Incidence, Risk Factors and Outcome of Pre-engraftment Gram-Negative Bacteremia after Allogeneic and Autologous Hematopoietic Stem Cell Transplantation: An Italian Prospective Multicenter Survey

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Summary

Background. Gram negative bacterial infections (GNBI) are a major cause of morbidity and mortality in Hematopoietic Stem Cell Transplant (HSCT) and updated epidemiological investigation is advisable.

Methods. We prospectively evaluated the epidemiology of pre-engraftment GNBI in 1,118 allogeneic HSCT (allo-HSCT) and 1,625 autologous HSCT (auto-HSCT) among 54 transplant centers during 2014 (SIGNB-GITMO-AMCLI study). Using logistic regression methods we identified risk factors for GNBI and evaluated the impact of GNBI on the 4-month overall-survival after transplant. SIGNB-GITMO-AMCLI is registered with ClinicalTrials.gov, number NCT02088840.

Findings The cumulative incidence of GNBIs was 18.6% in allo-HSCT and 10% in auto-HSCT. *E. coli*, *K. pneumoniae* and *P. aeruginosa* were the most common isolates. By multivariate analysis variables associated with pre-engraftment GNBI were a diagnosis of acute leukemia, a transplant from HLA-mismatched donor and from cord-blood, older age and duration of severe neutropenia in allo-HSCT, and a diagnosis of lymphoma, older age and no antibacterial prophylaxis in auto-HSCT. A pre-transplant infection by a resistant pathogen was significantly associated to an increased risk of post-transplant infection by the same microorganism in allo-HSCT. Colonization by resistant Gram-negative bacteria was significantly associated to an increased risk of pre-engraftment infection by the same pathogen in both transplant procedures. GNBIs were independently associated to increased mortality at 4-months from transplant both in allo-HSCT (HR 2.08, 95% CI 1.43-3.04; p=.0001) and auto-HSCT (HR 2.52, 95% CI 1.28-4.99; p=.008).

Interpretation. Pre-engraftment GNBIs are a major cause of morbidity and mortality in auto-HSCT and allo-HSCT. Previous infectious history and colonization monitoring represent major indicators of GNBI.

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Introduction

Gram negative bacterial infections (GNBI) are a leading cause of morbidity and mortality in hematologic patients including those submitted to Hematopoietic Stem Cell Transplant (HSCT) (1-9). A challenging aspect is represented by the continuous evolution of the incidence, susceptibility pattern to antibiotics and prognosis due to the change of the transplant populations, the global epidemiology of bacterial infections and the antimicrobial strategies. Therefore, updated epidemiological investigation in this high risk population is advisable.

Because of the poor outcomes associated with GNBIs in transplant patients, there is much interest in the identification of risk and prognostic factors which may help to determine tailored treatment strategies (5, 8,9). Risk factors for bacterial infections have been evaluated extensively in HSCT patients, but there are few studies describing specific variables related to outcome (4,6,7,10).

To assess the current incidence, risk and prognostic factors of pre engraftment GNBIs in allogeneic and autologous HSCT patients, data of patients undergoing allogeneic HSCT (allo-HSCT) and autologous HSCT (auto-HSCT) during 2014 were prospectively registered. These data provide the basis for promoting direct efforts in risk stratification, prevention and management of GNBIs in the HSCT population.

Methods

Study design. GITMO-AMCLI Severe Infections by Gram Negative Bacteria (SIGNB) study was a prospective epidemiological survey done in 54 transplant Centres in Italy along a period of one year: 1 January –31 December 2014. It was sponsored by the Italian Transplantation Network (Gruppo Italiano Trapianto di Midollo Osseo, GITMO) and the Italian Association of Clinical Microbiology (Associazione Microbiologi Clinici Italiani , AMCLI). Study start time could differ among Centres but all consecutive transplants were enrolled. Data collection of each case was interrupted 4 months after transplant, therefore the study end date was 30 April 2015. Data were entered into electronic case report forms and all reported results derive from the database locked at December 31, 2015. The results of this study were reported according to the STROBE (STrengthening the Reporting of OBservational studies in Epidemiology) statement (11). The study was approved by the ethical committee of each participating centre and informed consent was obtained from the patients.

Data collection. Variables included patients characteristics, diagnosis and phase of the underlying disease, prior auto-HSCT or allo-HSCT, neutropenia during the 30 days before transplant, GNBIs documented within the three months before transplant, stem cell donor, stem cell source, pretransplant conditioning regimen, the use of "in vivo" T cell depletion with antithymocyte globulin (ATG) or manipulation ex vivo, antibacterial prophylaxis during the engraftment period, colonization by resistant Gram-negative bacteria at the pre-transplant screening and during engraftment, development of grade II-IV mucositis, duration of pre-engraftment neutropenia, development of grade II-IV acute Graft versus Host Disease (GvHD) before engraftment, microbiologically documented bacterial, fungal and viral diseases and clinically documented infections before engraftment, survival at 4 months from transplant and cause of death. Information pertaining to the GNBIs included timing after HSCT, in vitro susceptibility data, and site of infection. Data on antibacterial treatments given at onset of febrile/infectious episode before

knowing the microbiological results (first line treatment) and any subsequent empirical or microbiologically driven modification of the treatment (second line treatment) in the cases of GNBI were also collected, however, they were not considered in the present paper but will be the subject of a following analysis.

Definition of infection and colonization. Cases of GNBI were defined as isolation of Gram negative bacteria from blood, other normally sterile body sites and from non-sterile body sites associated to signs of infection being a simple colonization excluded. The clinical and microbiological data reported to define cases of GNBI were reviewed to determine the reliability of the diagnosis. Onset of GNBI was defined as the day when the first positive culture was taken. For each GNBI isolate in vitro susceptibility data were collected, including sensitivity or nonsensitivity to third generation cephalosporins (ceph-S or ceph-NS) and sensitivity or nonsensitivity to carbapenems (imipenem and/or meropenem and/or ertapenem) (carba-S or carba-NS) for enterobacteria, (defined as a minimum inhibitory concentration, MIC, for imipenem and/or meropenem and/or ertapenem higher than 1 mg/L) and multi-drug-resistance (MDR) for Gram-negative non-fermenters (defined as resistance to three or more antimicrobial groups among piperacillin-tazobactam, ceftazidime, fluoroquinolones, aminoglycosides and carbapenems). The investigators were also asked to specify whether a surveillance of colonization by resistant bacteria (ceph-NS and carba-NS enterobacteria, and MDR non-fermenters), by culture of rectal/perianal swabs, was routinely performed at their center before and after transplant, and reported the cases of colonization documented in the 3 months before transplant and during the engraftment period after transplant. Therefore, for the colonization analysis, we excluded the population of the centers that never performed a screening for colonization by the above resistant pathogens.

