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Haploidentical

# Impact of Preemptive Granulocyte Infusions During Febrile Neutropenia in Patients Colonized with Carbapenem-Resistant Gram-Negative Bacteria Undergoing Haploidentical Transplantation

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A B S T R A C T

We prospectively studied the impact of preemptive granulocyte infusions (pGIs) in 69 patients colonized with carbapenem-resistant gram-negative bacteria (CRGNB) undergoing haploidentical hematopoietic cell transplantation (HCT) compared with a previous cohort of 33 patients who received only antimicrobials directed toward CRGNB at the onset of neutropenic fever (non-pGI group). All patients developed neutropenic fever at a median of day +8 (range, −4 to +12) after transplantation. Engraftment kinetics were similar for both groups. The median number of GIs was 2 (range, 1 to 7), and the median dose of granulocytes infused was $5 \times 10^{10}$ granulocytes per infusion (range, 1 to 30). The overall incidence of CRGNB bloodstream infections (BSIs) was 21.2% in non-pGI group (7/33) and 17.5% (12/69) in the pGI group ($P = .8$). However, the CRGNB-related mortality among those with BSI was 100% (7/7) in the non-pGI group versus 16.6% (2/12) in the pGI group ($P = .001$). The day 100 (4.4% versus 24.4%, $P = .002$) and 2-year nonrelapse mortality (7.5% versus 35.6%, $P = .0001$) were significantly reduced in the pGI group. The overall survival at 2 years was 75.6% in the pGI group versus 21.2% in the non-pGI group ($P = .0001$). Colonization and subsequent BSI with CRGNB are associated with a high incidence of mortality in patients undergoing HCT. pGI reduced early mortality associated with CRGNB in colonized patients undergoing post-transplant cyclophosphamide–based haploidentical HCT.



## INTRODUCTION

Infection-related mortality is a major obstacle to the success of allogeneic hematopoietic cell transplantation (HCT) since its inception. The first 2 decades of allogeneic HCT witnessed a high mortality associated with viral and fungal infections. With the improvement of diagnostic and therapeutic modalities, mortality related to fungal and viral infections has decreased over the years. However, multidrug-resistant bacteria have been on the rise in the recent times [1]. A major concern has been the global appearance of carbapenem resistance in gram-negative bacteria (GNB) in the last decade [2]. The dwindling spectrum of antimicrobial susceptibility for carbapenem-resistant GNB (CRGNB) has led to revocation of antibiotics such as polymyxins, which were shelved for several decades because of limited efficacy and worrying toxicities [3]. The spread of CRGNB is particularly worrisome in the most immunocompromised patients [4,5].

The outcome of allogeneic HCT for patients with hematologic malignancies is often compromised because of early infections with antibiotic-resistant GNB [6]. Allogeneic HCT produces a predictable but prolonged period of absolute neutropenia that might result in bloodstream infections (BSIs) with CRGNB. This is primarily seen in patients who were already colonized with such bacteria and in some instances through horizontal spread [7]. The outcome of such infections is often adverse in allogeneic HCT recipients [4,6]. In the absence of newer and effective antibiotics for CRGNB, the focus is on prevention of spread and colonization of CRGNB to the most vulnerable patients. However, despite all efforts, the spread and colonization in areas with high incidence of CRGNB are often unavoidable [8].

For the past 6 years we have carried out a prospective surveillance study on CRGNB colonization in patients with hematologic malignancies. We found a steep rise in CRGNB colonization and resultant mortality with increased hospital

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stay and intensive chemotherapy [8]. As a result, most patients undergoing allogeneic bone marrow transplantation after a phase of intensive treatment were colonized with CRGNB. Among the transplant recipients, those undergoing haploidentical (haplo-) family donor HCT are at the highest risk for CRGNB-BSI and mortality [9]. Because the mortality with CRGNB increases with the severity and duration of neutropenia [10], administration of granulocyte infusions (GIs) before the advancement of infection might be effective.

Data are scant in this regard, and the effect of GI in unselected neutropenic population has been equivocal in randomized studies, although the outcome was found to be superior in those with bacteremia compared with fungal infections [11,12]. There is no study of GI in this population of patients who are the highest risk of mortality. We carried out a prospective study evaluating the safety and efficacy of preemptive GIs (pGIs) in patients undergoing haplo-HCT in patients colonized with CRGNB.

## METHODS

This was a prospective comparative study evaluating the outcomes of CRGNB-colonized patients undergoing haploidentical peripheral blood stem cell transplantation as a part of an ongoing surveillance program for CRGNB. The initial cohort of 33 patients was treated with conventional antibiotics as per institutional protocols. The next 69 patients were enrolled for receiving pGIs, where granulocytes were infused within 48 hours of the onset of neutropenic fever. This study was approved by the Institutional Review Board, and informed consent was obtained from patients.

