BLOOD RESEARCH
VOLUME 54 · NUMBER 1
March 2019
ORIGINAL ARTICLE

Analysis of hematologic parameters of donors, patients, and granulocyte concentrates to predict successful granulocyte transfusion

Jong-Mi Lee[1], Seung Jun Choi[1], Hoon Seok Kim[1], Mina Yang[1], Yonggoo Kim[1], Jong Wook Lee[2], Jihyang Lim[1]

Departments of [1]Laboratory Medicine and [2]Hematology, College of Medicine, The Catholic University of Korea, Seoul, Korea

p-ISSN 2287-979X / e-ISSN 2288-0011
https://doi.org/10.5045/br.2019.54.1.52
Blood Res 2019;54:52-56.

Received on August 7, 2018
Revised on October 2, 2018
Accepted on October 19, 2018

Correspondence to
Jihyang Lim, M.D., Ph.D.
Department of Laboratory Medicine, College of Medicine, Seoul St. Mary's Hospital, The Catholic University of Korea, 222 Banpodaero, Seocho-gu, Seoul 06591, Korea
E-mail: ljh117@catholic.ac.kr

Background
Granulocyte transfusion (GTx) is performed as a supportive therapy in severe neutropenic patients caused by various conditions. The study aimed to analyze the hematologic parameters of donors, patients, and granulocyte concentrates to predict successful GTx.

Methods
This study was performed in 281 donors, with their granulocyte concentrates being collected through apheresis, and in 54 severe neutropenic patients who had various hematologic diseases. Complete blood cell counts of donors pre- and post-apheresis, granulocyte concentrates, and patients pre- and post-GTx were analyzed. Patients were divided into two groups according to survival at discharge (Group S, survival; Group D, dead) to compare various factors including age, infection status, pre- and post-GTx total white blood cell counts (TWBCC) and absolute neutrophil counts (ANC), total number of GTx, infused TWBCC and ANC per weight, and use of G-CSF during therapy.

Results
Overall data of patients showed that both TWBCC and ANC were significantly increased after GTx (median values at pre-GTx, TWBCC=0.40×10^9/L, ANC=0.14×10^9/L; post-GTx, TWBCC=0.57×10^9/L, ANC=0.29×10^9/L, both $P<0.0001$). After GTx, Group S (N=25) showed significantly higher TWBCC and ANC than Group D (N=29) ($P=0.01$ and $P=0.04$, respectively). Using different cutoff levels, post-GTx TWBCC greater than 0.5×10^9/L showed statistically significant difference between the two groups ($P<0.01$). None of the other factors showed statistically significant differences.

Conclusion
The TWBCC and ANC after GTx were significant factors to predict patients' outcome. Therefore, follow-up of those two parameters may be helpful to select or consider other therapeutic modalities including additional GTx.

Key Words Granulocyte transfusion, Neutropenia, Total white blood cell counts, Absolute neutrophil counts, Granulocyte colony-stimulating factor

INTRODUCTION

Neutropenia may occur due to decreased granulocyte production during treatment process such as high-dose chemotherapy or hematopoietic stem cell transplantation. Severe neutropenia occurs when the absolute neutrophil count (ANC) in the peripheral blood is less than 0.5×10^9/L [1]. Severe neutropenia patients often have poor prognosis. Although granulocyte transfusion (GTx) is known to be beneficial for recovery from infections and granulocytic regeneration in the bone marrow, the effect of GTx remains controversial [2-4]. Moreover, clinical evidences about the indications and treatment goals in GTx are insufficient. Therefore, the present study aimed to analyze the hematologic parameters of patients, donors, and granulocyte concentrates to predict successful GTx.

MATERIALS AND METHODS

Granulocyte donors

The subjects of this study included 281 healthy donors who visited Seoul St. Mary's Hospital from January 2015 to June 2017 and 54 patients with hematologic diseases who underwent GTx due to severe neutropenia. These donors were selected in accordance with the domestic blood management law in South Korea [5]. ABO and Rh-D blood types of all donors and patients were matched. For mobilization of granulocytes, 10 μg/kg of granulocyte colony-stimulating factor (G-CSF) was injected to donors subcutaneously about 12 hours before collecting granulocytes. After G-CSF administration, 650 mg of acetaminophen was administered orally when the following symptoms were present: fever, headache, muscle aches, and chills. This study received approval from the institutional review board (IRB).

