Original Article

HEALTH SCIENCES
MEDICINE

DOI: 10.32322/jhsm.1275159
J Health Sci Med 2023; 6(3): 674-679

The effect of granulocyte transfusion on engraftment in patients with allogeneic hematopoietic stem cell transplantation

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Cite this article as: İtmeç Y, Çelik S, Ünal A. The effect of granulocyte transfusion on engraftment in patients with allogeneic hematopoietic stem cell transplantation. J Health Sci Med 2023; 6(3): 674-679.

Received: 03.04.2023 ◆ **Accepted**: 23.05.2023 ◆ **Published**: 31.05.2023

ABSTRACT

Aim: Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is still one of the most effective treatments for many hematological malignancies. However, especially infections and neutropenic fever increase mortality during the engraftment development process after allo-HSCT. This study investigated the effect and safety profile of granulocyte transfusion (GT) on engraftment in patients with neutropenic fever after allo-HSCT.

Material and Method: We investigated 32 patients with hematological malignancies who had neutropenic fever following allo-HSCT between June 2018 and February 2020. Seventeen patients were given GT and defined as GT group (GTG). GT was given once daily until improvement in clinical and laboratory parameters (neutrophil >0.5 $\times10^3$/µL, platelet >20 $\times10^3$/µL). Fifteen patients who did not receive GT were included as a control group (CG).

Results: By comparing leukocyte levels between the start and end of GT, the median leukocyte increase was shown as 1.93 (0.37- 10.21) $\times10^3$/µL (p=0.001). Similarly, the median neutrophil increase was 1.14 (0.25-9.24) $\times10^3$/µL (p=0.001). A total of 65 GTs were administered, the average number of days was 4±1. The average dose of infused granulocyte was 4 $\times10^{10}$/unit. In GTG, neutrophil and platelet engraftments occurred on average at 14±2 and 10±2 days, respectively. In CG, neutrophil and platelet engraftments occurred on average 15±2 and 12±3 days, respectively. There was no statistically significant difference in neutrophil and platelet engraftment between the two groups (p=0.4, p=0.06, respectively).

Conclusion: GT was observed to be effective in managing complications such as neutropenic fever and sepsis after allo-HSCT by shortening the duration of neutropenia and increasing neutrophil and leukocyte values. Although statistical significance was not observed in our study, it was observed that the engraftment times were shortened with GT.

Keywords: Allogeneic transplantation, engraftment, granulocyte transfusion

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is a potentially curative treatment for many hematological malignancies. However, the success of allo-HSCT is often limited by delayed engraftment, which can lead to a higher risk of infection with severe morbidity and mortality (1). It is necessary for successful treatment in these patients to recover from neutropenia and use an appropriate and effective antimicrobial agent(s). Although broad-spectrum antibiotics and antifungal treatments have been introduced, infection remains a major cause of death in patients with allo-HSCT.

Granulocyte transfusion (GT) is a sensible therapeutic approach as a replacement therapy if infection control cannot be achieved despite these treatments (2). The survival effect of GT therapy in the presence of severe sepsis and invasive fungal infection in patients with an intrinsic defect in neutrophil function or neutropenia has been discussed in various studies and reported to be effective in most (1, 2).

It has been shown that GT administration against infections in neutropenic patients after allo-HSCT reduces infection-related morbidity and mortality (3). However, the effect of GT on engraftment in these patients was not evaluated. Therefore, we aimed to evaluate the effect of GT on hematological and clinical response in patients undergoing allo-HSCT, with a particular focus on its contribution to neutrophil and thrombocyte engraftment.

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MATERIAL AND METHODS

The study was carried out with the permission of Erciyes University Faculty of Medicine Ethics Committee (Date: 12.02.2020, Decision No:2020/126). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Thirty-two patients with neutropenic fever after allo-HSCT were included in this retrospective study between June 2018 and February 2020 at the Department of Bone Marrow Transplantation at Erciyes University. Seventeen patients were given GT and defined as GT group (GTG). GT was given once daily until improvement in clinical and laboratory parameters (neutrophil >0.5 $\times 10^3$/μL, platelet >20 $\times 10^3$/μL). Fifteen patients who did not receive GT were included as a control group (CG). Patients in both groups were treated with necessary antibiotics, antivirals, and antifungals.