Analyses. All the analyses were made separately for auto-HSCT and allo-HSCT patients. The pre engraftment cumulative incidence of GNBIs was calculated accounting for the competing risks of infection-free death. Cumulative incidence of GNBIs was calculated for any type and status of the underlying disease, any type of stem cell donor, stem cell source and pretransplant conditioning, history of GNBI before transplant, antibacterial prophylaxis, a-GVHD. All factors were evaluated in multivariate models to control potential confounders.

Survival analyses were performed according to the above variables. A death was attributed to the GNBI in patients who failed to respond to therapy (i.e., who had stable disease or disease progression) and in patients with a partial response to therapy who died as the result of an acute event involving any of the sites of infection and in the absence of other causes thought to have primarily contributed to death. Probability of 4 months survival from HSCT was calculated with Kaplan–Meier estimate, log-rank test was applied for univariate analysis and two-sided p-values $\leqslant 0.05$ were considered to represent statistical significance. Multivariate analyses were performed with Cox proportional hazards regression model. In this model variables related to GNBI and a-GvHD were considered as time-dependent covariates in order to evaluate their effect only in the period following their onset. All significant variables in univariate analysis were included in multivariate analysis; final models were also evaluated with backward and stepwise function. In all, 95% confidence intervals were reported for the main summary statistics and all statistical comparisons were based on two-tailed tests. Statistical analyses were done with SAS software version 9.4 and all comparisons are two-sided with a nominal significance level of 5%. GITMO-AMCLI-SIGNB is registered with Clinicaltrials.gov, number NCT02088840

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Results

Allogeneic HSCT

Patient Characteristics.

Overall 1,118 allo-HSCTs from 44 transplant centers were included in the study. Of these transplants 1013 were first and 105 second allo-HSCT, and 219 (19.6%) had been preceded by an auto-HSCT. The median number of transplants enrolled in the study among the 44 centres was 20 (range 2- 105 transplants). Demographics and patient characteristics at transplant are shown in Table 1.

Pre engraftment infections and details on GNBIs

The rates of bacterial infections, fungal infections and end organ viral diseases documented before engraftment are detailed in Table 2.

Overall, 157 GNBIs were documented in 148 of 1118 allo-HSCTs (13.2%) before engraftment (9 patients developed two distinct GNBIs by different bacterial species). Out of 157 isolates, 149 (94.9%), 7 (4.4%) and 1(0.6%) were isolated from blood, urinary tract and skin (subcutaneous abscess), respectively. The rate of GNBIs by transplant centre ranged from 0% to 39.3% (median 11.8%). The median rate of GNBI accounted for 11.1% and 13.3% (p=0.8) in centers with lower (≤ 20 transplants) and higher (>20 transplants) transplant activity, respectively. *Escherichia coli* was the most common Gram-negative pathogen (78 cases, 49.7%), followed by *Klebsiella pneumoniae* (32 cases, 20.4%) and *Pseudomonas aeruginosa* (23 cases, 14.6%).

The susceptibility pattern of the isolates is detailed in table 3. Ceph-NS/carba-S and ceph-NS/carba-NS was documented in 38.5% and 1.3% of *E. coli*, respectively, and in 25% and 53.1% of *K. pneumoniae*, respectively. MDR was documented in 36.4% of *P. aeruginosa*.

Risk factors for GNBI in allo-HSCT recipients

The cumulative incidence of GNBIs at 30 days from transplant was 18.6% (95% Confidence Interval 13.7 – 24 %) (figure 1A). GNBI free pre-engraftment death occurred in 25 (2.2%) cases. The risk of GNBI according to demographics, underlying disease and transplant variables are detailed in table 4. By multivariate analysis variables associated with pre engraftment GNBI were acute leukemia, a transplant from HLA mismatched donor (both related and unrelated) and from cord blood, older age and duration of severe (<100 PMN/cmm) neutropenia.

Risk of GNBI according to pre-transplant infection and colonization

A pre-transplant infection by one or more Gram-negative pathogens (88 isolates) was reported in 84 (7.5%) patients (ceph-S *E.coli*, 17 cases; ceph-NS/carba-S *E.coli*, 17 cases; ceph-S *K.pneumoniae*, 4 cases; ceph-NS/carba-S *K.pneumoniae*, 9 cases; carba-NS *K.pneumoniae*, 6 cases; non-MDR *P.aeruginosa*, 11 cases; MDR *P.aeruginosa*, 4 cases; *Enterobacter cloacae* complex, 6 cases; other pathogens, 14 cases). In 16 of these 84 patients (19%) an infection by the same species with the same susceptibility phenotype was documented after a median of 7 days (range 1-25 days) after transplant (ceph-S *E. coli*, 1 case; ceph-NS *E. coli*, 5 cases; ceph-NS *K. pneumoniae*, 2 cases; carba-NS *K. pneumoniae*, 4 cases; *Enterobacter cloacae* complex, 1 case; non-MDR *P. aeruginosa*, 3 cases). A significantly increased risk of pre-engraftment infection by the same species with the same susceptibility phenotype was documented in patients with a pre transplant infection by ceph-NS/carba-S *E. coli*, carba-NS *K. pneumoniae* and non-MDR *P. aeruginosa*. On the contrary pre-transplant infection by ceph-S *E.coli* did not represent a risk for a post-transplant infection by the same pathogen (figure 2).

A colonization by resistant Gram-negative bacteria was always associated to a significantly increased risk of pre-engraftment infection by the same pathogen. In particular, a high probability of a pre-engraftment infection by the same colonizing species was observed for carba-NS *K. pneumoniae* (32.5% in colonized *vs*. 0.8% in non-colonized, p<0.0001) and MDR *P. aeruginosa* (28.6% in colonized *vs*. 0.6% in non-colonized, p<0.0001) (figure 3).

Survival

The overall survival (OS) of the entire population at 4 months from transplant was 86.3 % (95% CI 84.3 – 88.4%). In multivariate analysis, acute leukemia, a previous auto HSCT, a disease not in complete remission at the time of transplant, older age, prolonged pre engraftment neutropenia, acute-GVHD, and pre engraftment GNBI (Hazard ratio 2.08, 95% CI 1.43-3.04; p=.0001) were factors independently associated to increased mortality (Table 5).

The mortality rate at 30 days from the diagnosis of GNBI was 13.5% (25 of 148 patients) and in 96% of cases (24 of 25) the infection was considered the primary cause of death. Of 46 patients who died before engraftment, the cause of death was a GNBI in 18 (39.1%) cases.

Probability of survival at 4 months from transplant in 970 patients who did not develop any GNBI was 88.4%, as compared to 80.4% in 46 patients with ceph-S *E. coli* infection (p=0.11), 86.7% in 30 patients with ceph-NS *E. coli* infection (p=0.72), 43.8% in 16 patients with carba-NS K. *pneumoniae* infection (p<.0001), 64.3% in 14 patients with non-MDR *P. aeruginosa* infection (p=0.003) and 25% in 8 patients with MDR-*P. aeruginosa* infection (p<.0001) (Figure 4).