### *Granulocyte Collection and Infusion*

pGIs were administered within 48 hours of the onset of fever once the absolute neutrophil count (ANC) was $<.5 \times 10^9$/L. This was planned to be administered on alternate days until subsidence of fever for greater than 48 hours or neutrophil engraftment above $.5 \times 10^9$/L.

For granulocyte collections, donors were either an HLA-haploidentical family member or unrelated volunteer donor with compatible ABO blood group. The former was preferred when available. The donors met all the criteria established by the Transfusion Medicine Department, following the national guidelines. All were cytomegalovirus (CMV) seropositive and were screened for transfusion-transmitted infections according to national guidelines. Donors received granulocyte colony-stimulating factor (G-CSF) subcutaneously not exceeding 10 μg/kg (rounded to prefilled syringes of 300 μg) 18 to 24 hours before donation. This was followed by dexamethasone 8 mg orally 4 hours before collection. The donation was done on a continuous flow apheresis machine (Spectra Optia; Terumo BCT, Lakewood, CO). Granulocytes were collected on the established software-based protocol with an intention to collect at least $1 \times 10^{10}$ granulocytes per collection. The granulocyte product was irradiated and infused at the earliest time possible.

### *Monitoring During Transfusion*

GI was initiated with injectable pheniramine and injectable hydrocortisone as per institutional protocol at gradually increasing doses with planned infusion time of 8 to 10 hours. All vital signs were recorded throughout the infusion, and all the adverse events were recorded. These adverse events were acted on as per institutional policies. Amphotericin B was avoided 4 hours before and after the infusion. No G-CSF was given along with GI.

### *Surveillance for CRGNB*

Rectal swabs from all patients were collected in an aseptic manner at the bedside the first day of admission and then repeated subsequently on a weekly basis for continuous hospital stay or in subsequent admissions. Surveillance continued throughout the entire duration of active treatment. After collection, the samples were transported to the microbiology department and subsequently cultured. Enterobacteriaceae were identified based on standard laboratory protocols [8]. All clinical specimens were inoculated on MacConkey agar and blood agar for isolation of GNB. After 18 to 24 hours of incubation, the MacConkey agar plates were examined for both lactose-fermenting (pink) colonies and non–lactose-fermenting (pale) colonies. More than 1 colony morphology may represent distinct species. Wherever there was difference in the colony morphology, colonies of each were subcultured in nutrient agar media (nonselective media). Isolates were subjected to a series of biochemical tests for identification, both manually or using an automated identification system (Vitek2; BioMérieux, Marcy-l'Étoile, France), if necessary. These colonies were identified up to species level using standard protocol [13]. Susceptibility testing was performed by the disc diffusion (Kirby-Bauer) method following Clinical and laboratory Standard Institute guidelines, version 2016 [13]. Isolates showing positive disc screen test with ertapenem (10 μg) and meropenem (10 μg) or imipenem (10 μg) were suspected as possible carbapenem-resistant Enterobacteriaceae (CRE), and these isolates were further subjected to the modified Hodge test for detection of carbapenemases with $ZnSO_4$ supplementation of culture media to increase the detection rate of New Delhi Metallo-Beta-Lactamase (NDM-1) [14,15]. Reference strains used as controls were *Escherichia coli* American Type Culture Collection (ATCC) 25922, *Klebsiella pneumoniae* ATCC 700603, and *Pseudomonas aeruginosa* ATCC 27853. CRGNB was defined as nonsusceptible to any 1 of 3 antibiotics tested.

### *Monitoring and Management of Patients with CRE Colonization*

Patients with a positive rectal swab screening on more than 1 sample before or within 1 week of admission for transplantation without any sign or symptoms of infection were defined as colonized. CRE-positive patients were put under barrier nursing precautions as per Centers for Disease Control and Prevention guidelines. Patients were advised for regular sitz bath and cleaning with chlorhexidine-based cleansing solutions.

### *CRE Infections and Therapy*

Paired blood and urine samples were sent for culture before starting empirical antibiotics for patients developing clinical pictures suggesting an infection. Patients were started on high-dose antipseudomonal carbapenems along with aminoglycosides and tigecycline. Antibiotics were modified as per susceptibility report and the clinical status of patients. However, colistin or polymyxin B was added if there were signs of progression of sepsis or if there was a lack of response within 24 hours. G-CSF at 10 μg/kg/day was administered to all patients in the non-pGI group starting at day +8 after transplant or at the onset of febrile neutropenia, whichever was earlier until engraftment. Those in the pGI group did not receive G-CSF until pGI was deemed to be unsuccessful in controlling sepsis.

### *Transplant Protocols*

Conditioning regimens for malignant and nonmalignant diseases have been described in detail in our previous publications [16–18]. All patients received post-transplant cyclophosphamide as described previously. Along with the introduction of pGI, the graft-versus-host disease (GVHD) prophylaxis protocol was also modified to include CTLA4Ig with either sirolimus (for nonmalignant diseases) [17] or cyclosporine (for malignant diseases) [16]. For patients with malignant diseases, in addition, CTLA4Ig primed donor lymphocyte infusions were administered as described previously [16].