Granulocyte collections

Granulocytes were collected following the Mononuclear Cell (MNC) collection protocols (LRS Turbo, version 7.0) of COBE Spectra (Terumo BCT, Lakewood, CO, USA). Only anticoagulant citrate dextrose solution, solution A was mixed with blood at a ratio of 1:15. Erythrocyte sedimentation agent with concentrated citrate was not used because of domestic reason. The collection speed was 30–45 mL/min, and the volume of the total blood circulation for single collection was 5,500–7,000 mL. WBC Colorgram (Terumo BCT, Lakewood, CO, USA) set hematocrit to an approximate range between 5.0 and 7.5%. The total collection volume of granulocyte concentrates was 350 mL, and the target granulocyte count was more than 1.8×10^9/unit according to the national standard guideline of blood centers. Collected granulocytes were irradiated with gamma rays of 25 Gy from a cesium source and were stored at room temperature.

Analytic parameters

We measured donors' total white blood cell count (TWBCC) before and after G-CSF administration and donors' ANC after G-CSF administration. General characteristics including gender, age, height, and weight were also analyzed. For granulocyte concentrates, TWBCC and ANC were measured. Patients' TWBCC and ANC before and after GTx, number of transfusions, and total infused TWBCC and ANC were measured. The performance of G-CSF for patients was also reviewed to analyze its effect. Clinical characteristics of patients including gender, age, weight, diagnosis, neutropenic cause, infection status, and survival at discharge were also analyzed.

Statistics

Pearson's correlation analysis was performed for TWBCC before and after G-CSF administration of donors and for TWBCC and ANC of donors and granulocyte concentrates after G-CSF administration. In this study, we divided patients into "Group S" for surviving patients and "Group D" for dead patients based on their survival at discharge. Various parameters of these two groups were compared using Mann-Whitney U test or Fischer's exact test. A *P*-value less than 0.05 was considered statically significant. MedCalc (v.16.4.3) was used for all statistical analyses.

RESULTS

Characteristics of granulocyte donors and granulocyte concentrates

Among the 281 granulocyte donors, 270 (96.1%) donors were males and 11 (3.9%) donors were females, respectively. Their mean age, height, and body weight were 28.3±7.7 years, 174.1±5.9 cm, and 71.6±9.5 kg, respectively. The mean TWBCC values of donors before and after G-CSF admin-

Table 1. Comparison of total white blood cell count and absolute neutrophil count of donors and their granulocyte concentrates.

Parameters	Mean±SD	Range	Pearson's correlation
Donors			
TWBCC ($\times10^9$/L) of donors			
Before G-CSF administration	6.30±1.34	4.06–10.00	$r=0.412$, $P<0.0001$[b]
After G-CSF administration	24.40±4.41	14.21–38.49	
ANC ($\times10^9$/L) of donors			
Before G-CSF administration	Not tested		Not available
After G-CSF administration	22.39±4.24	12.18–36.79	
Granulocyte concentrates (GC)			
TWBCC ($\times10^9$/L)[a] of GC	73.78±23.41	23.01–156.55	$r=0.291$, $P<0.0001$[c]
TWBCC after G-CSF administration of donors	24.40±.41	14.21–38.49	
ANC ($\times10^9$/L)[a] of GC	49.02±24.88	2.90–124.97	$r=0.258$, $P<0.0001$[c]
ANC after G-CSF administration of donors	22.39±4.24	12.18–36.79	

[a]Total collection volume of granulocyte concentrates was 350 mL. [b]TWBCC of donors' blood before and after G-CSF administration. [c]TWBCC and ANC of donors after G-CSF administration and their GC.
Abbreviations: ANC, absolute neutrophil count; GC, granulocyte concentrates; G-CSF, granulocyte colony-stimulating factor; TWBCC, total white blood cell count.

istration were 6.30±1.34×10^9/L and 24.40±4.41×10^9/L, respectively. ANC was measured only after G-CSF administration, with a mean value of 22.39±4.24×10^9/L. The mean values of TWBCC and ANC of granulocyte concentrates were 73.78±23.41×10^9/L and 49.02±24.88×10^9/L, respectively (Table 1). Donors' TWBCC and ANC showed significant correlation before and after G-CSF administration (r=0.412, P<0.0001). Both TWBCC and ANC of granulocyte concentrates also showed significant correlation with those of donors after G-CSF administration (TWBCC, r=0.291; ANC, r=0.258, respectively, both P<0.0001).

