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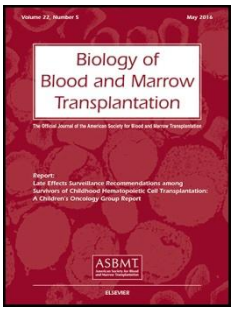

Title: Risk Factors and Outcome of Infections by Multidrug-Resistant Gram-Negative Bacteria in Patients Undergoing Hematopoietic Stem Cell Transplantation

Author: F Patriarca, C Cigana, D Massimo, D Lazzarotto, A Geromin, M Isola, ML Battista, M Medeot, M Cerno, A Sperotto, A Candoni, M Crapis, A Sartor, C Scarparo, M Bassetti, R Fanin

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RISK FACTORS AND OUTCOME OF INFECTIONS BY MULTIDRUG-RESISTANT GRAM-NEGATIVE BACTERIA IN PATIENTS UNDERGOING HEMATOPOIETIC STEM CELL TRANSPLANTATION

Patriarca F (1), Cigana C (1), Dozzo M (1), Lazzarotto D (1), Geromin A (1), Isola M (2), Battista ML (1), Medeot M (1), Cerno M (1), Sperotto A (1), Candoni A (1), Crapis M (4), Sartor A (3), Scarparo C (3), Bassetti M (4), Fanin R (1).

(1) Clinica Ematologica, Dipartimento di Scienze Sperimentali e Cliniche, Università di Udine, (2) Istituto di Statistica, Dipartimento di Ricerche Mediche e Biologiche, Università di Udine, (3) Istituto di Microbiologia, Azienda Sanitaria-Universitaria Integrata (ASUI) di Udine, (4) Clinica di Malattie Infettive, ASUI di Udine, Italy

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Correspondence to:

Francesca Patriarca, MD

Division of Hematology and Cellular Therapies Unit 'Carlo Melzi'

Azienda Sanitaria-Universitaria Integrata

p.zale S.Maria della Misericordia 1

33100 Udine

tel 0039 432 559662

fax 0039 432 559661

e mail: francesca.patriarca@asuiud.sanita.fvg.it

Highlights

- We reported the results of a 3-year surveillance program of infections by multidrug resistant Gram-negative (MDR GN) bacteria in a Transplant Unit.
- Cumulative incidence of MDR GN infections was 10,5%.
- Allogeneic transplant and colonization by MDR GN bacteria at admission to the Transplant Unit were significantly associated with an increased risk of infection.
- Patients who proceeded to allogeneic transplant and developed MDR GN infections had a significantly higher TRM and a poorer OS, in comparison with patients without MDR infections.
- The risk-benefit ratio of performing an allogeneic transplant could be carefully evaluated in patients colonized with MDR GN bacteria.

SUMMARY

Objective of this study was to determine risk factors and outcome of infections by multidrug-resistant Gram-negative (MDR GN) bacteria in 241 recipients of hematopoietic stem cell transplantation (HSCT).

The cumulative incidence of infections was 10.5% (95% CI,12.0%-25.8%), with 57% of infections occurring during the period of severe neutropenia (neutrophil count < 0.1 x 10^6/L).

In multivariate analysis, allogeneic transplant and colonization with MDR GN bacteria at admission to the Transplant Unit were significantly associated with an increased risk of infection.

While we observed neither transplant-related mortality (TRM) nor deaths due to infections by MDR GN bacteria after autologous transplant, in the allogeneic setting a significant difference was reported in terms of OS and TRM between patients who developed infections and those who did not (1-year OS, 39% vs 68%; 1-year TRM, 42% vs 19%). In multivariate analysis, refractory disease and development of grade III-IV GVHD were factors that affected both TRM and OS, while occurrence of infections by MDR GN pathogens significantly reduced OS.

We conclude that eligibility to allogeneic HSCT in MDR GN bacteria carriers should be carefully evaluated together with all other factors that independently influence outcome (disease status, donor, GVHD risk).