Patients under the age of 18 and over 65 were not included in the study. Age, gender, weight, height, and diagnoses of patients were recorded. The granulocyte donor was selected according to the criteria for being a standard blood donor from non-related relatives or voluntary healthy donors. All donors before transfusion, immuno-hematological acceptance tests (blood group and crossmatch), compatible and infection screening tests (HIV, hepatitis B, hepatitis C, CMV, syphilis serology), seronegative and complete blood count were in good health. The Spectra Optia device was used for granulocyte collection.

Donors were stimulated to collect granulocytes with granulocyte colony-stimulating factor (G-CSF) and oral 8 mg dexamethasone 12 hours before apheresis. No donor had any side effects that required the collection to be stopped. The granulocyte was irradiated with 25 Gy before being infused. The objective cell dose given to the patient by granulocyte infusion was at least 1×10^{10} per transfusion. In patients taking amphotericin B for fungal infection, it was noted that there was a minimum of six hours between GT and amphotericin B administration. Transfusion was usually performed between 30-45 minutes. No severe side effects were observed in the patient when applying granulocytes. GT was continued daily and as long as the donor could be provided until the absolute neutrophil number rose above 0.5×10^9/L or until clinically signs of infection were controlled. Patients who received granulocytes for consecutive days were included in our study. Granulocyte was given for at least three days, and no more than six days.

Allo-HSCT was performed to 101 patients in our center between June 2018 and February 2020. GT was given to 17 (17%) of them. GT administration is considered in all allo-HSCT patients when an adequate response is not achieved with antibiotic therapy in our center. However, GT cannot be given when an unrelated or voluntary donor suitable for GT cannot be found or if the donor found is not suitable as a result of the tests. In our study, GT was given to 17 of 32 patients, but GT could not be given to 15 (47%) of the aforementioned reasons, and they were taken as the control group.

Engraftment has at least three consecutive days of absolute neutrophil count >500/mm³ and platelet count $>20\times10^3$/μL (3). Leukocyte, platelet, and neutrophil values were noted every day until engraftment occurred. Granulocyte infusion dose, granulocyte day count, engraftment day, clinical response, hematological response, and mortality status were followed. Clinical response to granulocyte transfusion was defined as a clinical benefit of the patient, a decrease in fever, control of infection, and the inability to show reproduction in the culture previously in the control culture.

Statistical Analysis

In our study, the SPSS 22 (IBM) package program was used to evaluate the data analysis. Descriptive statistics (the Kolmogorov-Smirnov/ Shapiro-Wilk tests) and graphs (histogram and probability) were used to create Central and prevalence criteria such as percentage, number, minimum, maximum, mean, standard deviation. In descriptive analyses, mean and standard deviation (mean±standard deviation) were used for normally distributed variables, while median and minimum-maximum values (median± min-max) were used for normal non-distributed variables. The Mann-Whitney U test was used for independent variables compared to 2 variables that were found not to match the normal distribution. The Wilcoxon test was used to compare two dependent variables that do not match the Normal distribution. The Chi-square test was used to determine the difference between categorical variables. The correlation between numerical data was evaluated by Spearman correlation analysis, as the variables did not correspond to the normal distribution. Overall survival (OS) was measured as the time from allo-HSCT to death regardless of any cause. Results were considered in the 95% confidence range, statistical flood significance $p<0.05$.

RESULTS

The main characteristics of the 32 patients who had neutropenic fever after allo-HSCT are presented in **Table 1**. Seventeen (53.1%) patients were given GT and defined as GT group (GTG). The other 15 (46.9%) patients did not receive GT and were named the control group (CG). Of these, 16 (50%) were female, and 16 (50%) were male. The median age in the GTG and CG was 44 (29-62) and 50 (26-65) years, respectively (p=0.14).

