **Table 4.**

Multidrug-resistant Gram-negative bloodstream infections in HSCT

Patients were observed for one year after transplant and all the BSI caused by Gram-negative bacteria were recorded during this time. Overall, we registered 73 GNB-BSI in 54 patients. Fifteen patients experienced recurrent GNB-BSI either from the same or from a different GNB. Median time from transplant to GNB-BSI onset was 16 days (range 0-308). Among non-colonized patients (n=296), 41/296 developed a GNB-BSI: of these, 30/296 (10%) patients developed a non-MDR GNB-BSI (in details: 16 BSI were sustained by multi-sensible E. Coli, 8 by Pseudomonas spp, 2 by Klebsiella spp, 1 by

Acinetobacter baumanii, 3 by other GNB), while 11/296 (4%) developed a MDR-GNB BSI (in details: 6 BSI were sustained by ESBL-producing E. Coli, 3 by ESBL-producing Kp, 1 by CR-Enterobacter cloacae, 1 by CR-Kp).

Among pre-transplant colonized patients (n=52), 13/52 developed a GNB-BSI: of these, 4/52 (8%) patients developed a non-MDR GNB-BSI (in details: 3 BSI were sustained by multi-sensible E. Coli and 1 by multi-sensible Pseudomonas aeruginosa), while 9/52 (17%) patients had a MDR GNB-BSI (in details: BSI were sustained by CR-Kp, 2 by *Stenotrophomonas maltophilia*, 1 by ESBL-producing E. Coli, 1 by CR- *Pseudomonas aeruginosa*). According to these distribution, pre-transplant carriers had a significant increased number of MDR-GNB BSI compared to non-carriers (9/52 colonized vs 11/296 non-colonized, P=0.0009).

Twenty-seven of 31 (87%) MDR-GNB BSI occurred in allo-HSCT, while only 4/31 (12.9%) in auto-HSCT. In the entire population, MDR GNB BSI were sustained by carbapenem-resistant *Pseudomonas aeruginosa* (5/15) or CR-Kp (10/15), with an attributable mortality of 20% (only three patients died at day six, nine and ten, respectively from BSI onset). Details of microbial isolates and distribution of MDR-GNB BSI according to transplant type are summarized in **Figure 3**. Thirteen out of 31 (41.9%) of MDR-GNB BSI episodes occurred in acute leukemia patients and 70% had active diseases at the onset of sepsis. Median neutrophil count at MDR-GNB BSI onset was $0x10^9$/L (range 0-6.2).

Rectal swabs positivity associated with the pathogen causing subsequent MDR-GNB BSI in 13/31 (41.9%). In particular, 9/13 (69.2%) MDR-GNB BSI were sustained by CR-Kp. Overall, OS at four months from MDR-GNB BSI onset was of 67.9%, with a 14-day crude mortality from BSI onset of 12.9% (4/31). For CR-Kp BSI, OS at four months was of 63%, with a 14-day attributed mortality of 20% (2/10). Interestingly, 14-day attributed mortality after MDR-GNB BSI onset was not significantly different between carriers (n=13) and non-carriers (n=18) (P=0.207), despite in non-carriers, BSI were sustained by $ESBL^+$

E. Coli or ESBL$^+$ Kp (9/18) in 50% of episodes, while in carriers up to 76.9% (10/13) MDR-GNB BSI were caused by CR-Kp (9/10) or CR-*Pseudomonas aeruginosa* (1/10). Details of first and second-line antimicrobial treatments in carriers and non-carriers are detailed in **Table 5**.

Discussion

In this single-center study we analyzed the clinical outcome of 348 patients undergoing autologous or allogeneic HSCT from July 2012 to January 2016 according to their pre-transplant multidrug-resistant Gram-negative bacteria (MDR-GNB) colonization status, and collected all Gram-negative bloodstream infectious episodes (GNB-BSI) during the first year after transplant.

Although recent data suggest an important role also of Gram-positive bacteria such as VRE or methicillin-resistant *Staphylococcus aureus* in allo-HSCT[16], their incidence in our cohort was very low (<1%), making the epidemiological scenario largely dominated by Gram-negative rods, particularly MDR-GNB, the latter becoming the main focus of our study.