Hematologic effects of GTx and prognosis analysis

Among the 54 patients, 28 (51.9%) were males and 26 (48.1%) were females, respectively. Their mean age was 46.7±16.4 years. Regarding the diagnosis, acute myeloid leukemia was the most common (43, 79.6%) one, followed by aplastic anemia (4, 7.4%) and acute lymphoblastic leukemia (3, 5.6%). Additionally, mixed phenotype acute leukemia, myelodysplastic syndrome, essential thrombocytosis, and hemophagocytic lymphohistiocytosis each had one (1.9%) case. All patients were suffering from neutropenia (ANC <0.5×10^9/L), which was caused by chemotherapy (42 patients, 77.8%), hematopoietic stem cell transplantation (4 patients, 7.4%), or others (8 patients, 6.8%). The mean initial values of TWBCC and ANC were 0.41×10^9/L and 0.16×10^9/L, respectively. After GTx, they were significantly increased to 0.58×10^9/L and 0.29×10^9/L, respectively (Table 2).

Twenty-five (46.3%) patients belonged to "Group S," while 29 (53.7%) patients belonged to "Group D," respectively. Age, gender, underlying disorder, cause of neutropenia, and infection status at enrollment were compared between the two groups. There were no significant differences in indicators mentioned above (Table 3). Variables associated with response to GTx were analyzed including the following: G-CSF, TWBCC, ANC, the frequency of GTx, and total infused TWBCC and ANC per kilogram. Among these parameters, TWBCC (P=0.02) and ANC (P=0.04) showed significant difference between the two groups after GTx (Table 4).

There were no significant correlations between TWBCC after GTx and patients' age (r=0.055, P=0.7), frequency of GTx (r=-0.073, P=0.6), TWBCC before GTx (r=0.07, P=0.62), total infused TWBCC (r=-0.132, P=0.36), and total infused TWBCC per kilogram (r=-0.115, P=0.42). When post-GTx TWBCC and post-GTx ANC were divided into three categories: >0.1×10^9/L, >0.5×10^9/L and >1.0×10^9/L, only patients with post-GTx TWBCC >0.5×10^9/L (23/25 vs. 15/29, P<

Table 2. Median of patients' TWBCC and ANC changes according to granulocyte transfusion.

Parameter	Median (range)	P[a]
TWBCC (×10^9/L)		<0.0001
Before granulocyte transfusion	0.18 (0.01–6.85)	
After granulocyte transfusion	0.96 (0.02–14.36)	
ANC (×10^9/L)		<0.0001
Before granulocyte transfusion	0.02 (0.00–2.40)	
After granulocyte transfusion	0.61 (0.00–5.03)	

[a]P-values were determined using Mann-Whitney test.
Abbreviations: ANC, absolute neutrophil count; TWBCC, total white blood cell count.

Table 3. Baseline characteristics of the subjects.

Variables	Group S (N=25)	Group D (N=29)	P[a]
Median age of patients (yr)	46 (18–71)	53 (17–77)	0.99
Male gender, N (%)	14 (56%)	14 (48.3%)	0.60
Underlying disorder, N (%)			0.37
Acute leukemia	21 (84%)	26 (89.7%)	
Myelodysplastic syndrome	0	1 (3.4%)	
Aplastic anemia	3 (12%)	1 (3.4%)	
Essential thrombocytosis	1 (4%)	0	
Hemophagocytic lymphohistiocytosis	0	1 (3.4%)	
Cause of neutropenia			0.75
Chemotherapy	21 (84%)	25 (86.2%)	
HSC transplantation	2 (8%)	3 (10.3%)	
None	2 (8%)	1 (3.4%)	
Infection at enrollment			0.86
Proven bacterial infection	14 (56%)	12 (41.4%)	
Probable/possible bacterial infection	3 (12%)	4 (13.8%)	
Proven fungal infection	1 (4%)	1 (3.4%)	
Probable/possible fungal infection	4 (16%)	5 (17.2%)	
Proven bacterial and fungal infection	1 (4%)	1 (3.4%)	
Proven bacterial and probable/possible fungal infection	2 (8%)	6 (20.7%)	

[a]P-values were determined using Mann-Whitney test for continuous variables and Fisher's exact test for categorical variables.
Abbreviations: Group D, dead patients at discharge; Group S, survived patients at discharge.