Autologous HSCT

Patient Characteristics.

Overall 1,625 auto-HSCTs from 52 transplant centers were included in the study. Of these transplants, 1281 were first and 344 second auto-HSCT. The median number of transplants enrolled in the study among the 52 centres was 23 (range 4 - 101 transplants). Demographics and patient characteristics at transplant are shown in Table 1.

Pre engraftment infections and details on GNBIs

The rates of bacterial infections, fungal infections and end organ viral diseases documented before engraftment are detailed in Table 2.

Overall, 162 GNBIs were documented in 157 of 1,625 auto-HSCTs (9.7%) before engraftment (5 patients developed two distinct GNBIs by different bacterial species). Out of 162 isolates, 151 (94.9%), 6 (3.7%), 4 (2.5%) and 1 (0.6%) were isolated from blood, urinary tract, intestinal tract and skin (subcutaneous abscess), respectively. The rate of GNBIs by transplant center ranged from 0% to 33.3% (median 7.6%). The median rate of GNBI accounted for 6.2% and 8.8% (p=0.9) in centers with lower (≤ 23 transplants) and higher (>23 transplants) transplant activity, respectively. *E.coli* was the most common Gram-negative pathogen (94 cases, 58.0%), followed by *K. pneumoniae* (25 cases, 15.4%), and *P. aeruginosa* (13 cases, 8.0%). The susceptibility pattern of the isolates is detailed in table 3. Ceph-NS/carba-S and carba-NS phenotype was documented in 31.9% and 0% of *E. coli*, respectively, and in 40% and 28% of *K. pneumoniae*, respectively. MDR was documented in only one *P. aeruginosa* isolate (7.7%).

Risk factors for GNBI in auto-HSCT recipients

The cumulative incidence of GNBIs at 20 days from transplant was 10.0% (95% Confidence Interval 9 – 11%) (figure 1B). GNBI free pre-engraftment death occurred in 6 (0.4%) cases. The risk of GNBI according to demographics, underlying disease and transplant variables are detailed in table 6. By multivariate analysis, variables associated with pre-engraftment GNBI were a diagnosis of lymphoma, older age and no antibacterial prophylaxis.

Risk of GNBI by species according to pre-transplant infection and colonization

A pre-transplant infection by a Gram-negative pathogen was reported in 39 cases (2.4%) (ceph-S *E. coli*, 7 cases; ceph-NS/carba-S *E. coli*, 5 cases; ceph-S *K. pneumoniae*, 7 cases, ceph-NS/carba-S *K. pneumoniae*, 2 cases; carba-NS *K.pneumoniae*, 2 cases; *Enterobacter cloacae* complex 5 cases; other pathogens, 11 cases). In only 3 cases an infection by the same pathogen (ceph-S *E. coli*, ceph-S *K. pneumoniae*, carba-NS *K. pneumoniae*) was documented 2, 7 and 7 days after transplant, respectively.

Overall, 89/1307 (6.8%), 21/1307 (1.6%) , 21/1432 (1.5%) and 2/1307 (0.1%) evaluable patients were colonized by ceph-NS/carba-S *E. coli*, ceph-NS/carba-S *K. pneumoniae*, carba-NS *K. pneumoniae* and MDR *P. aeruginosa*, respectively. A significant correlation between colonization and pre-engraftment infection by the same species at 20 days from transplant was observed for ceph-NS/carba-S *E. coli* (10.5% in colonized *vs*. 4.5% in non-colonized, p=0.038), ceph-NS/carba-S *K. pneumoniae* (20.4% in colonized *vs*. 0.3% in non-colonized, p<0.0001), and carba-NS *K. pneumoniae* (19.0% in colonized *vs*. 0.01% in non-colonized, p<0.0001).

Survival

The OS of the entire population at 4 months from auto-HSCT was 97% (95% CI 96.2 – 97.9%). In multivariate analysis, a diagnosis of lymphoma, a prolonged neutropenia during the month preceding auto-HSCT, a disease not in complete remission at the time of transplant, prolonged pre engraftment neutropenia, and pre engraftment GNBI (Hazard ratio 2.52, 95% CI 1.28-4.99; p=.008) were factors independently associated to increased mortality (Table 7). The mortality rate at 30 days from the diagnosis of GNBI was 3.8% (6 of 157 patients) and in all cases the infection was considered the primary cause of death. Of 11 patients who died before engraftment the cause of death was a GNBI in 4 (36.4%) cases.

Probability of survival at 4 months from transplant in 1469 patients who did not develop any GNBI was 97.5%, as compared to 97.3% in 73 patients with ceph-S *E. coli* infection (p=0.91), 96% in 25 patients with ceph-NS/carba-S *E. coli* infection (p=0.63), 88.9% in 9 patients with ceph-S *K. pneumoniae* infection (p=0.09), 90% in 9 patients with ceph-NS/carba-S *K. pneumoniae* infection (p=0.14), 71.4% in 7 patients with carba-NS *K. pneumoniae* infections (p<.0001), and 76.9% in 13 patients with non-MDR *P. aeruginosa* infection (p<.0001).

Discussion

The increasing incidence of GNBIs resistant to antibiotics, including carbapenems, has become a public health problem of major concern worldwide (12-14).

Antimicrobial resistance data from invasive isolates reported to the European Antimicrobial Resistance Surveillance Network (EARS-Net) by 30 European countries (trend analyses for the period 2012–2015) showed wide variations depending on the bacterial species, antimicrobial group and geographical region (14). A worrying situation was represented by the high and increasing resistance percentages reported from many parts of Europe. The observed increase in combined resistance to multiple antimicrobial groups, as well as the high proportion of isolates resistant to third-generation cephalosporins [generally related to an Extended Spectrum Beta Lactamase (ESBL) production], lead to an increased use of carbapenems, thus further favouring the emergence and spread of carbapenem-resistant enterobacteria, particularly among *K. pneumoniae*. Carbapenem resistance and MDR were also common in *P. aeruginosa* and *Acinetobacter* spp. In Italy, over the period 2012–2015, the rate of isolates resistant to third-generation cephalosporins increased from 26.3% to 30.1 % for *E. coli* and from 47.9% to 55.9% for *K. pneumoniae*, while carbapenem resistance increased from 29.1% to 33.5% for *K. pneumoniae* and remained stably <1% for *E. coli*. For *P. aeruginosa* carbapenem resistance was stable around 25% and MDR decreased from 23.7% in 2012 to 20% in 2015.