INTRODUCTION

Bacterial infections are one of the most common complications in cancer patients and in recipients of hematopoietic stem cell transplantation (HSCT). Although published epidemiological data on resistance to antibiotics are scanty and mostly dated, important differences in infections etiology and drug resistance exist among centres and geographical areas. In recent years, a reduction in the incidence of Gram-negative (GN) in favour of Gram-positive infections and higher rates of multidrug-resistant (MDR) infections in South-Eastern vs North-Western European regions have been observed (1). Moreover, MDR GN infections have been recognized as one of the leading cause of mortality after solid organ transplantation (2-5), while their epidemiology and impact on patients with hematological diseases and HSCT recipients have been less studied (6-9). Theoretically, the emergence of antimicrobial resistance represents a challenging problem in patients undergoing HSCT for the following reasons: 1) the eligibility to transplant in patients who have acquired the bacteria before the procedure (colonized patients or carriers); 2) the risk of infection spread into the Transplant Center in spite of isolation procedures, leading to epidemic episodes (infection control); and 3) the high mortality associated with infectious complications during the aplastic period and after the engraftment in highly immunocompromised patients due to graft-versus-host-disease (GVHD) prophylaxis or treatments.

We conducted a study to analyse the epidemiological, microbiological and clinical factors associated with the acquisition of infections by MDR GN bacteria in our Transplant Center, with the objective to determine risk factors and outcome of these infections in recipients of HSCT.

PATIENTS AND METHODS

Patients

In this observational retrospective study, we collected the data of all 241 consecutive patients who underwent HSCT at the Transplant Center of Udine between January 2013 and June 2015.

Two thirds of the candidates to HSCT were treated at the Hematology Department located near the Transplant Center, whereas one third came from Centers located in the surrounding towns (Trieste, Treviso, Padua). One hundred twenty-three patients underwent allogeneic HSCT and 118 autologous HSCT.

Patient and transplant characteristics are summarized in Table 1. Median age was 56 years (range 15-77). Lymphoma and myeloma were the most common indication for autologous HSCT, accounting for 38% and 57% of cases, respectively, whereas 56% of patients undergoing allogeneic HSCT were affected by acute leukemia. An advanced disease after a heavy pretreatment, defined as at least 2 previous therapy lines, was reported in 37% of patients before autologous HSCT and in 68% of patients before allogeneic HSCT. Stem cell source was peripheral blood for all the autologous transplants and for 67% of allogeneic transplants. Reduced intensity conditioning regimens (10) were administered in 70% of the allogeneic transplants. Allogeneic stem cells came from HLA-matched sibling donors, haploidentical donors, and unrelated donors in 27%, 12% and 51% of the patients, respectively. For unrelated donors, HLA typing for HLA-A, HLA-B, HLA-C, and HLA-DRB1 loci was required. Forty-five out of 75 unrelated transplants (60%) were HLA-mismatched, since at least one allelic or antigenic mismatch between donor and recipient was present in class I.

GVHD prophylaxis consisted of cyclosporine plus 3-4 methotrexate courses for HLA-matched sibling transplants, with the addition of antithymocyte globulin (ATG) Thymoglobulin for unrelated transplants. GVHD prophylaxis in haploidentical transplants consisted of cyclosporine, mofetilmycophenolate, and post-transplant cyclophosphamide.

Acute GVHD was graded according to the "1994 Consensus Conference on Acute GVHD Grading" criteria (11). Chronic GVHD was staged according to criteria developed by the National Institute of Health (12).

Definitions

A bacterial colonization was defined as the isolation of the microorganism from any non-sterile body site (usually rectum, urine and oral cavity) in the absence of clinical findings of infection.

A bacterial infection was defined as the isolation of the microorganism from blood culture or other usually sterile body sites in association with clinical signs of systemic inflammatory response syndrome, and after the exclusion of other possible etiologies.

MDR was defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories (13).

Invasive fungal disease (IFD) was defined according to the revised definitions by the EORTC/MSG consensus group (14).

Monitoring and management of MDR GN infections

At admission to the Transplant Unit, history of previous infections and/or colonization was collected from all patients.

Throat, nasal, rectal swabs and urine culture were collected at admission and were repeated when necessary, as clinically required.