Table 1. Main characteristics of the study population

	Granulocyte group (n:17)	Control group (n:15)	P
Age, years, median (range)	44 (29-62)	50 (26-65)	0.143
Gender, n (%)			0.3
Female	10 (59%)	6 (40%)	
Male	7 (41%)	9 (60%)	
Disease, n (%)			1.0
Acute myeloid leukemia	9 (53%)	11 (73%)	
Acute lymphoid leukemia	3 (17%)	2 (13%)	
Hodgkin lymphoma	2 (12%)	-	
Aplastic anemia	2 (12%)	-	
Myelodysplastic syndrome	1 (5%)	1 (7%)	
Diffuse large B-cell lymphoma	-	1 (7%)	
Neutrophil engraftment (day)±standard deviation	14±2	15±2	0.403
Platelet engraftment (day)±standard deviation	10±2	12±3	0.058
Underlying infections, n (%)			
Bacterial	12 (70.5%)	11 (73%)	0.86
Fungal	8 (47%)	8 (53%)	0.72
Viral	5 (29%)	3 (20%)	0.54
Pneumonia	7 (41%)	8 (53%)	0.49
CMV viremia	5 (29%)	2 (13%)	0.27
Aspergillosis	2	1 (7%)	0.62
Mucositis	7 (41%)	8 (53%)	0.49
Colitis	4	2 (13%)	0.46
Donor, n (%)			0.26
Matched related	8 (47%)	10 (67%)	
Haploidentical related	9 (53%)	5 (33%)	
Stem cell source, n (%)			0.62
Peripheral blood	15 (88%)	14 (93%)	
Bone marrow	2 (12%)	1 (7%)	
Conditioning regimen, n (%)			0.89
Myeloablative	15 (88%)	13 (87%)	
Reduced intensity	2 (12%)	2 (13%)	
Antithymocyte globulin	7 (41%)	5 (33%)	0.65

Haploidentical allo-HSCT was higher in GTG (53% (n=9) versus 33% (n=5)), but no statistically significant difference was observed in terms of all allo-HSCT features and underlying infection types (p>0.05 in all) (**Table 1**). Except for grade 2 allergic reaction in 2 (12%) patients, no other side effects related to GT were observed. On the 20th day, leukocyte values in the CG and GTG were 0.9 (0.75-1.33) $\times 10^3/\mu L$ and 2.17 (0.31-6.24) $\times 10^3/\mu L$, respectively (p=0.9), and neutrophil values were 0.85 (0.26-2.14) $\times 10^3/\mu L$ and 1.93 (1.45-2.95) $\times 10^3/\mu L$, respectively (p=0.71). Leukocyte and neutrophil values after allo-HSCT were showed in **Figures 1** and **2**.

The average neutrophil and platelet engraftment was observed on the 15th and 11th day, respectively. A total of 65 GTs were performed, and the average number of days was 4 ±1. The infused granulocyte dose averaged $4x10^{10}$/unit. The time to start the GT was in the median of 11 (5-17) days. There was no statistically significant difference in leukocyte, neutrophil, platelet values on transplant day between the granulocyte and control groups (p=0.49, p=0.36, p=0.16, respectively).

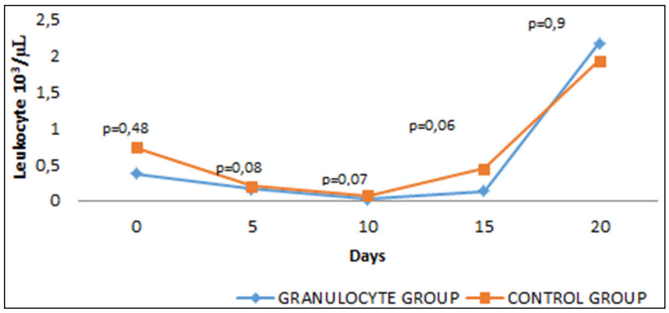

Figure 1. Distribution of leukocyte values of granulocyte and control groups according to day.

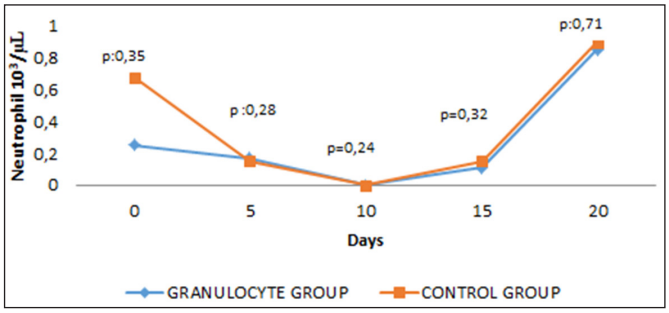

Figure 2. Distribution of neutrophil values of granulocyte and control groups according to day.