### *Donor Selection and Mobilization Protocol*

The methods followed for HLA typing, killer cell immunoglobulin-like receptor genotyping, and defining natural killer (NK) cell alloreactivity and the mobilization protocol for peripheral blood stem cells have been described in our earlier studies [18].

### *Supportive Care*

Antimicrobial prophylaxis was instituted per departmental guidelines. No anti-CRGNB treatment was initiated prophylactically. CMV prophylaxis was guided by preemptive monitoring of viral CMV load by quantitative PCR twice a week until day 100.

Acute GVHD was graded according to modified Glucksberg criteria [19], and chronic GVHD was scored based on National Institutes of Health global severity criteria [20]. Post-transplantation hemophagocytic syndrome was defined as in previous publications [21].

### *Statistics*

The primary endpoint of the study was nonrelapse mortality (NRM) and CRGNB-related mortality at 30 days, 100 days, and 2 years. Secondary endpoints were time to neutrophil and platelet engraftment, acute GVHD, and overall survival. Binary variables were compared between the 2 groups using the chi-square test, and continuous variables were analyzed using independent sample *t*-test considering Levenes test for equality of variances. Probabilities of survival were estimated using the Kaplan-Meier product-limit method. The cumulative incidences of NRM and CRGNB-related mortality and other outcomes were computed taking competing risks into account. Multivariate analysis was carried out using Cox regression analysis. An outcome was determined to be significantly different if the observed $P < .05$. All analyses were performed using statistical software IBM Armonk, NY: IBM Corp SPSS Statistics version 22.

## RESULTS

### *Patient Characteristics*

The details of patient and donor characteristics are shown in the Table 1. The median age of the entire study group was 22 years (range, 2 to 65) with a male predominance (61%).

**Table 1**
Characteristics of Patients and Donors in the pGI and Non-pGI Groups

| | Non-pGI Group (n = 33) | pGI Group (n = 69) | *P* |
|---|---|---|---|
| Median age, yr (range) | 32 (2-64) | 20 (2-65) | .1 |
| Gender, male/female | 25/8 | 37/32 | .05 |
| Median donor age, yr (range) | 33 (17-66) | 37 (12-62) | |
| Gender, male/female) | 18/15 | 34/35 | .6 |
| NK alloreactivity | 16 | 25 | .3 |
| ABO mismatched | 10 | 26 | .6 |
| Diagnosis | | | |
| Malignant | 29 | 54 | |
| Acute myeloid leukemia | 24 | 39 | |
| Acute lymphoblastic leukemia | 5 | 11 | |
| Lymphoma | 0 | 3 | |
| Myeloma | 0 | 1 | .2 |
| Nonmalignant | 4 | 15 | |
| Severe aplastic anemia | 3 | 10 | |
| Hemoglobinopathies | 1 | 5 | |
| Median CD34 cells, $\times 10^6$/kg (range) | 6.3 (3-14.4) | 8.2 (3.5-10) | .1 |
| Median CD3 cells, $\times 10^6$/kg (range) | 53 (9.7-110) | 46.7 (1.4-110) | .1 |

Malignant diseases were indications for HCT in 83 patients and nonmalignant diseases in 19 patients. All with malignant diseases had severe or very severe disease risk index [22]. Those in the nonmalignant cohort were categorized as high-risk or advanced disease as per individual disease criteria [23,24]. Fifty-four patients with malignant diseases and 15 with nonmalignant diseases received pGI. The median duration of follow-up for survivors was 24 months (range, 14 to 59) in the pGI group compared with 48 months (range, 36 to 85) in the non-pGI group. There was no statistical significance between the groups in terms of age, donor age, gender, ABO mismatch, degree of HLA mismatch, NK ligand mismatch, killer cell immunoglobulin-like receptor-B haplotype, and disease status.

### *Granulocyte Infusions*

Sixty-nine patients received infusion of apheresed granulocytes within the first 48 hours (range, 18 to 36) of neutropenic fever (pGI group), within 4 hours from the completion of collection. The infusions were continued on alternate days until subsidence of fever or engraftment. One hundred fifty-eight granulocyte collections were carried out. All procedures were uneventful. We did not observe any side effects precollection or in the 7 days after collection in the donors.

The median number of GIs was 2 (range, 1 to 7), and the median dose of granulocytes infused was $3.6 \times 10^{10}$granulocytes per infusion (range, .9 to 6.7) (Table 2). The median dose of granulocytes/kg infused was $2.5 \times 10^9$ (range, 1 to 7.8) for children and $.6 \times 10^9$ (range, .3 to 1.1) for adults. The median increment in ANC, 24 hours after pGI, was $.2 \times 10^9$/L (range, .1 to .9).