In case of fever>38°C, patients underwent 3 sets of blood cultures by peripheral veins and central catheters.

All the patients were isolated in single rooms and received anti-infective prophylaxis with intravenous levofloxacin, fluconazole, and acyclovir.

Patients with history of previous infection or colonization by MDR GN bacteria (independently of the results of swabs at admission) and patients with any positive swabs at admission were considered as MDR GN bacteria carriers. Hospital staff and all people getting in contact with carriers and their body fluids had to use additional contact precautions (such as gloves and disposable coats).

Patients who had previous infections by carbapenem-resistant *Klebsiella pneumoniae* and were still colonized before transplant received one or more courses of oral gentamicin at the dose of 80 mg q.i.d. for 7 days, to reach negative rectal swabs before admission (15).

If an MDR carrier developed fever >38° C during the neutropenia period, he/she was treated with an antibacterial combination effective against the MDR GN pathogen, until blood cultures results were available.

Two antimicrobial combinations against the most common carbapenem-resistant Enterobacteriacae were administered (16). Pre-emptive treatment directed to carbapenem-resistant K. pneumoniae was:

- meropenem 6 g/24 hours iv continuous infusion (ci) and
- tigecycline 200 mg iv, then 100 mg x 2/day and
- gentamicin 140 mg iv once daily.

Antibacterial combination against MDR Pseudomonas aeruginosa was:

- meropenem 6 g/24 hours iv ci or piperacillin/tazobactam 18 g iv ci and
- amikacin 20 mg/Kg iv daily and/or
- colistin 9.000.000 UI iv, then 4.500.000 bid iv, on the basis of pattern of sensitivity to antimicrobial agents of the previous isolations and severity of infection.

Once diagnosis of MDR GN bacteria infection was established, treatment followed in vitro antibacterial drugs testing in vitro.

Amikacin, meropenem, gentamicin and piperacillin/tazobactam dosage was driven by regular therapeutic drug monitoring (TDM). Treatment of documented infections by MDR GN bacteria was carried on until the infection was microbiologically eradicated, all clinical signs of infection were resolved and the patient was afebrile for at least 4 consecutive days.

Surveillance cultures

Rectal, nasal and throat swabs and urine were inoculated into a non-selective culture media and incubated for 24 hours. Colonies screening for MDR GN bacteria were identified by conventional phenotypic microbiological techniques (culture characteristics, Gram stain, biochemical reactions and susceptibility to antimicrobial agents). Antibiotic susceptibility confirmation was performed using both microdilution method and standardized criteria defined by CLSI (Clinical Laboratory Standards Institute) and EUCAST (European Committee on Antimicrobial Susceptibility Testing). A phenotypic resistance was determined by an increase of the minimum concentration of antimicrobial agent required to inhibit the growth of a microbe. Standard operating procedures and robust quality control guaranteed accuracy and reproducibility of the results.

Statistical analyses

Data were collected in an XLS database from the patients medical records and reviewed by a senior hematologist and an Infective Diseases consultant; then, data were imported into Stata/SE 9.0 for Windows for statistical analysis. The close-out date for analysis was December 31, 2015.

Transplant-related mortality (TRM) was defined as death due to all causes not related to the underlying disease. Overall survival (OS) was defined as the time (months) from transplantation to either death or last observation.

OS was described according to the Kaplan-Meier approach. Comparison between groups was performed using the log-rank test.

The cumulative incidence method was used to estimate TRM and MDR infection incidence accounting for the presence of competing risks. For TRM, relapse was the competing event; for MDR GN infection incidence, death was the competing event. We collected patient-related and transplant-related variables and explored which patient-related and transplant-related factors were associated with incidence of MDR GN infections, TRM and OS. We examined the potential association between variables and incidence of MDR GN infections in the overall sample of 241 patients. We limited the analysis of the potential association between variables and TRM and OS to the population of allogeneic transplant recipients, since no TRM event occurred in the group of autologous transplants.