In patients receiving auto-HSCT, a rapid immune recovery and lack of pharmacological immunosuppression make TRM and IRM generally low[17]. In the auto-HSCT cohort, transplant outcomes did not significantly differ according to the pre-transplant MDR-GNB colonization, at variance to what has been recently reported by Scheich et al[18] in the same setting. Noticeably, MDR-GNB pre-transplant carriers were only 9.6% of the transplanted population, a proportion significantly lower than that of allo-transplanted patients (16.9%). The reason for this may be due to the different characteristics of the two populations. Autologous HSCT is usually reserved to patients with MM and NHL, who receive the majority of chemotherapy cycles in an outpatient

regimen and are less exposed to prolonged hospitalization periods and broad-spectrum antibiotics.

During the past decades, allo-HSCT transplant activity has increased exponentially[19] compared to auto-HSCT, and is now a potentially curative option also for older patients without expecting a substantial increase in TRM even when HLA-haploidentical donors are used[20]. However, in allo-HSCT, IRM is a cause of major concerns, especially in the recent era of emerging MDR bacteria[21]. In our study, despite some high-risk factors both for TRM and for disease relapse (e.g. prevalence of AML patients and of patients with advanced diseases, use of HLA-haploidentical donors), the 2-yrs TRM in allo-HSCT was 25%, comparable to that reported by other Centers using standard allo-HSCT procedures[22,23] and 2-yrs IRM was 16%, without a substantial increase in MDR-GNB pre-transplant carriers compared to non-carriers and again not significant different from those reported in literature[24]. Nonetheless, the incidence of post-transplant MDR-GNB BSI in carriers was significantly higher than in carriers (17% vs 4%, P=0.0009), suggesting that the acquisition of a MDR GNB colonization may be a surrogate marker more advanced diseases.

In our experience, 42.4% of GNB-BSI were due to a MDR strain, with carbapenem-resistant *Klebsiella pneumoniae* (CR-Kp) and ESBL$^+$ *Escherichia coli* being the most frequent pathogens. The majority of patients with a MDR-GNB BSI had acute leukemia (50%) and grade III-IV neutropenia (74%). Recently, it has been reported that MDR bacterial colonization, evaluated through rectal swabs, decreases OS by increasing TRM and the probability of infectious complications after allo-HSCT, despite the use of antibiotics targeting the gut-colonizing pathogens[25]. On the other hand, Heidenreich et al. in a retrospective single-center study demonstrated that MDR-GNB carriers other than from *Pseudomonas aeruginosa* (evaluated also in urinary samples or other body regions) and treated with imipenem/cilastatin as first line therapy, did not a negative impact on TRM and OS[26].

For these reasons, several transplant centers still consider MDR-GNB colonization as a relative contraindication to transplant, especially in allo-HSCT, due to the high-mortality rate in case of neutropenic MDR-GNB BSI and the likelihood of pre-engraftment BSI development in carriers. At our Institution, when combinations of pre-emptive measures were applied, MDR GNB BS could be successfully managed and TRM and OS in carriers were not significantly increased compared to non-carriers. However, it should be noticed that MDR-GNB BSI often arose in patients showing concomitant dismal features (i.e. active disease (88%), severe aGVHD (60%), no neutrophil engraftment (20%). In this study, we showed that, both in auto-HSCT and allo-HSCT, a pre-transplant colonization is not *per se* significantly associated with long-term reduced OS, TRM or IRM. Moreover, the 14-day attributed mortality after MDR-GNB BSI onset was not significantly different between carriers and non-carriers (P=0.207), despite in non-carriers MDR-GNB BSI were mostly sustained by ESBL$^+$ *E. Coli* or ESBL$^+$ Kp, while in carriers up to 76.9% MDR-GNB BSI were caused by CR-Kp or CR-*Pseudomonas aeruginosa*, which are known from previous studies to be associated with higher mortality (ranging from 50-70%)[12] compared to ESBL$^+$ producing pathogens. This lack of a significant mortality difference may be related to an intensive Institutional policy of early de-escalation approach of antimicrobials in carriers, with the initiation of a targeted first-line therapy at the onset of neutropenic fever, whenever possible, in order control the infectious mortality due to MDR pathogens.

Therefore, we think that in this cohort, the adoption of pre-transplant active surveillance and the early initiation of targeted combined antimicrobial therapy in MDR-GNB carriers have successfully impacted on clinical outcomes, thus making OS, TRM and IRM not significantly different between carriers and non-carriers, while the onset of severe GVHD remains the major risk factor for a detrimental outcome.