Ten patients had a grade 1 febrile transfusion reaction during the first infusion and none with subsequent infusions. Two patients, both of whom died, had respiratory difficulty with the third and seventh GIs, respectively. They were both in septic shock from ongoing BSI with *Pseudomonas aeruginosa* (Table 3) and were on inotropes and noninvasive ventilation during the events. They both succumbed to ongoing sepsis within 4 and 6 hours of the event. Because of progressive sepsis and existent pulmonary compromise, it was difficult to adjudicate the contribution of GI to the events. They were the only patients who received G-CSF on the day of death because pGI was unsuccessful. The rest engrafted without administration of G-CSF. There was no difference in CMV reactivation between the groups (52.9% in pGI versus 57.6% in non-pGI, *P* = .37).

**Table 2**
Details of GI

| Characteristics | Value |
|---|---|
| Median no. of GI infusions | 2 (1-7) |
| Median time to infusion, hr | 24 (18-36) |
| Median dose of granulocytes infused, $\times 10^9$ kg/infusion | |
| Age group 2-16 yr | 2.5 (1-7.8) |
| Age group 17-65 yr | .6 (.3-1.1) |
| Median increment in ANC, 24 hr post-GI, $\times 10^9$ cells/mL | .2 (.1-.9) |

Values in parentheses are ranges.

### *Microbiology of CRGNB Colonization*

*Klebsiella pneumoniae* was isolated from rectal swab samples in 57 patients and *Escherichia coli* in 40 patients, with both organisms in 3 patients (Table 3). Three patients were colonized with *Pseudomonas aeruginosa* and 1 each with *Proteus mirabilis* and *Acinetobacter baumanii*. There was no difference in the pattern of colonization between the 2 groups.

### *CRGNB Bloodstream Infection*

The overall incidence of CRGNB-BSI was similar in both the groups, 17.4% (12/69; 95% confidence interval [CI], 12.8 to 22.0) in the pGI group and 21.9% (7/33; 95% CI, 14.6 to 29.2) in the non-pGI group (*P* = .7) (Figure 1A). The median time to documented CRGNB-BSI was 16 days (range, 10 to 92) in the non-pGI group and 12 days (range, 9 to 15) in the pGI group (*P* = .003). Two patients with Post-transplantation hemophagocytic syndrome (PTHPS) in the non-pGI group developed *K. pneumoniae* bacteremia late after transplant associated with severe pancytopenia at days 49 and 92.

CRGNB-BSIs were from the colonized organisms only (Table 3). All episodes of CRGNB-BSI in the non-pGI group were due to *K. pneumoniae* (n = 4) or *E. coli* (n = 3). In the pGI group, *K. pneumoniae* (n = 6), *E. coli* (n = 4), and *P. auerginosa* (n = 2) accounted for episodes of BSIs. All isolates had minimum inhibitory concentrations > 16 against meropenem, and none was susceptible to aminoglycosides. Of 10 *K. pneumoniae* isolates, all were susceptible to colistin and only 5 to tigecycline as well. Among 7 *E. coli* isolates, 5 were susceptible to both tigecycline and colistin and 2 were susceptible only to colistin. Both *P. auerginosa* isolates were not susceptible to any antibiotics including colistin and polymyxin B.

The median time to onset of the first episode of febrile neutropenia was day +8 (range, −4 to +12). This was not different between groups. However, the duration of overall antibiotic usage for febrile neutropenia was significantly shorter for surviving patients in the pGI group (median, 7 days [range, 5 to

**Table 3**
Microbiology of CRGNB Colonization, BSI, and Mortality

| Organisms | Non-pGI Group (n = 33) | pGI Group (n = 69) |
|---|---|---|
| *Klebsiella* spp. | 19/4/4 | 38/6/0 |
| *Escherichia coli* | 12/3/3 | 28/4/0 |
| *Pseudomonas aeruginosa* | 1/0/0 | 2/2/2 |
| *Acinetobacter baumanii* | 0/0/0 | 1/0/0 |
| *Proteus mirabilis* | 1/0/0 | 0/0/0 |

Values are colonization/bacteremia/death.