A median increase in leukocytes was 1.93 (0.37-10.21) $\times 10^3/\mu L$ compared to the beginning and end of GT. A statistically significant difference was found between GT and leukocyte increase (p=0.001). Similarly, the median neutrophil increase was 1.14 (0.25-9.24) $\times 10^3/\mu L$. A statistically significant difference was found between GT and neutrophil increase (p=0.001). In GTG, neutrophil and platelet engraftments occurred on average at 14±2 and 10±2 days, respectively. In CG, neutrophil and platelet engraftments occurred on average 15±2 and 12±3 days, respectively. There was no statistically significant difference in neutrophil and platelet engraftment between the two groups (p=0.4, p=0.06, respectively).

Clinical response after GT was evaluated. Eight (47%) of 17 patients had a clinical response, but 9 (53%) patients did not have. The hematological response was achieved in 15 (88%) of 17 patients. Clinical response was obtained in all 8 (53%) patients who received a hematological response. Only hematological response was observed in 6 (40%) patients without a clinical response (p=0.2).

The median follow-up time was 9 (1-48) months. The mean OS was 34.3±4.9 (24.5-44.1) months in CG and 18.4±4.9 (8.7-28.1) months in GTG (p=0.035) (**Figure 3**). Five (29%) patients died in the first month after GT, and 7 (41%) died in the first three months. In the

CG, four (26%) died in the first three months. More deaths in the GTG arm were thought to be due to having more haploidentical allo-HSCT than the CG arm. There was no statistically significant difference between neutrophil and platelet engraftment time and mortality (**Table 2**). Relapse was observed in 2 (11.8%) patients in GTG and not in any patient in CG. While infection-related mortality developed in 2 (11.8%) patients in GTG, it developed in 3 (20%) patients in CG. Graft versus host disease-related mortality was observed in 17.6% (n=3) of the patients in GTG, and in 1 (6.7%) of the patients in CG.

Table 2. Effect of neutrophil and platelet engraftment durations on mortality

		Neutrophil Engraftment Day			Platelet Engraftment Day		
		≤15	>15	p	≤11	>11	p
Granulocyte Group	Non-survivors	7	3	0.45	9	4	0.67
	Survivors	2	2		3	1	
Control Group	Non-survivors	1	3	0.46	1	3	0.34
	Survivors	5	6		6	5	

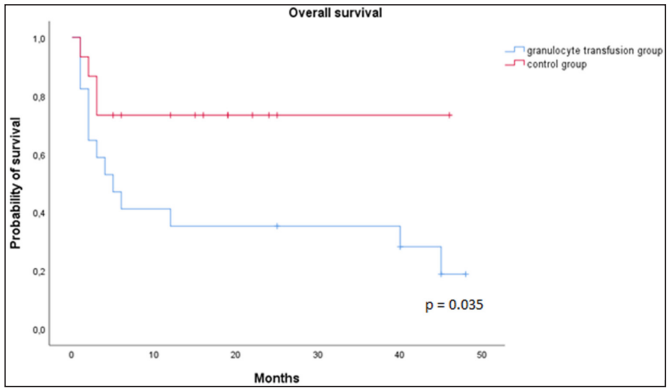

Figure 3. The cumulative survival chart of control and granulocyte group.

Sepsis was present in 7 (41.1%) patients in GTG, and in 6 (40%) patients in CG. In GTG, 23.5%(n=4) of the patients needed intensive care unit (ICU), 11.8%(n=2) needed mechanical ventilator (MV), 17.6%(n=3) needed vasopressors. In CG, ICU need developed in 5 (33.3%) patients, MV need developed in 4 (26.7%) patients, and vasopressor need developed in 5 (33.3%) patients.

DISCUSSION

Modern intensive chemotherapy for patients undergoing stem cell transplants often results in prolonged neutropenia periods, which is a significant risk factor for severe bacterial and fungal infections. In patients with febrile neutropenia, infection is the leading cause of morbidity and mortality, despite broad-spectrum antibacterial and antifungal agents and the necessary support treatments. The occurrence of multidrug-resistant bacterial and invasive fungal infections with the change in the spectrum of infections has led to problems in immunosuppressive patients over the past two decades. GT is a life-saving treatment method by increasing the number of peripheral granulocytes and reducing infection risk (5).