Conflict of interests

The authors declare no competing financial interests.

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Author contribution

Conception and design: Alessandra Forcina, Francesca Lorentino, Vincenzo Marasco and Fabio Ciceri

Provision of study material or patients: All authors

Collection and assembly of data: Alessandra Forcina, Vincenzo Marasco, Francesca Lorentino, Magda Marcatti

Data analysis and interpretation: All authors

Manuscript writing: All authors

Final approval of manuscript: All authors

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Figure 1. Autologous HSCT. (**A**) Kaplan-Meier curves of the estimated 2-yrs OS for pre-transplant MDR-GNB carriers (dotted line) compared non-carriers (black line); (**B**) Kaplan-Meier curves of the estimated 2-yrs TRM for pre-transplant MDR-GNB carriers (dotted line) compared non-carriers (black line); (**C**) Kaplan-Meier curves of the estimated 2-yrs IRM for pre-transplant MDR-GNB carriers (dotted line) compared non-carriers (black line).

Figure 2. Allogeneic HSCT. (**A**) Kaplan-Meier curves of the estimated 2-yrs OS for pre-transplant MDR-GNB carriers (dotted line) compared non-carriers (black line); (**B**) Kaplan-Meier curves of the estimated 2-yrs TRM for pre-transplant MDR-GNB carriers (dotted line) compared non-carriers (black line); (C) Kaplan-Meier curves of the estimated 2-yrs IRM for pre-transplant MDR-GNB carriers (dotted line) compared non-carriers (black line). (**D**) Cumulative incidence of 100-day acute GVHD for pre-transplant MDR-GNB carriers (dotted line) compared non-carriers (black line).

Figure 3. Distribution of MDR-GNB BSI in auto-HSCT (n=4) and in allo-HSCT (n=27) patients according to microbiological etiology. $ESBL^+$ EC = extended-spectrum beta lactamase producing *E. Coli*; CR-Kp = Carbapenem-resistant *Klebsiella pneumoniae;* CR-PA = carbapenem-resistant *Pseudomonas aeruginosa*; STN = *Stenotrophomonas maltophilia*. Other MDR-GNB included: 1 CR-*Enterobacter cloacae*; 1 *Ochrobactrum antrhopi*; 1 *Sphingomonas paucimobilis*.

Table 1. Patients characteristics (n=348)

Characteristic	**Allo-HSCT (n=254)**	**Auto-HSCT (n=94)**
Age, median years (range)	53 (16-77)	54 (19-78)
Male, sex n (%)	154 (60.6)	58 (61.7)
Underlying disease: n. (%)		
Acute myeloid leukemia	138 (54.3)	6 (6.3)
Acute lymphoid leukemia	13 (5.1)	0 (0)
Myelodisplastic syndromes	19 (7.4)	1 (1)
Chronic myeloproliferative	9 (3.5)	1 (1)
Non Hodgkin's lymphoma	27 (10.6)	35 (37.2)
Hodgkin's lymphoma	16 (6.2)	7 (7.4)
Multiple myeloma	25 (9.8)	35 (37.2)
Other diseases	7 (2.7)	9 (9.5)
Sorror HCT-CI, median (range)	2 (0-9)	/
Donor type: n. (%)		
HLA-Identical	43 (16.9)	/
Matched Unrelated	86 (33.8)	/
HLA-Haploidentical	120 (47.2)	/
Cord blood	5 (1.9)	/
CMV host/donor serostatus		
Negative/Negative	26 (10.2)	/
Positive/Positive	149 (58.6)	/
Positive/Negative	12 (4.7)	/
Negative/Positive	66 (25.9)	/
Disease Risk Index		
Very High	18 (7)	/
High	85 (33.4)	/
Low-intermediate	144 (56.6)	/
Not applicable	7 (2.7)	/
Pretransplantation conditioning intensity: n, (%)		
Myeloablative	165 (64.9)	68 (72.3)
Reduced intensity	89 (35)	26 (27.6)
Phase of the underlying disease at transplant: n. (%)	110 (43.3)	42 (44.7)
Malignancies in complete remission	113 (44.4)	46 (48.9)
Malignancies not in complete remission/active	31 (12.2)	6 (6.4)
Non-malignant stable/ chronic diseases		
T-cell depletion		
No	23 (9)	/
ATG	96 (37.8)	/
PT-Cy	121 (47.6)	/
ATG + PT-Cy	14 (5.5)	/
Pre-transplant MDR-GNB carriers: n, (%)	43 (16.9)	9 (9.6)

Abbreviations: ATG = antilymphocyte globulin; PTCy= post-transplant Cyclophosphamide; MDR-GNB= multi-drug resistant Gram-negative bacteria.