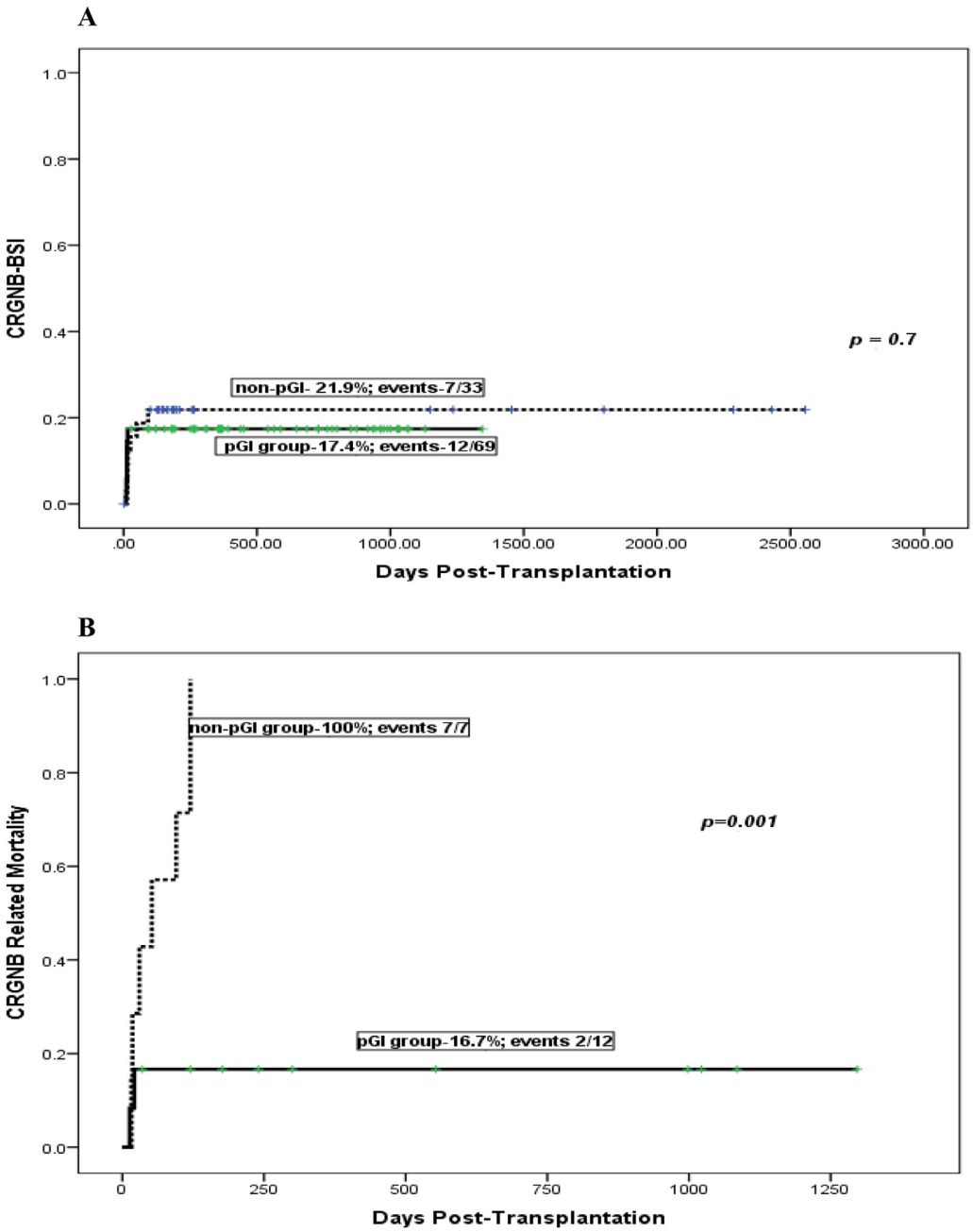


**Figure 1.** Cumulative incidences of (A) CRGNB-BSI and (B) CRGNB-related death among patients with CRGNB-BSI. The solid line represents the pGI group, and the dotted line represents the non-pGI group.

12]) compared with the non-pGI group (median, 13 days [range, 10 to 22]; $P$ = .01). The kinetics of C-reactive protein and procalcitonin levels indicated a faster decline in the pGI group. The median times to 50% decrease in the C-reactive protein and procalcitonin values in responding patients were 5 days (range, 3 to 7) and 3 days (range, 2 to 5), respectively, after the first GI in the pGI group compared with 7 days (range, 6 to 14) and 6 days (range, 5 to 10), respectively, in the non-pGI group ($P$ = .03).

### *Engraftment*

The median time to neutrophil engraftment was 15 days (range, 10 to 20) and platelet engraftment 14 days (range, 8 to 23), with no difference among groups (Table 4). However, it is worth noting that patients in the pGI group did not receive G-CSF and yet engrafted with similar kinetics. Patients receiving pGI from haploidentical family donors (n = 51) had a faster engraftment compared with those receiving pGI from third-party donors (n = 18) (median 14 days versus 16 days, $P$ = .03).

### *Acute and Chronic GVHD*

The incidence of acute GVHD was 13.7% in the pGI group compared with 35.9% in the non-pGI group ($P$ = .005). However, chronic GVHD was similar in the 2 groups (Table 4).