Hematologic patients are considered at high risk for these severe infections, but only few information is specifically available regarding the incidence and prognostic factors of GNBI in this population of immunocompromised subjects, particularly those submitted to HSCT. To our knowledge, only two multicenter studies, both from Italy, prospectively evaluated the epidemiology of GNBI in onco-hematologic populations, mainly in non-transplant patients, during the recent years, and confirmed the above resistance patterns observed in the general population (7,15). Literature data on the recent epidemiology of GNBIs specifically in HSCT populations are limited

to retrospective, mainly single center, experiences.(16-24). These studies, while showing a variable rate of resistance patterns according to local epidemiology, patient age and type of transplant, were not able to provide reliable data on the epidemiological patterns of GNBI in the HSCT populations.

The SIGNB survey, which includes about 64% of allo-HSCTs and 51% of auto-HSCTs performed in Italy in 2014, is a prospective study designed to critically assess not only the incidence during the pre-engraftment period but also the risk factors and the prognostic role of GNBI at four months from transplant. A valuable characteristic of this study is the availability of complete denominator data prospectively collected for consecutive patients who received transplant at each Centre.

The overall pre-engraftment cumulative incidence of GNBI was 18.6% at 30 days from allo-HSCT and 10% at 20 days from auto-HSCT. *E. coli*, *K. pneumoniae* and *P. aeruginosa* were the most common isolates in both transplant procedures. In auto-HSCT patients, the resistance rates to third-generation cephalosporins and to carbapenems in *E. coli* and *K. pneumoniae* and the MDR rate in *P. aeruginosa* were similar to those reported in the general Italian population by the EARS-net survey (14). Conversely, in allo-HSCT patients the rate of resistance was significantly higher. Indeed, in allo-HSCT ceph-NS isolates were documented in about 40% of *E. coli* and 78% of *K. pneumoniae*, carba-NS isolates accounted for 53% of *K. pneumoniae*, and MDR was detected in about 36% of *P. aeruginosa*. While the resistance patterns of GNBI observed in auto-HSCT patients reflect the epidemiology of hospital acquired infections in standard-risk populations, in allo-HSCT the hospitalizations and exposition to multiple antibiotics courses during the pre-transplant period presumably justify the selection of microorganisms with reduced susceptibility phenotypes.

We found that diagnosis of acute leukemia, a transplant performed from an HLA mismatched donor (both related and unrelated) and from cord blood, older age, and prolonged neutropenia were independent risk factors of pre-engraftment GNBI in allo-HSCT recipients. In the

setting of auto-HSCT a diagnosis of lymphoma, older age, and no antibacterial prophylaxis were significantly predictive of GNBI.

Of interest is the different effect of fluoroquinolone prophylaxis in the two transplant populations since it halved the rate of GNBI in auto-HSCT but had no significant impact on allo-HSCT. If antibacterial prophylaxis continues to be indicated in neutropenic patients in an era of antibiotic resistance is a debated issue and some centers have already chosen to not administer it in view of the high rate of fluoroquinolones resistance and of the possible negative impact of prophylaxis in the selection of resistant intestinal flora (25-27). Our data seem to show that in high risk patients with a history of antimicrobial pressure, as those submitted to allo-HSCT, fluoroquinolone prophylaxis is no more protective at least against GNBI, while low-risk, antibiotic treatment-free subjects, such as multiple myeloma patients who receive auto-HSCT immediately after a non-intensive remission induction therapy, still appear to benefit from selective intestinal decontamination with fluoroquinolones during neutropenia. Our data are in agreement with those reported in a recent retrospective, cohort study in neutropenic patients with multiple myeloma undergoing auto-HSCT in which levofloxacin prophylaxis was associated with a decreased risk of bloodstream infections and febrile neutropenia episodes (28).

A pre-transplant infection in allo-HSCT and a colonization by resistant Gram-negative bacteria in both transplant procedures were highly predictive of a pre-engraftment infection by a pathogen with the same susceptibility phenotype (6). In view of the lack of a molecular characterization of the isolates we were not able to demonstrate if the same pathogen was responsible of the pre-transplant infection or colonization and of the post-transplant GNBI; however, this correlation seems highly reliable in the majority of cases. Indeed, the high rate of early infection relapses (median 7 days from transplant), observed in allo-HSCT recipients with a pre-transplant infection by a resistant pathogen may suggest the failure of previous antibiotic treatment to eradicate the multiresistant infection, particularly by carba-NS pathogens.

Overall mortality at 4 months from transplant was 13.7% and 3% in allo-HSCT and auto-HSCT, respectively. In addition to the well-known factors predicting poor outcome (acute leukemia, a previous auto-HSCT, a disease not in complete remission at the time of transplant, older age, prolonged pre-engraftment neutropenia, acute-GVHD in allo-HSCT; a diagnosis of lymphoma, a prolonged neutropenia during the month preceding transplant, a disease not in complete remission at the time of transplant, prolonged pre engraftment neutropenia in auto-HSCT), GNBIs represented an independent prognostic factor in both populations and a GNBI was the cause of death in 39.1% of allo-HSCT recipients who early died before the achievement of engraftment. However, the poor prognostic impact of GNBI was mainly related to infections by carba-NS enterobacteria and by *P. aeruginosa*, regardless of the susceptibility pattern, while the outcome of patients who developed an infection by carba-S enterobacteria was not significantly different from that of patients who did not experience any GNBI (29).

In conclusion, our study identifies incidence and risk factors for GNBIs during the pre-engraftment phase in a real life HSCT scenario. It confirms that the phenomenon of antimicrobial resistance is relevant in this high-risk population, particularly in allo-HSCT recipients, and dramatically impacts on the overall outcome of the patients both in auto-HSCT and allo-HSCT. Previous infectious history and colonization monitoring represent major indicators of the GNBI risk during the pre-engraftment phase. The results of the present study may be useful to identify the sub-populations of transplant recipients who might benefit from targeted antibiotic treatments and underline the crucial importance of the continuous epidemiology monitoring in the definition of appropriate infection-control strategies in high-risk hematologic populations.

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Table 1. Characteristics of patients at transplant.

Characteristic	**Allo-HSCT (n = 1,118)**	**Auto-HSCT (n= 1,625)**
Age, median years (range)	44 (1-72)	56 (1-75)
Pediatric patients (age ≤ 18 years) n. (%)	224 (20)	92 (5.7)
Male sex n (%)	652 (58.3)	958 (58.9)
Underlying disease: n. (%)		
Acute myeloid leukemia	420	47
Acute lymphoid leukemia	205	15
Other acute leukemias	6	/
Myelodisplastic syndromes	96	1
Chronic myeloproliferative	64	2
Non Hodgkin's lymphoma	99	432
Hodgkin's lymphoma	66	168
Chronic lymphoid leukemia	14	3
Multiple myeloma, plasmacellular leukemia, amyloidosis	59	846
Aplastic anemia	34	/
Hemoglobinopaties	28	/
Solid tumors	4	88
Other diseases	23	23
Phase of the underlying disease at transplant: n. (%)		
Malignancies in complete remission	617	668
Malignancies not in complete remission/active	416	934
Non malignant stable/ chronic diseases	85	23
Previous hematopoietic stem cell transplant: n. (%)		
Autologous alone	204	343
Allogeneic alone	90	2
Autologous and Allogeneic	15	1
Donor type: n. (%)		
HLA Matched related	296	/
HLA MisMatched related*	352	/
HLA Matched Unrelated volunteer	295	/
HLA MisMatched Unrelated volunteer	145	/
Unrelated cord blood	30	/
Stem cell source: n. (%)		
Bone Marrow	476	9
Peripheral blood	612	1616
Cord blood	30	/
Pretransplantation conditioning: n.(%)		
Myeloablative	826	1472
Reduced intensity	251	32
Non myeloablative	41	121
T cell depletion		
No	636	1625
Yes in vivo (ATG or Alemtuzumab)	386	/
Yes ex vivo (graft manipulation)	96	/
Prolonged neutropenia during the month before transplant (PMN <500/mmc for at least 7 days)	219	82
Rituximab therapy before autologous transplant	NA	367
Antibacterial prophylaxis during engraftment		
No	141	214
Yes	977	1411