In CG patients, neutrophil and platelet engraftment occurred at average at 15 and 12 days, respectively; and in the GTG, neutrophil and platelet engraftment occurred at 14 and 10 days, respectively. Although there was no statistically significant difference, probably due to small population of the study, engraftment occurred faster in GTG.

The clinical response was defined as the return of fever to normal after transfusion and the clinical recovery. The hematological response was defined as improved laboratory parameters and was achieved in 15 (88%) of 17 patients. Differences in infection severity or infection factor may have caused this condition in patients where the clinical response was not achieved, although there was a hematological response. In a retrospective study conducted in 2016, a total of 25 patients were given GT, and the hematological response was observed in 21 (84%) of the patients, and no hematological response was observed in 4 (16%) (6). After GT in studies, hematological response and recovery rate of clinical symptoms were observed as 68,2%, 50%, and 40% respectively (7-9). In these studies, the occurrence of a lower hematological response may be due to the use of relatives as donors or the inclusion of patients with both allogeneic and autologous hematopoietic stem cell transplants.

In our study, neutrophils increased by 1.14 (0.25-9.24) $\times 10^3/\mu L$ after GT. This increase is higher than reported in other studies, showing a 0.6-2.6 $\times 10^3/\mu L$ neutrophil increase (10, 11). In another study, 84% of patients reported an increase in the number of neutrophils to 1 $\times 10^3/\mu L$ (6). The reason for the increase in neutrophil value at various levels in studies may be that patient groups' characteristics are different and affected by treatment methods other than GT.

Ikegawa et al. (12) administered GT 40 times in 13 patients after allo-HSCT and showed a one-month survival of 84.6% after GT. In the Resolving Infections in Neutropenia with Granulocytes (RING) study, adult and pediatric patients planned a collection of at least 4 $x10^{10}$ granulocytes per transfusion at a high target transfusion dose (13). For this purpose, before collecting, donors were given 480 µg G-CSF and 8 mg dexamethasone. Study groups were formed as high-dose granulocyte-receiving, low-dose granulocyte-

receiving, and a control group that did not receive granulocytes. At the end of 42 days, survival in the high-dose group was better than in the low-dose group (59% vs. 15%, p=0.01), while the control group was no better than in the high-dose group (59% vs. 37%, p=0.11) (13). In our study, 1-month survival with granulocyte administration was 70.5%.

In the study of Lee et al. (15) 54 patients had a pre-granulocyte leukocyte value of 0.18 (0.01-6.85) $\times 10^3/\mu L$, post-granulocyte leukocyte value increased to 0.96 (0.02-14,36) $\times 10^3/\mu L$ ($p<0.0001$) (14). In our study, the leukocyte value was 0.04 (0-0.25) $\times 10^3/\mu L$, 1.96 (0.61-10.4) $\times 10^3/\mu L$, respectively, and the increase was greater. The leukocyte response may have been different due to the use of dexamethasone only in the donors. In another retrospective study, GT obtained using dexamethasone combined with G-CSF was found to be more efficient than that obtained using G-CSF alone (16).

Ang et al. (17) showed that neither granulocyte dose nor neutrophil increase was associated with improved infection control or decreased mortality. In our study, a statistically significant difference was found between GT and neutrophil increase (p=0.001). This may be because the granulocyte collection from each donor was different once in the study. The dose of C-GSF used with dexamethasone in donor preparation was different, and the infused granulocyte dose and the pathogens causing the infection were different.

There were some limitations in our study, such as the number of patients, single center experience, and retrospective nature. Besides, unlike other studies, although it is advantageous to select only patients with allo-HSCT in terms of homogeneous distribution, all patients' primary diagnosis was not the same.

CONCLUSION

GT was observed to effectively manage complications such as neutropenic fever and sepsis after allo-HSCT by shortening the duration of neutropenia and increasing neutrophil and leukocyte values. Prospective, multi-center, further studies involving more homogeneous patient groups are needed to investigate the effects of GT on engraftment.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Erciyes University Faculty of Medicine Ethics Committee (Date: 12.02.2020, Decision No:2020/126).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

Financial Disclosure: The authors declared that this study has received no financial support.

Author Contributions: All the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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