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ORIGINAL ARTICLE

Hematopoietic Stem Cell Transplantation in a Very High Risk Group of Patients with the Support of Granulocyte Transfusion

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Abstract High risk patients with active fungal infection who had undergone hematopoietic stem cell transplantation (HSCT) with the support of granulocyte transfusions (GTX) as an adjunct to antifungal agents are reviewed retrospectively. Patients requiring immediate allogeneic HSCT for their primary hematological disorders (two severe aplastic anemia, one T cell acute lymphoblastic leukemia (ALL) in second complete remission, one acute myeloid leukemia (AML)-in first complete remission, one T-ALL in refractory relapse) but were denied by other transplant programs due to active invasive fungal infections had undergone HSCT with the support of GTX at the stem cell transplantation unit of Gazi University. Five patients who had undergone six transplants were included in the study and received a total of 38 (3–13) granulocyte transfusions during these six transplants. The median granulocyte concentration was 3.4×10^{11} per apheresis bag. Full clinical and radiological recovery was achieved in three of the five high risk patients with active invasive fungal infection with the combination of antifungal agents and GTX. Even a very high risk patient with aplastic anemia who had undergone two consecutive transplants due to secondary graft failure was also cured of his primary disease despite the presence of multiple pulmonary fungus balls. Three of the five patients with very high risk features due to the underlying hematological disease and the associated active fungal infection were rescued with allogeneic HSCT performed with the support of GTX combined with antifungal agents. Despite the limitations of this report due to its retrospective nature, it suggests that GTX might be an alternative in patients with active fungal infections who otherwise are denied by the transplant programs. However, prospective randomized studies are required to draw a solid conclusion regarding the role of GTX in HSCT recipients in desperate situations such as active fungal infections.

Keywords Stem cell transplantation · Granulocyte transfusion · Fungal infection · Anti-fungal treatment

Introduction

Increasing use of more aggressive treatment procedures in patients with hematological diseases leads to an increase in the frequency of invasive fungal infections, which remains to be the major cause of transplant related mortality in hematopoietic stem cell recipients [1, 2]. Presence of active invasive fungal infection (IFI) does not seem to be an absolute contraindication for HSCT, particularly in high risk patients in whom delaying the treatment could be fatal [3]. Success rates might be lower than expected in this group of patients even with the most recently developed broad spectrum antifungal agents, which leads transplant physicians to search for adjunct alternative treatment methods [4]. Since duration of neutropenia has a major impact on transplantation, boosting the host defense system by granulocyte transfusions (GTX) might improve the outcome of neutropenia-associated infections. Data that confirm the value of GTX are limited, and results of the

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studies are heterogeneous and inconclusive [5–7]. Absence of randomized studies, limited number of cases, and diversity of granulocyte harvesting methods precludes drawing a conclusion on the efficacy of granulocyte transfusions. The routine use of granulocyte transfusions is not recommended by the Infectious Disease Society of America [8]. Adequately powered prospective trials, with clinically relevant endpoints, precise patient selection criteria with generally accepted definitions of neutropenia are required before granulocyte transfusion can become a part of routine clinical practice. However, there are certain conditions, associated with neutropenia, where the use of granulocyte colony stimulating factor (G-CSF)-stimulated granulocyte transfusions may be reasonable. These include:

1. A resistant severe clinical infection in a neutropenic (neutrophil count $<0.2–0.5 \times 10^9$/l) patient that has shown no response to aggressive antibiotic treatment with no recovery in neutrophil count expected for more than 7 days.
2. Severe infections, e.g., systemic fungal infections/necrotizing fasciitis or severe neutropenic typhilitis progressing on appropriate anti-fungal or broad spectrum antibiotics, in neutropenic patients, and no recovery in neutrophil count expected for more than 7 days [9].

Several recent clinical studies have shown clinical benefits of GTX in HSCT recipients with severe bacterial or fungal infections [7, 10, 11]. Patients with active invasive fungal infections with a very high risk primary disease without HSCT received antifungal agents combined with GTX and the results were presented retrospectively.