* The 352 transplants from HLA MisMatched related donors included 311 haploidentical transplants

Table 2. Infections documented before engraftment

	Allo-HSCT (n=1,118)	Auto-HSCT (n=1,625)
No fever, no infection, n. of patients (%)	329 (29.5)	755 (46.5)
Fever of unknown origin only, n. of patients (%)	395 (35.3)	472 (29.0)
Clinically documented infections, n of episodes/ n. of patients (%)	68/67 (6.0)	87/85 (5.2)
- Pneumonia,	39/39 (3.5)	53/53 (3.3)
- Skin infections,	14/14 (1.2)	12/12 (0.7)
- Gastrointestinal tract,	6/6 (0.5)	20/18 (1.1)
- Other,	10/9 (0.8)	2/2 (0.1)
Microbiologically documented infections, n of episodes/ n. of patients (%)	412/331 (30.1)	355/320 (19.2)
- Gram negative bacteria	157/148 (13.2)	162/157 (9.7)
- Gram positive bacteria	209/193 (17.3)	182/172 (10.6)
- Fungi	24/24 (2.1)	9/9 (0.5)
- Viral diseases	22/22 (2.0)	2/2 (0.1)

Table 3. Distribution of Gram-negative species and antimicrobial susceptibility patterns

Pathogen and resistance pattern	Allo-HSCT (n=157)	Auto-ASCT (n=162)
Escherichia coli, total n (%)	78 (49.7)	94 (58.0)
Ceph-S, Carba-S, n (% of *E. coli*)	47 (60.3)	64 (68.1)
Ceph-NS, Carba-S, n (% of *E. coli*)	30 (38.5)	30 (31.9)
Ceph-NS, Carba-NS, n (% of *E .coli*)	1 (1.3)	0
Klebsiella pneumoniae, total n (%)	32 (20.4)	25 (15.4)
Ceph-S, Carba-S, n. (% of *K. pneumoniae*)	7 (21.9)	8 (32.0)
Ceph-NS, Carba-S, n. (% of *K. pneumoniae*)	8 (25.0)	10 (40.0)
Ceph-NS, Carba-NS, n. (% of *K. pneumoniae*)	17 (53.1)	7 (28.0)
Other enterobacteriaceae, total n (%)	10 (6.4) a	12 (7.4) b
Ceph-S, Carba-S, n. (% of other enterobacteriaceae)	9 890.0)	12 (100)
Ceph-NS, Carba-S , (% of other enterobacteriaceae)	0	0
Ceph-NS, Carba-NS, (% of other enterobacteriaceae)	1 (10.0)	0
Pseudomonas aeruginosa, total n (%)	22 (14.6)	13 (8.0)
Non-MDR *P. aeruginosa*, (% of *P. aeruginosa*)	14 (63.6)	12 (92.3)
MDR-*P. aeruginosa*, (% of *P. aeruginosa*)	8 (36.4)	1 (7.7)
Other Gram negative bacteria, total n (%)	15 (9.5) c	18 (11.1) d
No-MDR , (% of other Gram negative)	11 (73.3)	16 (88.9)
MDR, (% of other Gram negative)	4 (26.7)	2 (11.1)

Ceph-S: susceptible to third generation cefhalosporins; Carba-S: susceptible to carbapenems, defined as a MIC for imipenem, meropenem and ertapenem $\leq$ 1 mg/L; Carba-R: resistant to carbapenems, defined as a MIC for imipenem and/or meropenem and/or ertapenem > 1 mg/L; MDR= multi drug resistant, resistance to at least three of the following antibiotics: ciprofloxacin, amikacin, piperacillina-tazobactam, ceftazidime and meropenem or imipenem

a: Enterobacter spp, 6 cases; Klebsiella oxytoca, 2 cases; Proteus mirabilis, 1 case; Serratia marcescens, 1 case.

b: Enterobacter spp, 5 cases; Proteus mirabilis, 3 cases; Morganella morganii, 2 cases; Serratia marcescens, 1 case; Yersinia enterocolitica, 1 case

c: Stenotrophomonas maltophilia, 4 cases; Acinetobacter spp, 3 cases; Pseudomonas spp, 4 cases; Capnocytophaga spp, 2 cases; Rhizobium radiobacter, Citrobacter sp, 1 case each.

d: Acinetobacter spp, 6 cases; Campylobacter jejunii, 4 cases; Capnocytophaga spp, 2 cases; Bacteroides fragilis, Citrobacter sp, Fusobacterium sp, Sphingomonas sp, Pseudomonan sp 1, Stenotrophomonas maltophilia, one case each

Table 4. Cumulative Incidence and risk factors for GNBIs during the engraftment period after allo-HSCT according to the demographic, underlying disease and transplant variables.