Patient-related variables included age (as continuous variable), sex (males vs females), underlying disease (acute leukemias, lymphoma or myeloma or other diagnoses), disease status at HSCT (responsive vs resistance/progression), phase of transplant (early vs advanced). Transplant-related variables included donor type (HLA-identical sibling vs unrelated or partially matched), stem cell source (bone marrow vs peripheral blood), conditioning regimen (myeloablative vs reduced intensity), duration of severe neutropenia (number of days with neutrophil count $< 0.1 \times 10^6$/L, as continuous variable), duration of severe thrombocytopenia (number of days with platelets count $< 20 \times 10^9$/L, as continuous variable), GVHD prophylaxis (ATG or not), acute GVHD (grade 0-II vs III-IV). Other factors that were correlated with incidence of MDR infections, TRM and OS were: previous history of infection or colonization with MDR GN bacteria before transplant (yes or no), colonization with MDR GN bacteria at transplant (yes or no), infections by Gram-positive bacteria (yes or no), infections by GN bacteria sensitive to antibacterial agents (yes or no), proven or probable IFD (yes or no).

Univariate e multivariate Cox regression were used to estimate which patient-related and transplant-related factors were associated with OS.

Based on the method of Fine and Gray (1999), univariate and multivariable backward stepwise competing-risk regression were used to explore which patient-related and transplant-related factors were associated with incidence of infections by MDR GN bacteria and TRM. This model is based on the hazard of the subdistribution and provides a simple relationship between covariates and cumulative incidence (17).

Multivariate analyses included all variables significant at $p \leq 0.10$ in univariate analysis. Retention in the stepwise model required the variable to be significant at $p \leq 0.05$ in a multivariate analysis.

RESULTS

Monitoring of colonization and/or infection by MDR GN bacteria

Eighteen out of 241 patients (7%) had a previous history of colonization (5 patients) or infection (13 patients) by MDR GN bacteria occurring at a median time of 135 days (range 28-352) before admission. At the time of admission to the Transplant Unit, 13 patients (5%) were MDR GN bacteria carriers: 8 patients had positive rectal swabs, 4 patients had positive urine cultures, 1 patient had both positive rectal swab and urine culture. Nine out of 13 patients (69%) were colonized by K. pneumoniae (Table 2). Only 1 out of 4 patients infected and colonized with carbapenem-resistant *K. pneumoniae* had obtained a negative rectal swab after a course of oral gentamicin before entering the Transplant Center. One hundred forty-three out of 241 patients (59%) developed at least one infective episode due to a documented bacterial or fungal etiology: 54 patients had infections by Gram positive bacteria, 64 patients showed infections by GN bacteria, 15 patients developed IFDs, while 10 patients presented both bacterial infections and IFDs.

Twenty-five out of 241 patients (10%) developed at least one infective episode by MDR GN bacteria at a median of 9 days (range 0-75) after HSCT, with 57% of infections occurring during the period of severe neutropenia (neutrophil count < 0.1 x 106/L). With a median follow-up of 11 months (range 1-30) after transplant, the cumulative incidence infections by MDR GN pathogens was 10.5% (95% CI,12.0%-25.8%) overall and it was 2.5% (95% CI, 0.7%-6.7%) and 18.4% (95% CI, 12.0%-25.8%) after autologous and allogeneic HSCT, respectively. A documented infection by a MDR GN bacteria with the same pattern of sensitivity to antimicrobial drugs occurred in 6 out of 13 colonized patients (46%): infections developed in none of the 3 recipients of autologous transplant and in 6 out of 10 recipients of allogeneic transplant. Twenty-five patients presented 33 infective episodes. MDR GN bacteria were isolated from 13 blood cultures (40%), 11 urine cultures (33%), 4 stool cultures (12%), and 5 broncholavage fluids (15%). The MDR GN microorganism was P. aeuruginosa in 20 samples (61%), K. pneumoniae in 12 (36%), and Escherichia coli in the remaining case (3%) (Table 2). The pattern of sensitivity of the MDR GN bacteria to antimicrobial drugs is provided in the Supplementary Table.