Table 4. Variables associated with response to granulocyte transfusion.

Variables	Group S (N=25)	Group D (N=29)	*P*
	Median (range)		
G-CSF administration (number of cases)	17 (68%)	14 (48.3%)	0.18
Pre-GTx TWBCC of patients ($\times10^9$/L)	0.17 (0.01–1.01)	0.18 (0.01–6.85)	0.67
Pre-GTx ANC of patients ($\times10^9$/L)	0.05 (0.0–0.6)	0 (0.0–2.4)	0.29
Post-GTx TWBCC of patients ($\times10^9$/L)	1.8 (0.12–14.36)	0.61 (0.02–5.78)	0.01
Post-GTx ANC of patients ($\times10^9$/L)	0.68 (0.04–5.03)	0.35 (0.0–3.54)	0.04
Total number of granulocyte transfusions	4.0 (1–17)	4.0 (1–13)	0.67
Infused total WBC content ($\times10^9$)	91.41 (25.84–416.46)	130.58 (11.10–374.76)	0.89
Infused total WBC content/each transfusion ($\times10^9$)	24.92 (18.43–35.7)	25.75 (11.10–49.50)	0.98
Infused total WBC content/each transfusion/body weight ($\times10^9$/kg)	0.40 (0.14–1.28)	0.41 (0.15–1.35)	0.99
Infused total WBC content/body weight ($\times10^9$/kg)	1.76 (0.42–8.30)	2.12 (0.17–9.37)	0.80
Infused total ANC content ($\times10^9$)	60.16 (17.08–180.49)	31.16 (4.57–200.80)	0.68
Infused total ANC content/each transfusion ($\times10^9$)	18.22 (8.23–25.80)	18.38 (4.57–25.54)	0.54
Infused total ANC content/each transfusion/body weight ($\times10^9$/kg)	0.26 (0.02–0.87)	0.28 (0.05–1.06)	0.54
Infused total ANC content/body weight ($\times10^9$/kg)	1.00 (0.28–2.85)	0.62 (0.06–4.00)	0.70

Abbreviations: ANC, absolute neutrophil count; Group D, dead patients at discharge; Group S, survived patients at discharge; GTx, granulocyte transfusion; TWBCC, total white blood cell count.

0.01) showed significant correlation with the survival or death based on Fisher's exact test. There was no significant difference in TWBCC (*P*=0.16) or ANC (*P*=0.43) between the group that used G-CSF and the group that did not use it.

DISCUSSION

In this study, 10 μg/kg of G-CSF was administered to donors for stimulation. The mean value of TWBCC in each donor after G-CSF administration was increased four times from 6.3×10^9/L to 24.4×10^9/L. According to the donors' TWBCC increment after GSF-administration, it also correlated with the TWBCC of their apheresis granulocyte concentrates. We could obtain granulocyte concentrates at an average TWBCC of 73.78×10^9/L. It is generally known that when granulocyte collection is performed using 5–10 μg/kg of G-CSF, it is possible to obtain granulocyte concentrates at an average of $40–60\times10^9$/L [6]. Our results exceeded those of the previous studies.

In this retrospective analysis performed on 54 patients with hematologic diseases, chemotherapy was the most common cause of neutropenia (42 people, 77.8%), followed by hematopoietic stem cell transplantation (7 people, 7.4%). This is similar to a previous study showing that chemotherapy is the most common cause of decreased neutrophil counts in the majority (73–80%) of cases [7].

In average, a total of 73.8×10^9/L granulocytes were transfused, and ANCs were significantly increased as much as 0.59×10^9/L. This increment is lower than that reported in the previous studies which showed increase of approximately $0.6–2.6\times10^9$/L of ANCs after an average GTx of $40–80\times10^9$/L [6, 8-10]. A domestic study has also reported that with an average transfused granulocyte count of approximately 50×10^9/L, neutrophil counts are increased to 1.0×10^9/L in 84% of cases [7]. This percentage is higher than that (37%, 20/54) in the present study. Considering that several studies have reported various levels of increase in neutrophil counts, features of patient groups and treatment modalities other than GTx might have affected the degree of granulocyte increase.