Variables		Univariate analysis		Multivariate analysis	
		HR (95% CI)	**P**	**HR (95% CI)**	**P**
Gender	Male	1.00			
	Female	1.023 (0.74-1.42)	0.87		
Age (increased by 10 years)		1.19 (1.09-6.14)	<.0001	1.15 (1.05-1.25)	0.016
Underlying disease	Acute leukemia	1.00			
	Other diseases	0.70 (0.50-0.97)	0.03	0.64 (0.46-0.89)	0.009
Phase of the underlying disease at transplant	Complete remission	1.00			
	No complete remission	1.13 (0.82-1.56)	0.46		
Previous auto-HSCT	No	1.00	0.95		
	Yes	0.99 (0.66-1.48)			
Previous allo-HSCT	No	1.00			
	Yes	1.11 (0.67-1.85)	0.69		
Pre-transplant neutropenia	No	1.00			
	Yes	1.06 (0.71-1.56)	0.79		
Stem Cell Source_	Peripheral blood	1.00			
	Bone marrow	1.71 (1.23-2.40)	0.001		
	Cord blood	2.76 (1.24-6.13)	0.013		
Donor type	Matched related	1.00			
	Mismatched related	3.73 (2.17-6.42)	<.0001	3.74 (2.15-6.50)	<.0001
	Matched Unrelated volunter	1.96 (1.07-3.58)	0.03	1.64 (0.87-3.10)	0.12
	Mismatched unrelated volunter	3.49 (1.87-6.51)	<.0001	2.91 (1.50-5.64)	0.0015
	Cord blood	5.11 (20.7-12.65)	0.0004	3.77 (1.50-9.45)	0.005
Conditioning_regimen	Myeloablative	1.00			
	Non myeloablative/ reduced intensity	1.73 (1.25-2.41)	0.001		
T cell depletion	No	1.00			
	Yes, ATG in vivo	1.08 (0.77-1.51)	0.67		
	Yes, manipulation ex vivo	0.16 (0.04-0.63)	0.009		
Antibacterial prophylaxis	No	1.00			
	Yes	0.77 (0.49-1.22)	0.27		
Acute GVHD during engraftment	Grade 0-I	1.00			
	Grade II-IV	1.13 (0.29-4.39)	0.86		
Days of pre-engraftment neutropenia	PMN <500/cmm	1.02 (1.01-1.03)	<.0001		
	PMN <100/cmm	1.03 81.02-1.04)	<.0001	1.02 (1.01-1.03)	0.0004
Mucositis	CTC Grade 0-I	1.00			
	CTC Grade II-IV	1.07 (0.77-1.49)	0.68		

Table 5. Probability of mortality at 4 months from allo-HSCT

	Univariate		Multivariate	
	HR (95% CI)	P	HR (95% CI)	P
female vs male	0.82(0.59-1.14)	0.24		
Age (increased by 10 years)	1.15(1.06-1.25)	0.0014	1.10 (1.01-1.21)	0.026
Underlying disease, no acute leukemia vs acute leukemia	0.81(0.59-1.12)	0.21	0.42 (0.28-0.62)	<.0001
Phase of the underlying disease at transplant , no CR vs CR	1.82(1.32-2.51)	0.0003	2.18 (1.49-3.19)	<.0001
Prior auto-HSCT, yes vs no	1.43 (0.10-2.06)	0.051	1.76 (1.18-2.63)	0.006
Prior allo-HSCT, yes vs no	1.60 (1.01-2.54)	0.045		
Pre-transplant neutropenia, yes vs no	1.34 (0.93-1.94)	0.12		
Stem cell source				
Bone marrow vs Peripheral	1.10(0.79-1.53)	0.55		
Cord blood vs Peripheral	1.93(0.89-4.19)	0.09		
Donor type				
Mismatched related vs matched related	1.93(1.25-2.97)	0.003		
Matched Unrelated volunteer vs matched related	1.19(0.73-1.93)	0.48		
Mismatched unrelated volunteer vs matched related	1.11(0.60-2.03)	0.74		
Cord blood vs matched related	2.51 (1.10-5.71)	0.028		
Non Myeloablative/RIC vs myeloablative conditioning	1.55(1.11-2.16)	0.01		
T cell depletion				
In vivo ATG vs no T cell depletion	1.04 (0.74-1.46)	0.82		
Ex vivo manipulation vs no T cell depletion	1.0 (0.26-1.79)	0.99		
Antibacterial prophylaxis, yes vs no	0.76 (0.49-1.17)	0.21		
Mucositis, CTC grade II-IV vs CTC grade 0-I	0.94 (0.68-1.31)	0.73		
Days of pre engraftment neutropenia,				
ANC < 500/cmm	1.02 (1.01-1.04)	<.0001		
ANC < 100/cmm	1.03 (1.02-1.04)	<.0001	1.03(1.01-1.04)	<.0001
Acute GVHD, grade II-IV vs grade 0-I	2.07 (1.17-3.65)	0.01	2.16 (1.21-3.84)	0.009
Gram negative bacterial infection, yes vs no	2.86 (1.97-4.05)	<.0001	2.08 (1.43-3.04)	0.0001

ATG: anti thymocyte globulin; CTC= Common Toxicity Criteria ANC= absolute neutrophil count; HSCT= hematopoietic stem cell transplant; HR= hazard ratio; CI= Confidential Interval; CR=complete remission; AL= acute leukemia; GVHD= graft versus host disease;
RIC= reduced intensity conditioning

Table 6. Cumulative Incidence and risk factors for GNBIs during the engraftment period after auto-HSCT according to the demographic, underlying disease and transplant variables

Variables		Univariate analysis		Multivariate analysis	
		HR (95% CI)	**P**	**HR (95% CI)**	**P**
Gender	Male	1.00			
	Female	10.9 (0.78-1.50)	0.57		
Age (increased by 10 years)		1.09 (0.99-1.21)	0.09	1.18 (1.05-1.33)	0.006
Underlying disease	Multiple myeloma	1.00			
	Lymphoma	1.50 (1.08-2.10)	0.015	1.84 (1.31-2.61)	0.0005
	Acute leukemia	1.58 (0.77-3.24)	0.21	1.69 (0.82-3.50)	0.15
	Other diseases	1.09 (0.56-2.11)	0.80	2.08 (0.93-4.64)	0.07
Phase of the underlying disease at transplant	Complete remission	1.00			
	No complete remission	1.58 (0.84-1.60)	0.37		
Previous auto-HSCT	No	1.00			
	Yes	0.85 (0.60-1.27)	0.43		
Pre transplant rituximab	No	1.00			
	Yes	1.48 (1.05-2.08)	0.03		
Pre-transplant neutropenia	No	1.00			
	Yes	1 (0.49-2.03)	1		
Antibacterial prophylaxis	No	1.00			
	Yes	0.48 (0.33-0.68)	0.0001	0.46 (0.32-0.68)	<.0001
Days of pre-engraftment neutropenia	PMN <500/cmm	1.03 (1.004-1.048)	0.02		
	PMN <100/cmm	1.03 (1.007-1.05)	0.01		
Mucositis	CTC Grade 0-I	1.00			
	CTC Grade II-IV	1.07 (0.78-1.47)	0.65		