Table 2. Patients characteristics according to the colonization status in allo-HSCT and in auto-HSCT

	Allo-HSCT			Auto-HSCT		
Characteristic	**Carriers (n=43)**	**Non-carriers (n=211)**	***P value***	**Carriers (n=9)**	**Non-carriers (n=85)**	***P value***
Age, median (range)	47 (21-77)	53 (16-77)	*0.315*	61 (31-71)	53 (18-77)	*0.148*
Sorror HCT-CI, median (range)	2 (0-9)	2 (0-8)	*0.166*	-	-	-
Sex, Male	26	128	*1*	6	52	*1*
Underlying disease			*0.058*			*0.45*
Acute lymphoid leukemia	1	12		-	-	
Acute myeloid leukemia	31	107		1	5	
Myelodisplastic	0	19		-	1	
syndromes	1	8		-	1	
Chronic	1	24		2	33	
myeloproliferative	3	13		-	7	
Multiple myeloma	6	21		6	29	
Hodgkin's lymphoma	-	-		-	9	
Non Hodgkin's lymphoma						
other						
Donor type			*0.725*	-	-	-
HLA-Identical	9	34				
Matched Unrelated	13	73				
HLA-Haploidentical	21	99				
Cord blood	0	5				
Disease Risk Index			*0.749*	-	-	-
Low-intermediate	24	120				
High	15	70				
Very high	4	14				
Disease status at			*0.078*			*1*
transplant	1	30		0	6	
Upfront	21	89		4	38	
Malignancies in CR	21	98		5	41	
Malignancies not CR						
CMV host/donor			*0.586*	-	-	-
serostatus	3	23				
Negative/negative	40	188				
Others						

Table 3. Distribution of MDR-GNB genus and species and resistance patterns in pre-transplant carriers in auto-HSCT and allo-HSCT

Pathogen and resistance pattern	Allo-HSCT (n=43)	Auto-HSCT (n=9)
ESBL-producing Escherichia coli, total n (%)	10 (23.2)	/
CR Klebsiella pneumoniae, total n (%)	16 (37.2)	4 (44.4)
Other CR Enterobacteriacee, total n (%)	2 (4.6)	1 (11.1)
MDR Pseudomonas aeruginosa, total n (%)	7 (16.2)	1 (11.1)
Stenotrophomonas maltophilia, total n (%)	4 (9.3)	1 (11.1)
Other MDR-GNB, total n (%)	4 (9.3)	2 (22.2)

Abbreviations: ESBL = extended-spectrum beta lactamase producing; MDR = multi-drug resistant; MDRO-GN= multi-drug resistant Gram-negative organisms; CR: resistant to carbapenems, defined as a MIC for imipenem and/or meropenem and/or ertapenem > 1 mg/L;

Table 4. Multivariate analysis for 2-yrs OS, 2-yrs TRM, 2-yrs IRM and 100-days severe acute GVHD in the allo-HSCT population (n=254)

OS	**HR**	**(95 % CI)**	**P-value**
aGVHD ≥ grade III	3.177	(2.060-4.901)	< 0.0001
DRI			
High vs low-intermediate	2.661	(1.781-3.977)	< 0.0001
Very high vs low-intermediate	5.361	(2.928-9.816)	< 0.0001
MDR-GNB carrier	1.417	(0.904-2.221)	0.129
TRM	**HR**	**(95 % CI)**	**P-value**
aGVHD ≥ grade III	7.933	(4.666-13.487)	< 0.0001
Disease type			0.055
Myeloid vs lymphoid	0.580	(0.333-1.011)	
MDR-GNB carrier	1.428	(0.760-2.682)	0.268
Age ≥ 53yrs	1.029	(1.009-1.050)	0.005
IRM	**HR**	**(95 % CI)**	**P-value**
MDR-GNB carrier	1.690	(0.737-0.737)	0.215
Age > 53 yrs	1.022	(0.998-1.046)	0.074
aGVHD ≥ grade III	18.690	(7.867-44.401)	< 0.0001
Disease type			
Myeloid vs Lymphoid	1.450	(1.029-2.044)	0.034
aGVHD ≥ grade III	**HR**	**(95 % CI)**	**P-value**
MDR-GNB carrier	1.864	(0.914-3.800)	0.087
Donor type		(0.086-1.574)	0.178
HLA-Haploidentical vs CBU	0.368		
MUD vs CBU	0.275	(0.062-1.219)	0.089
HLA-identical vs CBU	0.034	(0.003-0.382)	0.006