Materials and Methods

Patients

Five patients aged 16–53 (median 27) received granulocyte transfusions during severe neutropenia periods associated with active invasive fungal infections. All patients were denied by other transplant programs and met the following inclusion criteria at time of transfusion: (i) neutrophil counts below 500/μl, (ii) life-threatening infection progressing despite antimicrobial and antifungal therapy, and (iii) an underlying primary hematological disorder which requires immediate HSCT and is fatal otherwise. Patient characteristics at the time of GTX, including age, sex, basic hematological disease, status of disease, type of HSCT and conditioning regimen are shown in Table 1. All the patients received HLA-matched transplants; four from sibling donors and one from an unrelated donor. Fungal infections were classified according to the EORTC (European Organization for Research and Treatment of Cancer) criteria [12].

Granulocyte Donors

Donors were recruited from the friends and family members of the patients. Prerequisites for donation were informed consent, ABO and Rhesus compatibility, normal values in blood tests and negative serology for viral hepatitis and HIV. Except the family stem cell donors who were allowed to subsequently undergo granulocyte apheresis, granulocyte donors were not HLA-matched. All donors received 8 mg dexamethasone per oral and 300–450 μg of G-CSF subcutaneously 12 h prior to the apheresis procedure. Donor characteristics, demographics, body mass index, white blood cell (WBC), and neutrophil count prior and after stimulation, side effects at time of stimulation and apheresis were recorded.

Granulocyte Collection

The granulocyte collection procedure was performed using a continuous flow apheresis device (Com. Tec., Fresenius® Medical Care, Bad Homburg v.d.H., Germany) on which the granulocyte collection program was set. 'Hydroxyethyl starch' of 6% was utilized to weigh down the red cells

Table 1 Patient characteristics

#	Age/ gender	Diagnosis	Status of disease	Donor type (All HLA-matched)	Conditioning regimen
1	26/M	SAA	–	Sibling	Cy
1	26/M	SAA	–	Sibling	Bu/Flu/ATG
2	16/F	SAA	–	Sibling	Cy/ATG
3	27/M	T-ALL	CR2	Sibling	Bu/Cy/Thiotepa
4	30/M	T-ALL	Refractory, relapse	MUD	IV Bu/Cy
5	53/M	AML-M6	CR1	Sibling	Flu/TBI

Patient # 1 underwent two consecutive transplants due to secondary graft failure

MUD matched unrelated donor, *Bu* busulphan, *Cy* cyclophosphamide, *Flu* fludarabine, *TBI* total body Irradiation, *ATG* antithymocyte globuline, *SAA* severe aplastic anemia, *ALL* acute lymphocytic leukemia, *AML* acute myeloblastic leukemia, *CR* complete remission

(HES; Eczacibasi, İstanbul, Turkey). Trisodium citrate of 33.3% was used for anticoagulation. Anticoagulant and HES solutions were mixed at a ratio of 1:12 and were utilized throughout the procedure. Depending on the venous capacity of the donors, a mean flow rate of 50 ml/min was provided. Maximum 500 ml of HES was utilized. The final volume of the product was approximately 270 ml.

Granulocyte Transfusions

All granulocyte concentrates were irradiated at 25 Gy to prevent transfusion-induced graft versus host disease (GvHD). The product was transfused within 2 h of collection after cross-matching. Monitoring during GTX included continuous monitoring of oxygen saturation, blood pressure, respiratory, and pulse rate every 15 min. GTX was performed within 1–4 h. The minimum interval between GTX and the last amphotericin B infusion was 4 h. The transfusion protocol varied slightly from one patient to the other. In general, we attempted an every other day schedule (three GTX per week), which proved to be the easiest strategy to prevent organizational difficulties. The transfusions were terminated with the resolution of neutropenia or death of the patient.

The data regarding granulocyte dose administered per transfusion, time interval from granulocyte harvesting to administration, adverse events observed in granulocyte transfusion recipients, neutrophil counts 24 h after transfusion and infection-related outcome were collected from patient and donor charts. All the data reported herein were retrospectively analyzed.

Results

Five patients received a total of 38 (3–13) GTX's in six different treatment periods. The median concentration of granulocytes was 3.4×10^{11} per apheresis bag. All patients shared the diagnosis of active invasive fungal infection. Features of the infections are presented in Table 2.

Granulocytes were collected from 30 donors, aged 24–48 years (median = 31 years) eight donors donated twice, at least 1 week apart. The median WBC count of the patients was 127/μl (all with neutrophil counts below 200/μl). Data regarding the granulocyte transfusions are presented in Table 3.