Table 7. Probability of mortality at 4 months from auto-HSCT

	Univariate		Multivariate	
	HR (95% CI)	P	HR (95% CI)	P
female vs male	0.78 (0.43-1.41)	0.4		
Age (increased by 10 years)	0.99 (0.83-1.18)	0.9		
Underlying disease, Multiple myeloma Lymphoma Acute leukemia Other diseases	 1.00 5.1 (2.44-10.7) 3.01 (0.65-13.94) 4.1 (1.38-12.3)	 <.0001 0.16 0.01	 5.07 (2.35-10.9)	 <.0001
Phase of the underlying disease at transplant, no CR vs CR	3.55 (1.7-7.6)	0.001	4.8 (2.23-10.4)	<.0001
Prior auto-SCT, yes vs no	0.86 (0.41-1.77)	0.68		
Pre-transplant rituximab, yes vs no	2.9 (1.7-5.2)	0.0002		
Pre-transplant neutropenia, yes vs no	5.2 (2.6-10.5)	<.0001	3.39 (1.62-7.12)	0.001
Antibacterial prophylaxis, yes vs no	1.31 (0.52-3.31)	0.57		
Mucositis, CTC grade II-IV vs CTC grade 0-I	1.36 (0.76-2.44)	0.30		
Days of pre engraftment neutropenia, PMN < 500/cmm PMN < 100/cmm	 1.09 (1.06-1.12) 1.09 (1.07-1.12)	 <.0001 <.0001	 1.08 (1.04-1.12)	 <.0001
Gram negative bacterial infection, yes vs no	2.93 (1.49-5.74)	0.0018	2.53 (1.28-50.)	0.008

CR=complete remission; AL= acute leukemiaGVHD= graft versus host disease;

Figure 1. Cumulative incidence curve for Gram Negative Bacterial Infection among allo-HSCT (A) and auto-HSCT (B) recipients in the GITMO epidemiological survey.

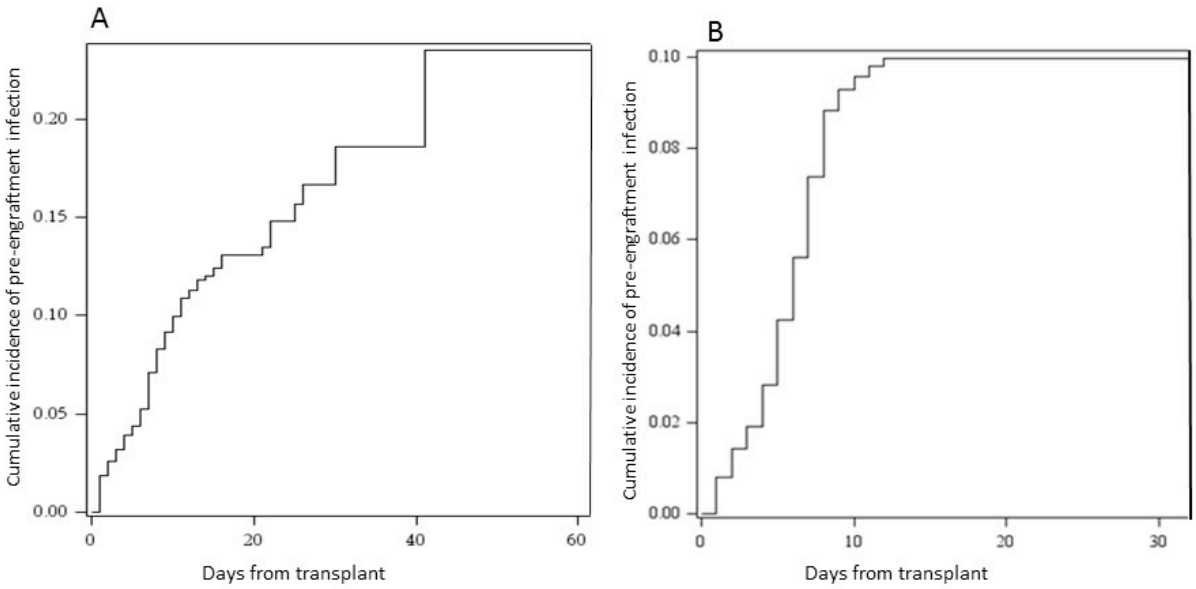
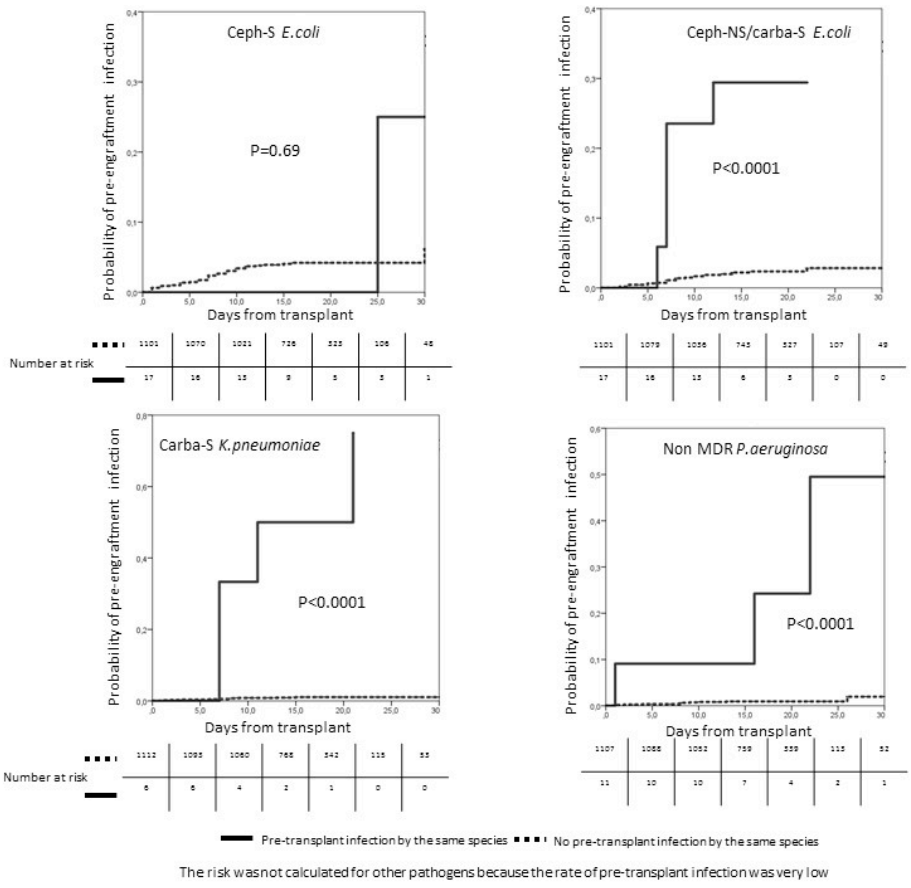
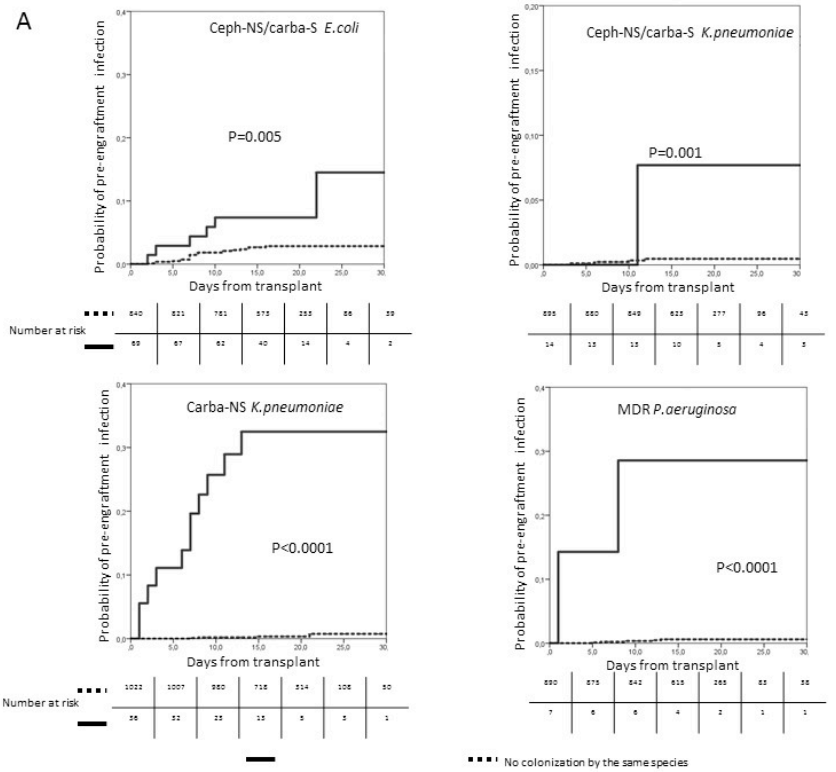