Abbreviations: OS= overall survival; TRM= transplant-related mortality; IRM= infection-related mortality; MDR-GNB= multi-drug resistant Gram-negative bacteria; aGVHD = acute graft versus host disease; DRI = disease risk index; MUD= matched unrelated donor; CBU=cord blood unit

Table 5. Details of microbiological isolates on blood cultures and antimicrobial therapy in pre-transplant carriers and non-carriers

Pt n°	Type of HSCT	Rectal swab isolate	BC isolate	I line therapy	II line therapy	Outcome	Cause of death
1	Allo	No	ESBL$^+$ EC	PIP/TAZ	AMIK	alive	
2	Allo	CR-KP	CR-KP	MER, TIGE, COL, CLINDA		dead	PD
3	Allo	No	CR-KP	MER, GENTA, TIGE	COL	alive	
4	Allo	STN	STN	MER, VANCO	AMIK	dead	GVHD
5	Allo	No	ESBL$^+$ EC	PIP/TAZ	MER, TIGE, AMIK	alive	
6	Allo	No	ESBL$^+$ EC	PIP/TAZ	BBK8	alive	
7	Allo	No	ENTB	CIPRO		dead	PD
8*	Allo	CR-KP	CR-KP	MER, TIGE, COL	ERT, GENTA	alive	
8	Allo	CR-KP	CR-KP	MER, TIGE, COL, ERT, GENTA		dead	sepsis
9	Allo	ESBL$^+$ EC	ESBL$^+$ EC	MER, AMIK		dead	sepsis
10	Allo	No	ESBL$^+$ EC	PIP/TAZ, METRO	MER	dead	PD
11	Allo	STN	STN	MER, LIN, AMIK	TIGE, TMP/SMZ, PIP/TAZ	dead	PD
12*	Allo	CR-KP	CR-KP	AMIK, TIGE, GENTA, MER	ERT, COL	alive	
12			ESBL$^+$ KP	CEFT, MER		alive	
13*	Allo	No	MDR-PA	MER, AMIK, COL		alive	
13			MDR-PA	CEF, CIPRO, AMIK		dead	sepsis in PD
14	Allo	No	ESBL$^+$ EC	MER		dead	GVHD
15	Allo	No	MDR-PA	LEVO	AMIK, IMIP	dead	GVHD
16	Allo	No	*Sphingomonas*	LEVO	CEFT, VANCO,	dead	sepsis

			paucimobilis		MER		
17	Allo	CR-KP	CR-KP	COL, GENTA, MER, LIN	TIGE	dead	PD
18	Allo	No	*Ochrobactrum antrhopi*	TIGE, MER		alive	
18	Allo	No	STN	AMOX/CLAV		alive	
19	Allo	No	MDR-PA	CEF	MER	dead	sepsis in PD
20	Allo	No	ESBL$^+$ KP	MER	DAPTO, AMIK	alive	
21*	Allo	CR-KP	CR-KP	MER, ERT, GENTA	TIGE, COL, RIF	alive§	
21			CR-KP	MER, ERT, GENTA, COL, RIF		alive§	
21			CR-KP	MER, ERT, GENTA, COL, RIF		dead	sepsis
22	Auto	MDR-PA	MDR-PA	PIP/TAZ, GENTA		alive	
23	Auto	No	ESBL$^+$ KP	PIP/TAZ	AMIK, MER	dead	sepsis in PD
24	Auto	CR-KP	CR-KP	MER, TIG, GENTA, COL		alive	
25	Auto		ESBL$^+$ EC	LEVO, CEFT	MER	alive	

Abbreviations: BC = Blood culture; ESBL$^+$= Extended-spectrum beta-lactamase producing; CR = Carbapenem-resistant; MDR = multidrug-resistant; EC = *Escherichia coli*: KP=*Klebsiella pneumoniae*; STN = *Strenotrophomonas maltophilia*; ENTB =Enterobacteriaceae; PIP/TAZ = piperacillin/tazobactam; AMIK= amikacin; MER = meropenem, CLINDA = clindamycin, TIGE = tigecycline; COL = colistin; GENTA = gentamycin; VANCO = vancomycin; CIPRO= ciprofloxacin; ERT = ertapenem; METRO = metronidazole; LIN = linezolid; TMP/SMZ = trimethoprim/sulfamethoxazole; CEFT = ceftazidim; CEF = cefepim; LEVO = levofloxacin; IMIP = imipenem; AMOX/CLAV = amoxicillin/clavulanate; RIF = rifampicin; PD = progression of disease; GVHD = graft-vs-host-disease.