Factors associated with development of MDR GN infections (Table 3)

Among factors related to patient and disease characteristics, an advanced disease phase and resistant disease at transplant significantly increased the incidence of MDR infections, whereas younger age and lymphoma or myeloma as indications for transplant were associated with a significant reduction of MDR infection incidence . The factors related to transplant procedure that were significantly ($p \leq 0.10$) associated with the development of infections by MDR GN bacteria were: type of transplant, intensity of conditioning, stem cell source, duration of severe neutropenia, and thrombocytopenia. We did not observe any significant correlation between donor type or GVHD occurrence and MDR infections. Moreover, history of previous MDR infections or colonization before transplant, positive swabs at admission to the Transplant Unit, and concomitant or subsequent IFDs were significantly associated with increased infection incidence. In multivariate analysis, allogeneic transplant and colonization with MDR GN bacteria were significantly associated with a higher risk of infections (SHR 18.822; 95% CI, 2.477-143.008; p = 0.005; SHR 8.256; 95% CI, 3.374-20.203; p = 0.000, respectively).

Outcome

With a median follow-up of 11 months (range 1-30) after transplant, 184 out of 241 patients (76%) were alive with a 1-year OS of 79% (95%CI, 73%-84%). In total, 57 patients died: 30 of disease progression and 27 of transplant-related complications. All TRM events occurred after allogeneic transplant. Among 122 evaluable patients after allogeneic transplant, 51 (42%) developed acute GVHD (grade I-II in 42 patients and grade III-IV in 9 patients). We did not observe a significant association between colonization or infection by MDR GN bacteria and incidence of acute GVHD,

that occurred in 4/10 carriers (40%) compared with 47/112 non colonized patients (42%) (p=0.590) and in 9/21 MDR infected patients (43%) in comparison with 42/101 patients without MDR infection (41%) (p=0.550). Moreover, there were no significant differences in rates of grade III-IV acute GVHD and visceral organ involvement [MDR positive patients: grade III-IV acute GVHD 2/9 (22%), gut involvement 2/9 (22%), liver involvement 1/9 (11%) ; MDR negative patients: grade III-IV acute GVHD 7/42 (17%), gut involvement 11/42 (26%), liver involvement 8/42 (19%); p =0.504,p=0.586,p=0.496, respectively].

Cumulative incidence of 1-year TRM was 0% after autologous transplant and 23% (95%CI, 16%-32%) after allogeneic transplant. Among the 27 patients dying of TRM, infection by MDR GN bacteria was considered the primary cause of death in 9 patients (33%), followed by GVHD (6 patients), organ toxicities (5), CNS hemorrhage (3), and other infections (4). Nine of the 25 patients infected by MDR GN microorganisms died because of the infection, with an overall infection-related mortality rate of 36%. Deaths were due to septic shock in 4 patients and respiratory failure secondary to pneumonia in 5 patients.. *P.aeruginosa* and *K.pneumoniae* equally contributed to mortality (5 and 4 deaths, respectively).

Since neither TRM events nor deaths due to MDR infections occurred after autologous transplant, we focused our analysis on factors affecting the outcome only in the setting of allogeneic transplant. There was a statistically significant difference in terms of OS and TRM between patients who developed MDR GN infections after allogeneic HSCT and those who did not (1-year OS 39% vs 68%; 1-year TRM 42% vs 19%; log-rank test p= 0.014; Gray test p=0.003) (Figs.1 and 2). In univariate analysis, factors significantly associated with TRM were: resistant disease at transplant (p=0.002), duration of severe neutropenia (p=0.005), development of grade III-IV acute GVHD (p=0.012), need for secondary treatment for acute GVHD (p=0.014), development of infections by MDR GN bacteria (p=0.000), and diagnosis of IFD (p=0.057). The variables that showed a significant association with OS in the univariate analysis were: leukemia as underlying hematological disease (p=0.055), resistant disease at transplant (p=0.000), duration of severe neutropenia (p=0.001), development of grade III-IV acute GVHD (p=0.033), need for secondary treatment of acute GVHD (p=0.001), history of previous infections or colonization by MDR GN bacteria before transplant (p=0.012), positive swabs at admission (p=0.035), and MDR infections after transplant (p=0.014). In multivariate analysis, a refractory disease at transplant and development of grade III-IV acute GVHD were factors that independently affected both TRM and OS after allogeneic transplant, while occurrence of MDR infections significantly reduced OS (Table 4).