We analyzed the effect of GTx based on the mortality at discharge. In this study, 46.3% of patients survived to hospital discharge, suggesting that GTx is a helpful supportive therapy in severe neutropenic patients. When we compared factors between "Group S" and "Group D" after treatment with GTx, TWBCC (*P*=0.01) and ANC (*P*=0.04) showed statistically significant differences. Additionally, the number of patients with TWBCC higher than 0.5×10^9/L after GTx in Group S was significantly higher than that in Group D. Therefore, a good prognosis may be expected if TWBCC is higher than 0.5×10^9/L. Interestingly, there were no significant differences in patients' TWBCC or ANC before GTx, the frequency of GTx, or combined use of GTx with G-CSF between the two groups. Moreover, ANC after GTx did not have any significant correlation with patient age, the frequency of GTx, or TWBCC and ANC before GTx. These findings are in line with the previous studies. Rutella *et al.* [11] have reported that only the recovery of neutrophil counts (≥500/μL) after GTx is associated with therapeutic response to infections. Ofran *et al.* [12] have also reported that continuous recovery of neutrophil counts (≥700/μL) has significant correlation with infection-related mortality ($P<0.02$). A secondary analysis in the controlled trial, showing negative result for the efficacy of GTx, pointed out that the most important parameter was the total dose of granulocytes per patients' weight per transfusion [3]. However, our result was not significantly related to patient survival as opposed to the previous result. There is no clear explanation as to why these factors, except TWBCC and ANC

after GTx, which can increase the granulocyte counts and productivity of the patients', are not related to their outcomes. This study provided the basic data for the development of effective treatments by revealing that patients' post transfusion granulocyte counts are important predictive factors for patients' clinical outcomes. The age, number of GTx, pre-GTx TWBCC and ANC, and use of G-CSF during therapy were not prognostic factors for survival to hospital discharge in severe neutropenic patients. Therefore, follow-up of TWBCC and ANC after GTx may be helpful in selecting or considering other therapeutic modalities, including additional GTx.

Authors' Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

REFERENCES

1. Elebute M, Massey E, Benjamin S, Stanworth S, Navarrete C, Lucas G. Clinical Guidelines for the Use of Granulocyte Transfusions. Bristol, UK: NHS Blood and Transplant, 2012.
2. Strauss RG. Role of granulocyte/neutrophil transfusions for haematology/oncology patients in the modern era. Br J Haematol 2012;158:299-306.
3. Price TH, Boeckh M, Harrison RW, et al. Efficacy of transfusion with granulocytes from G-CSF/dexamethasone-treated donors in neutropenic patients with infection. Blood 2015;126:2153-61.
4. Teofili L, Valentini CG, Di Blasi R, et al. Dose-dependent effect of granulocyte transfusions in hematological patients with febrile neutropenia. PLoS One 2016;11:e0159569.
5. Ministry of Health and Welfare. The administration law of blood management. Sejong, Korea: Ministry of Health and Welfare, 2016.
6. Price TH. Granulocyte transfusion therapy: it's time for an answer. Transfusion 2006;46:1-5.
7. Lee SN, Hu Y, Eom HS, Lee H, Lee E, Kong SY. Analysis of granulocyte transfusions in patients with infections and neutropenia: a single center experience. Korean J Blood Transfus 2016;27:247-56.
8. Hester JP, Dignani MC, Anaissie EJ, Kantarjian HM, O'Brien S, Freireich EJ. Collection and transfusion of granulocyte concentrates from donors primed with granulocyte stimulating factor and response of myelosuppressed patients with established infection. J Clin Apher 1995;10:188-93.
9. Adkins D, Spitzer G, Johnston M, Velasquez W, Dunphy F, Petruska P. Transfusions of granulocyte-colony-stimulating factor-mobilized granulocyte components to allogeneic transplant recipients: analysis of kinetics and factors determining posttransfusion neutrophil and platelet counts. Transfusion 1997; 37:737-48.
10. Price TH, Bowden RA, Boeckh M, et al. Phase I/II trial of neutrophil transfusions from donors stimulated with G-CSF and dexamethasone for treatment of patients with infections in hematopoietic stem cell transplantation. Blood 2000;95:3302-9.
11. Rutella S, Pierelli L, Sica S, et al. Efficacy of granulocyte transfusions for neutropenia-related infections: retrospective analysis of predictive factors. Cytotherapy 2003;5:19-30.
12. Ofran Y, Avivi I, Oliven A, et al. Granulocyte transfusions for neutropenic patients with life-threatening infections: a single centre experience in 47 patients, who received 348 granulocyte transfusions. Vox Sang 2007;93:363-9.