*patients experiencing recurrent BSI.

§ alive until subsequent BSI.

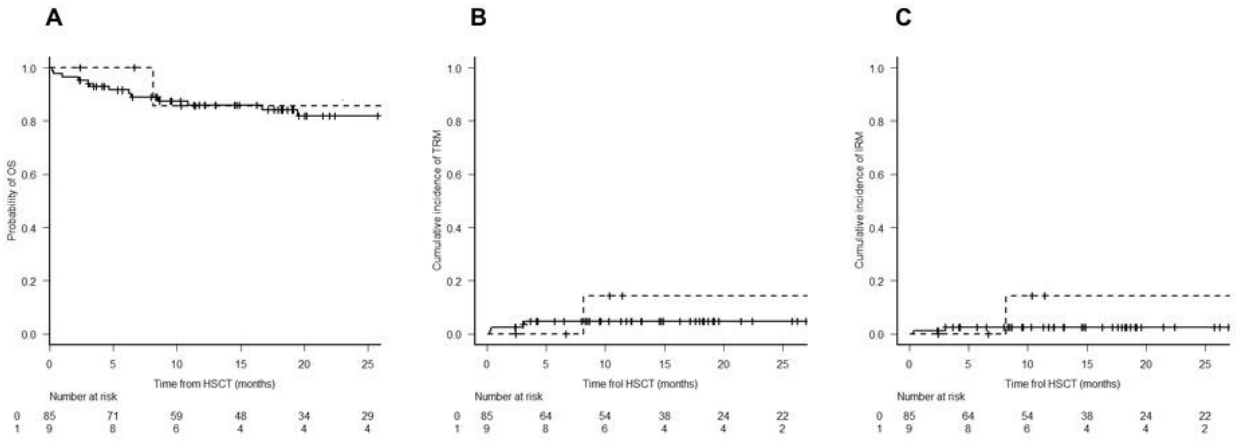
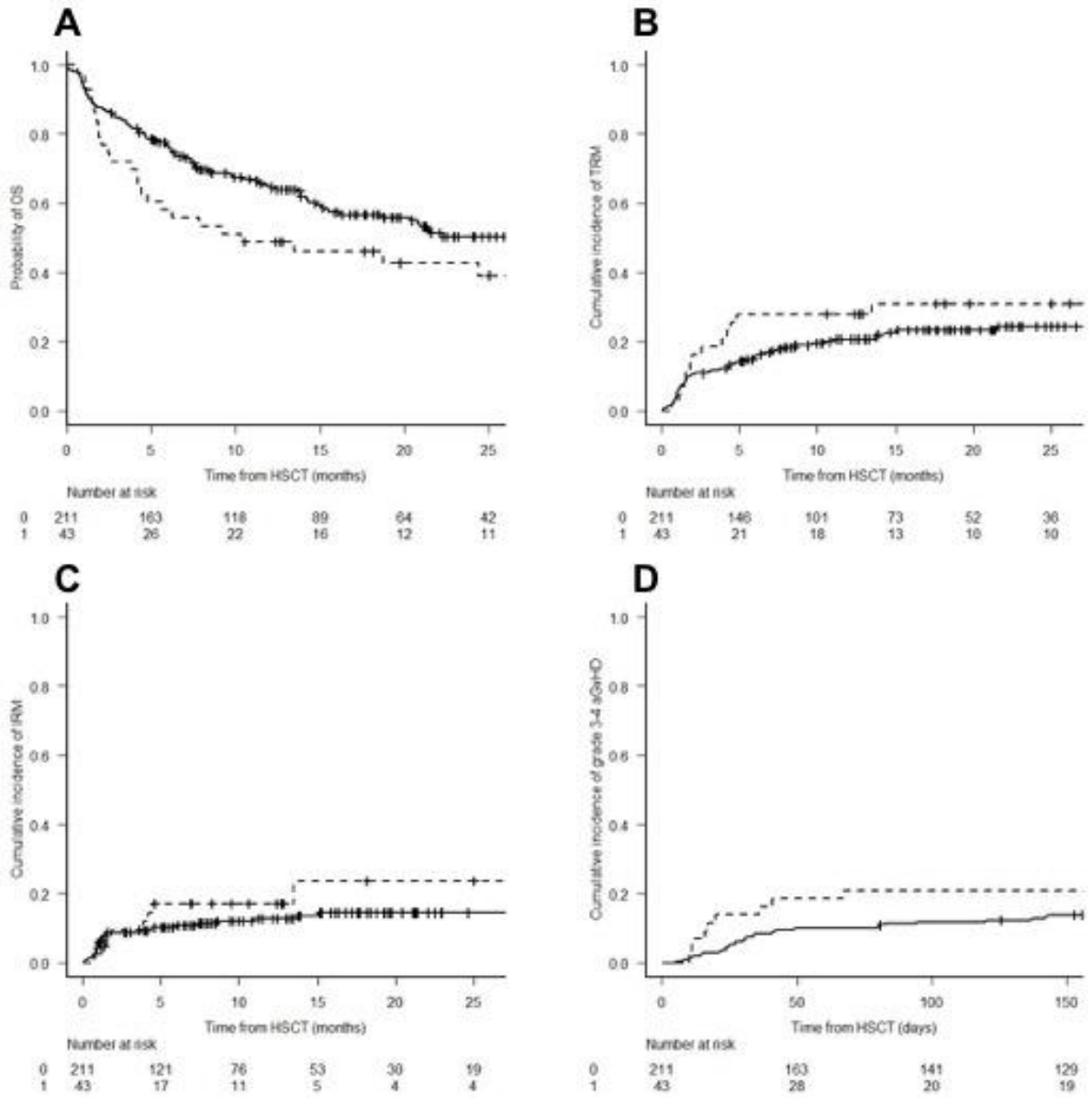