DISCUSSION

Antimicrobial resistance among GN bacteria isolated in hospitalized patients is a growing problem, with a heterogeneous distribution around the world. This phenomenon has reached Northern Italy only in recent years (18), with few cases identified recently in our hospital. The emergence of MDR GN bacteria among some HSCT candidates and the risk of spread in the Transplant Unit have led us to develop a strict program for infection control. The protocol was based on: 1) identification of all MDR GN bacteria carriers before admission to the Transplant Unit; 2) increased contact precautions in colonized and/or infected patients; and 3) prompt treatment with antimicrobial associations of all febrile episodes in MDR GN bacteria carriers, stopping the treatment in case of negative microbiological cultures.

At the time of admission, colonization was identified in 5% of patients: rectal swabs were more informative than urine cultures and identified K.pneumoniae more often than P.aeruginosa. We tried to decolonize patients with swabs positive for carbapenem-resistant K.pneumoniae, but only 1 out of 4 patients reached negativity, confirming that this treatment cannot represent a standard practice and needs further investigation. The incidence of infections by MDR GN bacteria was 10% after transplant, with the majority of infections occurring before engraftment. Forty six percent of the patients with positive swabs at transplant developed an overt infection, confirming the infection rate observed by a recent Italian survey in patients colonized with carbapenem-resistant *K.pneumoniae* undergoing HSCT (19). Factors predictive for risk of infection by MDR GN bacteria in multivariate analysis were allogeneic transplant and colonization at transplant. The association with allogeneic transplant probably reflects the clinical characteristics of recipients of this procedure, who were more likely affected by acute leukemias and were more often heavily pretreated compared to patients receiving autologous transplant. Moreover, these patients were more likely exposed to antimicrobial drugs before transplant and had more severe immunosuppression and alteration of the endogenous flora, that increased the susceptibility to different pathogens.

Development of infections by MDR GN bacteria had no impact on the outcome of autologous SCT recipients, since the few episodes resolved without clinical consequences. In contrast, MDR infections significantly increased TRM and reduced OS of allogeneic SCT recipients, being an independent predictor for poor OS. We found that development of infections by MDR GN microorganisms was an independent risk factor for mortality, along with other well known predictors for negative transplant outcome, such as active disease and severe acute GVHD. Moreover, in our study, the rates and severity of acute GVHD were similar in patients with and without colonization or infection by MDR GN bacteria, differently from what reported by Bilinski et al, who found that gut colonization with antibiotic-resistant bacteria predisposed to more frequent and severe GVHD, especially of the gastrointestinal tract (20).

Our results may support the position of some transplant experts, who do not consider MDRGN bacteria colonization 'per se' a contraindication to allogeneic transplant (21). In fact, eligibility to transplant in patients who have acquired the pathogen before the procedure should be evaluated in the context of all the other factors known to influence the outcome, such as disease status, HLA-matching of donor, and risk for acute GVHD, in order to weight the overall risk-benefit ratio of performing HSCT. Since deaths secondary to MDR infections were more common before engrafment, in the clinical practice, if we plan a transplant in MDR GN bacteria carriers, we should adopt all the measures to speed up neutrophil engrafment, such as use of peripheral blood stem cells,infusions of a large amount of cells and reduced-intensity conditioning, even if these measures have not been yet proven to reduce mortality .

A comparison of our infection-related mortality rate with previously reported data is hard, because of differences in study designs, patient populations, and multidrug-resistant microorganisms involved. Most of the published studies are on infections by carbapenem-resistant *K. pneumoniae* and were collected in intensive care units, in solid organ

transplants, and in patients with hematological malignancies (22-24). Recently, an Italian survey by Girmenia et al. (19) reported the outcome of colonizations and infections by carbapenem-resistant *K. pneumoniae* in recipients of HSCT. Comparing the mortality rate reported in that survey with our study, we note a reduction after both autologous and allogeneic transplants (from 16 to 0% and from 64 to 36%, respectively). It can be hypothesized that our infection control strategy, based on screening of MDR carriers and administration of first-line MDR bacteria-targeted therapy, contributed to lower the mortality rate after transplant in comparison with patients enrolled in a multicenter survey and treated with empirical antimicrobial regimens. .