### *CRGNB-Related Mortality*

The CRGNB-related mortality in the overall population was 9.1% (range, 16.0% to 23.8%). CRGNB-related mortality in the

**Table 4**
Outcomes of Patients in the Non-pGI and pGI Groups

| | Non-pGI Group (n = 33) | pGI Group (n = 69) | *P* |
|---|---|---|---|
| Median ANC, days (range) | 14 (12-19) | 15 (10-20) | .7 |
| Median platelet > 20,000, days (range) | 14 (8-22) | 14 (9-23) | .9 |
| Acute GVHD grades II-IV | 11 | 9 | .005 |
| Cumulative incidence, % (95% CI) | 35.9 (27.2-44.6) | 13.7 (9.4-18.0) | |
| Chronic GVHD | 4/21 | 16/63 | .7 |
| Cumulative incidence, % (95% CI) | 20 (11.1-28.9) | 29 (22.5-35.5) | |
| CMV reactivation | 19 | 36 | .3 |
| Cumulative incidence, % (95% CI) | 57.6 (49-66.3) | 52.9 (46.8-59) | |
| CRGNB infection | 7 | 12 | .7 |
| Cumulative incidence, % (95% CI) | 21.9 (14.6-29.2) | 17.4 (12.8-22) | |
| CRGNB-related death | 7 | 2 | .002 |
| Cumulative incidence, % (95% CI) | 21.2 (14.1-28.3) | 2.9 (.9-4.9) | |
| NRM at day 100 | 8 | 3 | .002 |
| Cumulative incidence, % (95% CI) | 24.4 (16.9-31.9) | 4.4 (1.9-6.9) | |

pGI group was 2.9% (2/69; 95% CI, .9 to 4.9) compared with 21.2% (7/33; 95% CI, 14.1 to 28) in the non-pGI group ($P = .002$). More importantly, CRGNB-related mortality in patients who had CRGNB-BSIs was 100% (7/7) in the non-pGI group compared with 16.7% (2/12; 95% CI, 9.9 to 27.5) in the pGI group ($P = .001$) (Figure 1B). Mortality from CRGNB occurred at a median of 4 days (range, 2 to 9) from the onset of CRGNB-BSI. The median time post-transplant for CRGNB-related death in the non-pGI group was 16 days (range, 12 to 96) and was 12 and 20 days for the pGI group. Both deaths in the pGI group occurred before engraftment. In the non-pGI group, 2 patients with ongoing post-transplant hemophagocytic syndrome developed late CRGNB-BSI (*K. pneumoniae*).

### *NRM and Overall Survival*

Day 100 NRM was 10.9% and 2-year NRM was 16.2%. Day 100 NRM was 4.4% (95% CI, 1.9% to 6.9%) in the gGI group and 24.4% (95% CI, 16.9% to 31.9%) in the non-pGI group ($P = .002$). Two-year NRM was 7.5% (5/69; 95% CI, 4.3 to 10.7) and 35.6% (11/33; 95% CI, 26.8 to 44.2) in the pGI and non-pGI groups, respectively ($P = .0001$) (Figure 2A). Overall survival was 75.6% (95% CI, 70.3% to 80.9%) in the pGI group compared with 21.2% (95% CI, 14.1% to 28.3%) in the non-pGI group ($P = .0.0001$) (Figure 2B). This was contributed by a reduction both in CRGNB-related mortality and, in disease progression in malignant diseases with novel transplant protocols introduced in the pGI group (77.4% in the non-pGI group versus 27.3% in the pGI group, $P = .0001$).

## DISCUSSION

In a prospective surveillance study on gut colonization with CRGNB in 225 patients with hematologic malignancies, we had observed colonization in 21% of newly diagnosed patients that doubled with hospital stays of 4 weeks or more [8]. More importantly, in patients with acute myeloid leukemia who were colonized during the hospital stay, CRE-related mortality was 55%. In the current study, of the initial cohort of 33 CRGNB colonized patients undergoing haplo-HCT, 22% developed BSI with CRGNB, which was associated with 100% mortality and NRM of 24.4% at 100 days and 35.6% at 2 years.

In a retrospective analysis of 533 patients, of which 330 patients received haplo-HCT, a higher incidence of BSI was noted in haplo-HCT recipients treated on post-transplant cyclophosphamide–based protocols [9]. CRGNB-related mortality was 55%. In several other studies BSI was reported to be higher after haplo-HCT [25–27]. Colonization with CRGNB has been an independent risk factor for BSI and mortality after allogeneic HCT [10,27–29]. Apart from the Indian subcontinent, a very high incidence of CRGNB colonization as well as BSI has been reported from Italy, with CRGNB accounting for about 60% of all GNB bacteremia in HCT recipients [5]. The mortality from CRGNB-BSI likewise was over 50% across all such reports [4,30].

In this context, carrying out haplo-HCT with post-transplant cyclophosphamide in patients colonized with CRGNB invokes a very high risk of infection-related mortality, which is not influenced by the initiation or choice of antibiotics as shown in our study. The same has been highlighted in a multicenter study in non-HCT patients from New York as well, which is supposed to be the CRGNB epicenter of the United States [31]. Even though some studies have claimed success of early initiation of CRGNB-directed antibiotics [26], our experience has not been gratifying with a similar approach. This was primarily because the GNB species responsible for colonization and BSI were often sensitive to colistin alone or even pan-resistant. In an attempt to address this problem, we initiated GI in the subsequent 69 patients with similar characteristics. This resulted in a dramatic improvement in CRGNB-related mortality. Against the backdrop of equivocality that has emerged from randomized studies on GI [11,32], these findings assume greater importance. The failure of GI to demonstrate any definite advantage in survival is probably because of the small cohort size in the randomized studies along with heterogenous patient populations with different underlying causes for neutropenia. Moreover, GI is unlikely to make any major impact if there are good antimicrobial agents available to treat the pathogens responsible for BSI. In addition, prospective studies on the use of GI in the setting of allogeneic HCT in patients who are at a high risk of BSI are lacking. Randomization becomes ethically untenable in a situation where the mortality with standard of care is of such magnitude as seen with CRGNB-BSI. Hence, we did not consider randomization and used the prior non-pGI arm as the comparator.