Figure 1.jpg



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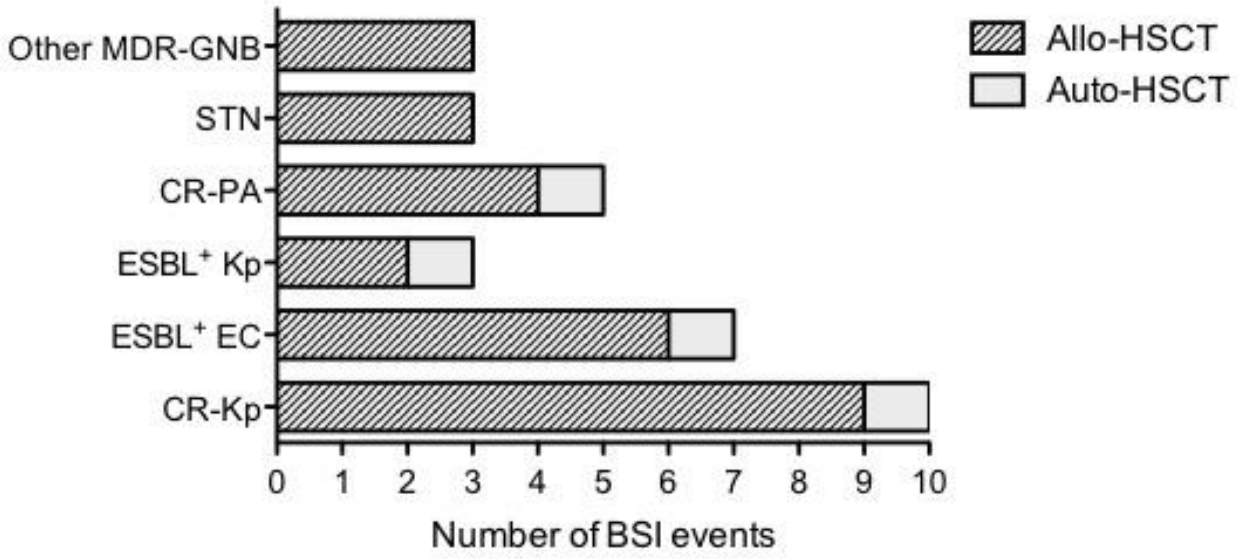

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