We aknowledge that our study has several limitations. First, the data were collected in a single Center of Southern Europe, where incidence of infections by MDR GN pathogens has been increasing; therefore, the results cannot be generalized to other Centers with different epidemiological data. Second, we studied both autologous and allogeneic transplant recipients, who had different risk of infection and mortality. However, this study suggests a positive impact of a strict infection control strategy, that every Transplant Center should adopt, on the basis of its own epidemiological data.

In conclusion, in a 3-year surveillance program of MDR infections in the HSCT setting all eligible patients could proceed to autologous transplant with a negligible risk of toxicity. In case of allogeneic transplantation, patients who developed MDR infections had a poorer outcome than patients without infection, suggesting that eligibility to allogeneic transplant in colonized patients should require a careful evaluation of the risk-benefit ratio.

All the authors state that they have no conflict of interest to disclose.

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Figure 1. Overall survival (OS) according to infections by multidrug resistant (MDR) Gram-negative bacteria after allogeneic stem cell transplantation (allo-SCT). p = 0.014 (dotted line: patients without infections; solid line: patients who developed infections by MDR Gram-negative bacteria).

Figure 2. Cumulative incidence of transplant-related mortality (TRM) according to infections by multidrug resistant (MDR) Gram-negative bacteria after allogeneic stem cell transplantation (allo-SCT). p = 0.003 (dotted line: patients without infections; solid line: patients who developed infections by MDR Gram-negative bacteria).

Table 1. Characteristics of patients and transplants *(SCT: stem cell transplantation; early phase: a single line of treatment before transplant; advanced phase: at least 2 lines of treatments before transplant*).

	Auto-SCT	Allo-SCT	All
N° patients	118	123	241
Age (years), median (range)	58 (22-77)	52 (15-69)	56 (15-77)
Sex (male/female)	67/51	69/54	136/105
Diagnosis			
Leukemia	5 (4%)	69 (56%)	74 (31%)
Lymphoma	45 (38%)	19 (15%)	64 (26%)
Multiple Myeloma	67 (57%)	9 (7%)	76 (32%)
Other	1 (1%)	26 (22%)	27 (11%)
Disease status at transplant			
Responsive	110 (93%)	84 (68%)	194 (80%)
Resistance/progression	8 (7%)	39 (32%)	47 (20%)
Disease phase			
Early	75 (63%)	39 (32%)	114 (47%)
Advanced	43 (37%)	84 (68%)	127 (53%)
Donor and HLA-matching			
HLA-identical sibling	/	33 (27%)	
HLA-haploidentical	/	15 (12%)	
Matched unrelated	/	30 (24%)	
Mismatched unrelated	/	45 (37%)	
Stem cell source			
Bone marrow		41 (33%)	41 (17%)
Peripheral blood	118 (100%)	82 (67%)	200 (83%)
Conditioning			
Myeloablative	118 (100%)	37 (30%)	155 (64%)
Reduced-intensity		86 (70%)	86 (36%)

Table 2. Characteristics of the colonizations at admission to Transplant Center and the infections after transplant by multidrug resistant (MDR) Gram-negative bacteria *(*GN:gram negative; SCT: stem cell transplantation;*the number includes patients with more than 1 site of colonization or infection).