In specific high-risk scenarios, pGI could make an impact on reducing mortality as shown in our study. However, several aspects need to be considered while determining the possible impact of GI. With G-CSF and dexamethasone mobilization, large number of granulocytes can be collected by a single apheresis, which helps in raising the ANC promptly [33,34]. At the same time, mortality from CRGNB-BSI is often rapid, making it imperative that granulocytes are infused early in the course of infection to make any impact on the outcome. This is particularly relevant in CRGNB-BSI because the time to death from the onset is often rapid, varying from 24 hours to 7 days [5,8,9,31]. It is worth noting that surveillance protocols and antibiotic policies were not different across the pGI and non-pGI cohorts. The incidence of BSI associated with CRGNB remained unchanged in the pGI group as the intervention in the form of pGI was preemptive and not prophylactic. There was a constant effort to administer granulocytes within 24 hours of onset of neutropenic fever. We believe that this, along with the higher doses of granulocytes infused, were the key factors in improving the outcomes in the current study. This was exemplified by prompter reduction in C-reactive

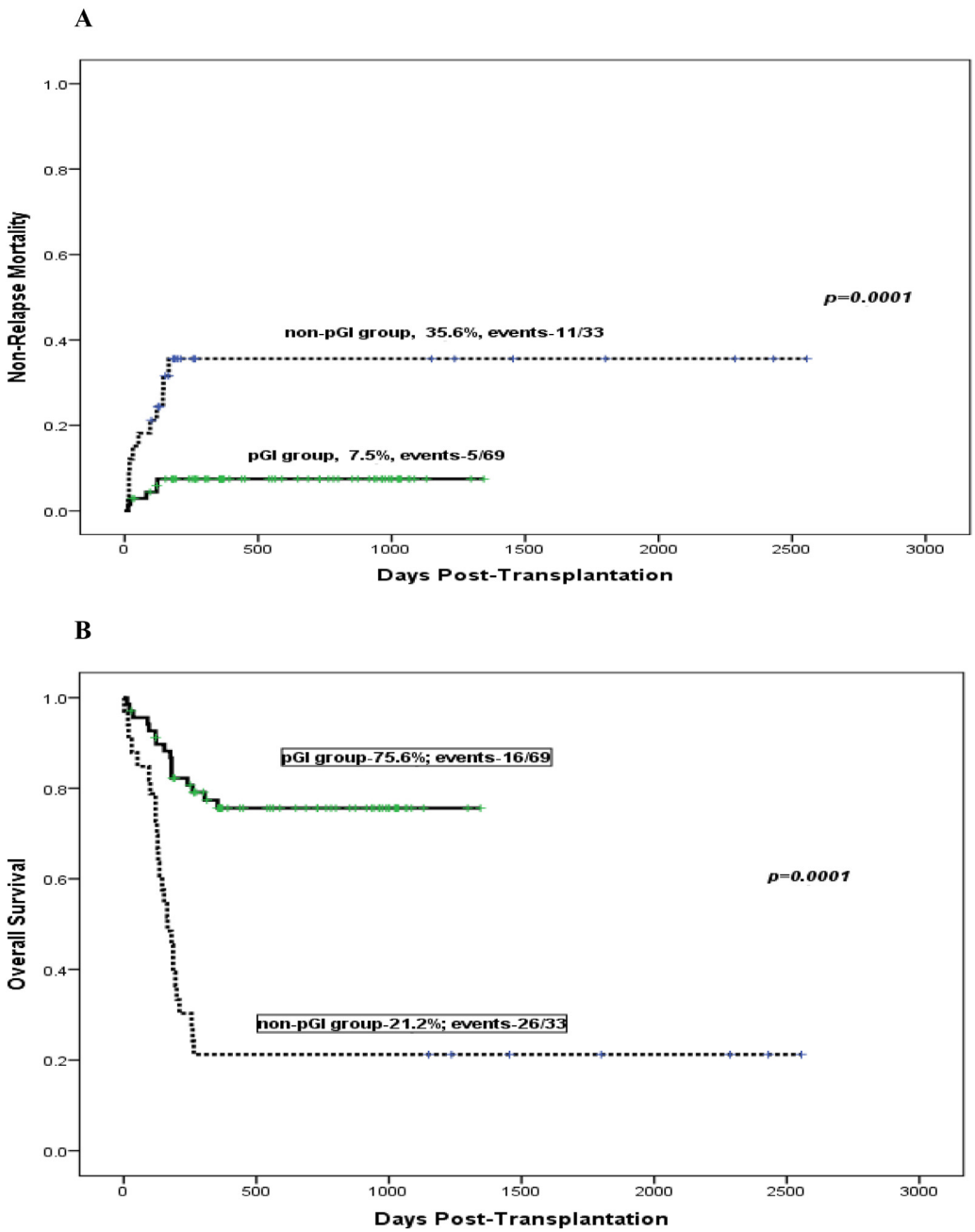


**Figure 2.** Cumulative incidences of (A) NRM and (B) overall survival. The solid line represents the pGI group and the dotted line represents the non-pGI group.

protein and procalcitonin in the pGI group and a reduced duration of antibiotic usage.

In addition, virtually no significant toxicities were encountered with pGI apart from those described above, which were primarily attributable to the fulminant nature of pseudomonal sepsis. There could be several reasons for the lack of toxicity. First, granulocytes were infused at a much slower rate than that described previously [11]. This did not compromise the efficacy because most responded to the first dose of pGI. Despite the prevailing practice of rapid infusion of granulocytes, existing data suggest that G-CSF–mobilized granulocytes are stable for over 24 hours and retain complete functional capability at or beyond this period [35,36]. Second, most granulocyte transfusions were from HLA-haploidentical family members, which could have contributed to better longevity and the attenuation of possible allogeneic immune effects. GI in the context of HLA-mismatched HCT before engraftment might have very different immunologic consequences than that of a matched donor HCT or a non-HCT setting [37]. Most of our patients were heavily pretransfused and alloimmunized against a wide array of HLA antigens. A valid concern is triggering of an alloimmune response in either a graft-versus-host or a host-versus-graft direction from a third-party GI in the context of haplo-HCT. This prompted us to use an HLA-haploidentical family donor in most cases. Even though we did not encounter any adverse event with the use of unrelated donor GI, caution should be exercised regarding repeated random donor GI in the setting of haplo-HCT in the periengraftment period.

The other striking finding was prompt engraftment in the pGI group without G-CSF support, which was more prompt in