Table 2 Characteristics of the invasive fungal infections (IFI)

#	IFI status	Clinical appearance[a]	Histo/cytopathological evidence	Anti-fungal treatment	IFI etiology	GM/Asp Ag
1	Proven	Pneumonia with multiple nodules, and cavities within area of consolidation	Positive lung biopsy	Amphotericine B Caspofungin	*Aspergillus* spp.	−/−
1	Proven	Pneumonia with multiple nodules, and cavities within area of consolidation	Positive lung biopsy	Amphotericine B Caspofungin	*Aspergillus* spp.	±
2	Probable	Pneumonia with nodular infiltration Nasopharyngeal soft tissue Right fronto-parietal abscess formation (fungal?)	No biopsy performed	Amphotericine B Voriconazole	*C. albicans*	−/−
3	Possible	Pneumonia with multiple nodules and cavity within area of consolidation Sinusitis	No biopsy performed	Amphotericine B	–	+/+
4	Proven	Pneumonia with multiple consolidation areas and peripheral ground-glass opacity Sinusitis	Positive sinus biopsy	Voriconazole Caspofungin	*Aspergillus* spp.	∓
5	Proven	Pneumonia Sinusitis with erosion of sinus walls	Sinus biopsy	Amphotericine B	*Aspergillus* spp.	−/−

Patient #1 had positive blood culture for *E. coli* and typhlitis during his first transplantation

Patient #1 had urinary pseudomonas infection and catheter related *Corynebacterium jeikeium* infection during his second transplantation

Patient #2 had an additional blood stream infection with *Pseudomonas aeruginosa*

Patient #3 had an additional peri-anal infection and positive blood culture for *Acinetobacter baumani*

GM galactomannan, *Asp Aspergillus* antigen

[a] All patients had positive host factors which included neutropenia, body temperature >38°C and persistent fever >96 h

Table 3 Data of the patients receiving the granulocyte products

#	GTX dosage/kg (median)	ANC count prior to GTX (μ/l)	ANC increment (median μ/l)	Radiological improvement	Survival status (days)
1	4.3×10^9 (2.59–6.4)	46 (first course) 0 (second course)	403 (105–565)	Full radiological recovery	+1723 Alive
2	2.2×10^9 (1.39–8.62)	0	137 (100–215)		+11 Death due to infection
3	3.9×10^9 (2.1–7.6)	127	146 (100–530)	Full radiological recovery	+170 Death due to relapse
4	1.2×10^9 (0.9–1.63)	59	211 (190–310)		+11 Death due to infection
5	2.3×10^9 (0.9–8.38)	65	545 (133–723)	Full radiological recovery	+1330 Alive

GTX granulocyte transfusion, *ANC* absolute neutrophil count

Adverse Effects in Donors

None of the donors developed a serious adverse event following stimulation or during apheresis.

Adverse Effects in Recipients

None of the patients developed a serious adverse event following granulocyte transfusions.

Characteristics of GTX's

Granulocyte dosage per kg of body weight is presented in Table 3. The granulocyte count of the product was found to be significantly associated with the donor's WBC count after stimulation ($P < 0.05$). Granulocyte count had no correlation with the donor's age and weight. The impact of gender was not assessed since all donors were male.

Hematological Response to GTX's

Increase in neutrophil count after granulocyte transfusions is presented in Table 3. An evaluation of the relationship between the increase in granulocyte count and age, body weight, type of disease, status of disease, features of the conditioning regiment could not be done due to the low number of patients. However, an impact of the administered dose of granulocytes is apparent (non-statistical data).

Clinical Efficacy

Two of the patients died due to progressive fungal infection (Table 3). It should be noted that these two patients received hemodynamic support together with mechanical ventilation. Besides, patient # 4 had undergone matched unrelated donor (MUD) transplantation due to refractory acute lymphoblastic leukemia (ALL). The second patient of this series suffered from a more extensive fungal infection (see Table 2). Patient #3 in whom complete recovery of fungal infection was documented clinically and radiologically, died in less than 6 months (+170 days status post transplantation) due to disease relapse.

When the post-GTX increment in the absolute neutrophil count (ANC) and dosages of granulocytes per kg of body weight were taken into account, these values were found to be higher in surviving patients.