Figure 2. Risk of pre-engraftment Gram Negative Bacterial Infection recurrence according to pre-transplant infection by the same species with the same susceptibility phenotype in allo-HSCT. The risk was calculated only for species with significant rate of pre-transplant infection.



The risk was not calculated for other pathogens because the rate of pre-transplant infection was very low

Figure 3. Risk of pre-engraftment Gram Negative Bacterial Infection according to colonization by resistant gram negative bacteria in allo-HSCT (A) and auto-HSCT (B) patients. The risk was calculated only for species with significant rate of colonization.



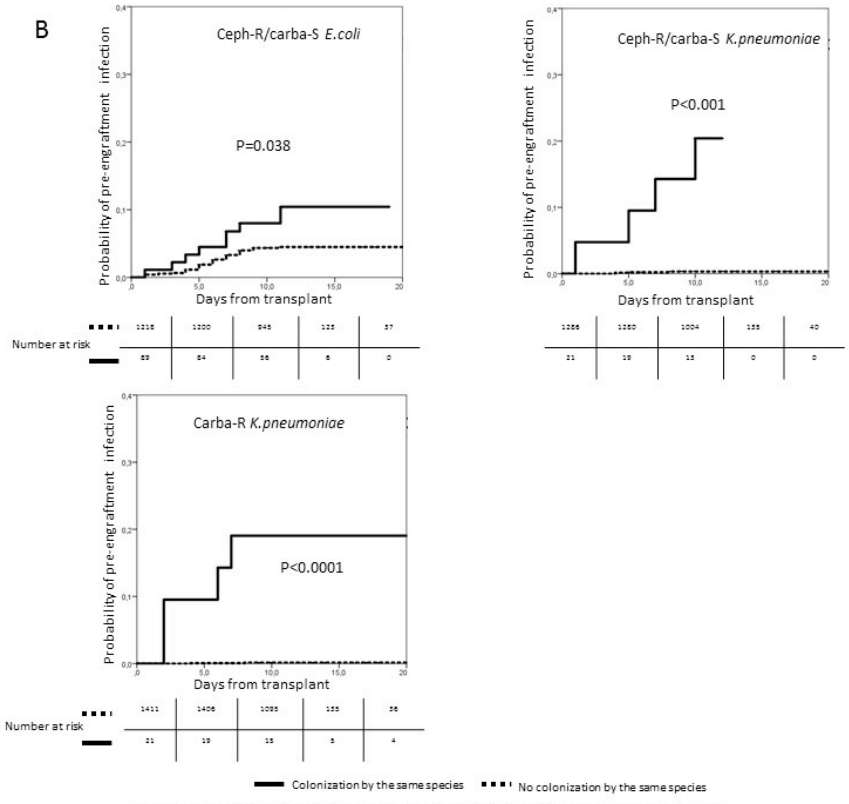
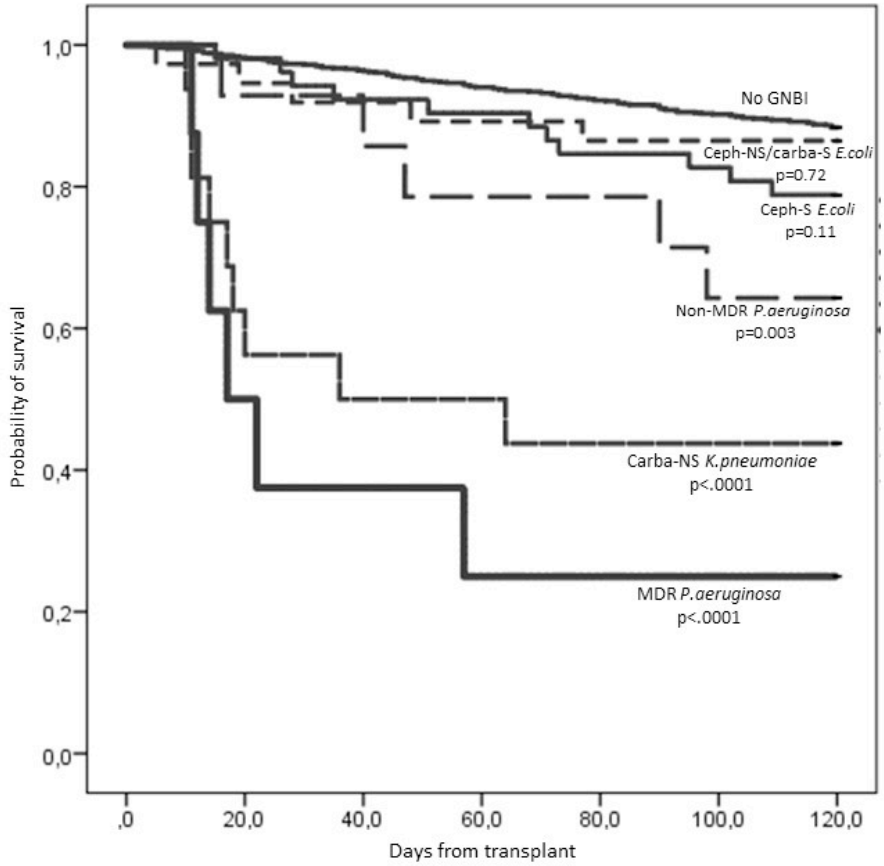


Figure 4. Probability of survival at 4 month from allo-HSCT according to the development of a pre-engraftment Gram Negative Bacterial Infection (GNBI) by different species. Survival was calculated only for species with significant rate of infection.



Probability of survival was not calculated for other pathogens because the number of episodes was very low