those receiving pGI from haploidentical family donors. In the current protocol of NK cell–based immunotherapy with CTLA4Ig-primed donor lymphocyte infusions [16], we avoided prophylactic administration of G-CSF, which is known to interfere with NK cell function [38]. It is possible that natural cytokines mobilized with the donor granulocytes could have influenced the engraftment process. Despite being purely conjectural, this is interesting enough to deserve further attention.

On the downside, granulocyte collection imposes significant logistic burden on transfusion services in terms of staff and resources [39], which includes precollection treatment and monitoring of the donor, a 3-fold increase in collection time compared with platelet pheresis and postcollection follow-up of the donor. On the other hand, a 5-fold decrease in early mortality and reduced usage of antibiotics, as achieved in this study, might justify this measure.

The key to realization of the potential of a transplant protocol lies primarily in reducing early mortality because of infections and GVHD. Our current protocols using CTLA4Ig have resulted in excellent progression-free survival with significant reduction in early alloreactivity in both malignant and nonmalignant diseases [16,17]. We attribute a significant reduction in early infection-related mortality with the use of pGI in reducing CRGNB-related mortality and the modification of GVHD prophylaxis in reducing acute GVHD in this cohort. Thus, these combined approaches helped in realizing the full potential of this approach, accounting for overall survival of more than 75% in this group of patients who are otherwise deemed to have an extremely poor outcome.

Despite significant reduction in CRGNB-related mortality, we cannot overlook the fact that both deaths in the pGI group occurred with pan-resistant *P. aeruginosa*. The virulence of the species and fulminant clinical courses with complete absence of effective antibiotics against the same remain a major concern [40]. With emergence of resistance against colistin among GNB, even introduction of ceftazidime-avibactam is unlikely to offer much hope becasue it is ineffective against metallo-$\beta$-lactamase–producing CRGNB [4]. Indiscriminate use of antimicrobials has undoubtedly led to the current situation. The encouraging results of pGI with respect to both safety and efficacy might prompt the prophylactic use of GI (ie, at the onset of neutropenia before onset of fever or infection). This might be the only option for colonization with pan-resistant GNB in this setting.

CRGNB is no longer geographically limited but a global phenomenon. In the absence of robust preventive measures and effective antibiotics, CRGNB would continue to be a major cause of infection-related mortality after allogeneic HCT, particularly after HLA-mismatched grafts. Our study suggests that pGI in febrile neutropenia after haplo-HCT might be effective in reducing mortality from CRGNB. The best way to exploit the efficacy of donor granulocytes in the postallograft setting is yet to be resolved. Further exploration and expansion on the modalities used in this study might help us to optimize the efficacy of GI in patients at the highest risk of mortality from CRGNB.

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