	K. pneumoniae	P. aeruginosa	E. Coli	Total
GN MDR colonization at transplant				
Rectal swabs	**7**	**2**	**0**	**9**
Urine cultures	**3**	**2**	**0**	**5**
Throat swabs	**0**	**0**	**0**	**0**
Total sites (total patients)	**10 (9*)**	**4 (4)**	**0**	**14 (13*)**
Site of GN MDR infections				
Blood	**6**	**7**	**0**	**13**
Lung	**1**	**4**	**0**	**5**
Gut	**1**	**3**	**0**	**4**
Urogenital	**4**	**6**	**1**	**11**
Total sites (total patients)	**12 (9*)**	**20 (15*)**	**1**	**33 (25*)**

Table 3. Univariate analysis of risk factors for incidence of infections by multidrug resistant (MDR) Gram-negative bacteria (*early phase: a single line of treatment before transplant; advanced phase: at least 2 lines of treatments before transplant; RIC:reduced-intensity conditioning; ATG: antithymocyte globulin; MDR:multidrug resistant*; GN: gram negative; GP: gram positive; *IFD: invasive fungal disease; SHR: subdistribution hazard ratio).*

Risk factor	SHR	95% conf. interval	p
Sex			
Male	**1**	**0.916-4.516**	**0.081**
Female	**2.034**		
Age (years) at transplant (median) modeled as continuous variable	**0.971**	**0.943-0.999**	**0.048**
Diagnosis			
Leukemia	**1**	**0.079-0.424**	**0.000**
Other diagnosis	**0.184**		
Disease status			
Responsive	**1**	**1.125-5.790**	**0.025**
Resistant	**2.552**		
Disease phase			
Early	**1**	**1.003-5.687**	**0.049**
Advanced	**2.390**		
Procedure			
Autologous transplant	**1**	**2.301-26.015**	**0.001**
Allogeneic transplant	**7.737**		
Preparative regimen			
Myeloablative	**1**	**1.340-6.573**	**0.007**
RIC	**2.966**		
Stem cell source			
Bone Marrow	**1**	**0.182-0.958**	**0.039**
Peripheral blood	**0.417**		
Days of neutrophils< 0.1 x 10^6/L (median) modeled as continuous variable	**1.174**	**1.107-1.246**	**0.000**
Days of platelets<20 x10^9/L (median) modeled as continuous variable	**1.051**	**1.023-1.080**	**0.000**
Donor			
HLA-identical sibling	**1**	**0.409-2.725**	**0.911**
Unrelated or partially matched	**1.056**		
GVHD prophylaxis			
No ATG	**1**	**0.375-2.132**	**0.802**
ATG	**0.894**		
acute GVHD			
grade 0-II	**1**	**0.449 -2.474**	**0.903**
grade III+IV	**1.054**		
Infections or colonizations with MDR GN bacteria before transplant			
No	**1**	**3.849-20.904**	**0.000**
Yes	**8.971**		
Colonizations with MDR GN bacteria at transplant			
No	**1**	**2.808-17.178**	**0.000**
Yes	**6.945**		
non MDR GN infections			
No	**1**	**0.587-3.555**	**0.423**
Yes	**1.444**		
GP infections			
No	**1**	**0.734-3.664**	**0.227**
Yes	**1.640**		
Proven/probable IFD			
No	**1**	**1.733-9.880**	**0.001**
Yes	**4.137**		

Table 4. Multivariate analysis of risk factors for transplant-related mortality (TRM) and overall survival (OS). (*MDR:multidrug resistant*;GN: gram negative; HR*: hazard ratio)*

	TRM			OS		
Factor	**HR**	**p**	**95% CI**	**HR**	**p**	**95% CI**
Disease status at transplant: **Responsive** **Refractory/progressive**	**1** **3.101**	**0.006**	**1.386-6.938**	**1** **3.148**	**0.001**	**1.649-6.011**
Acute GVHD **Grade 0-II** **Grade III-IV**	**1** **4.702**	**0.000**	**2.187-10.108**	**1** **2.685**	**0.033**	**1.085-6.640**
Infections by MDR GN bacteria after transplant **No** **Yes**	**/**	**/**		**1** **2.757**	**0.006**	**1.344-5.657**

Supplementary table. Pattern of sensitivity to antimicrobial agents of infections by multidrug resistant (MDR) Gram-negative bacteria (MIC: mean inhibitory concentration; P.a.:Pseudomonas aeruginosa; K..p: Klebsiella pneumoniae; E.c: Escherichia coli; S: sensitive; I: intermediate;R: resistant)