Discussion

Combined antimicrobial therapy is the standard of care for many serious infections such as pseudomonas infections and tuberculosis. However, the combination of different antifungal agents has been a matter of debate in fungal infections, and there is, so far, no evidence of superiority of combining different antifungal agents over monotherapy. However, even the so-called 'gold standard'-amphotericin B- results in poor survival rates, especially during HSCT [13–15]. Protracted neutropenia seems to be one of the most important causes of low success rate [16]. Infections can be more easily managed with the resolution of neutropenia. Raad et al. [17] in his report on patients with fusariosis treated with posaconazole, demonstrated a success rate of 20% in neutropenic patients, whereas this rate raised to 67% in non-neutropenic patients. This evidence suggests that supportive measures might increase the efficacy of antifungal agents until bone marrow recovery. Kerr et al. [10] in a case–control study including nine allogeneic HSCT recipients with high risk invasive aspergillosis demonstrated the feasibility of prophylactic GTX's which resulted in a significant reduction in the period of post-transplant neutropenia. Price et al. [7] documented 19

patients with fungal or bacterial infections who received GTX before and after HSCT, and they concluded that GTX had more favorable impact on survival rate in cases with bacterial infections compared to invasive fungal infections, though without a statistical significance. Five high risk patients with active invasive fungal infections received antifungal treatment in combination with GTX during stem cell transplantation in the presented study and three of the patients achieved long term clinical and radiological recovery.

Previous studies reveal that stimulation with G-CSF and dexamethasone increases granulocyte counts in donors distinctively [18]. Our donors did not experience any major side effects due to stimulation except mild and self limited bone pain. In healthy donors a dosage of 5 μg/kg of G-CSF is within acceptable toxicity levels. Mild pain limited to the musculoskeletal system which responds to acetaminophen or ibuprophen is the major side effect. However, data regarding the long term effects of stimulation is not available. The use of G-CSF for mobilization of normal stem cells in donors has been undertaken for more than 10 years and there is no evidence of associated leukemogenic potential so far [18]. There has also been a recent report suggesting that corticosteroids may increase the risk of cataracts in granulocyte donors which suggests, if confirmed the use of steroids in this setting should be with caution [19].

None of the patients developed a serious adverse event following granulocyte transfusions in our patients. However it should be emphasized that due to the retrospective nature of the study which analyzed severely ill patients whose main medical problem at the time of GTX was an overwhelming infection, it was almost impossible to determine whether some of the 'adverse events' reported during or early after transfusion (e.g., increase in body temperature, tachycardia) were caused by GTX or were a part of the ongoing infection.

A minimum of 70% of the granulocyte transfusions must contain 1×10^{10} neutrophils for an adult patient for an effective GTX. With the use of modern apheresis techniques, products containing $4–8 \times 10^{10}$ granulocytes per transfusion can be obtained [18].We were able to collect and transfuse the required dosages of granulocytes during all apheresis sessions (Table 3).

We used hetastarch (HES) in our collections without any side effects. We limited the use of HES to 500 ml to minimize potential side effects. While HES is more effective in collecting granulocytes, it persists in the circulation and might cause side effects such as transient hypertension, flushing, and headache [18].

Previous studies demonstrate a linear relation between donor and product granulocyte counts. The targeted granulocyte count was >20,000/μl which was met in all donors in our study and products were transfused within 1–4 hours.

Ofran et al. [11] showed that a sustained increase in neutrophil count following GTX's (>700 cells/μl) was the only independent prognostic factor for infection-related survival. This observation might reflect a 'favorable' subgroup of patients, whose granulocyte consumption was modest due to a less severe infection that therefore attained a sustained 'high' neutrophil count. On the contrary, high increment in the neutrophil counts might suggest a true clinical efficacy of daily GTX's as well. Our study revealed a lower increment in ANCs after granulocyte transfusion compared to other studies. The increment in ANC was higher in surviving patients suffering from active invasive infection as Ofran et al. previously reported. Higher granulocyte counts in the product in the surviving patients can be explained by higher increment in ANC after GTX in surviving patients than those who died. The two patients who died in our series might possibly have had more severe fungal infection which led to a higher neutrophil consumption. One other explanation could be that the two patients who died received a lower dosage of granulocytes which actually was the case in the presented study with higher median granulocyte numbers in the surviving patients. Finally an important concern is the possibility of alloimmunization to HLA class I or granulocyte-specific antigens or both after transfusion. Although rapid alloimmunization does not seem to occur in patients who are severely immunosuppressed it may result in refractoriness to transfusions [20, 21]. More effective rises in ANC, could also have been obtained by daily administration of granulocytes which was not possible due to the difficulties in recruiting donors.

To conclude, despite the limitations of this report and the need for controlled studies granulocyte transfusions might be considered in allogeneic HSCT candidates with severe invasive fungal infections who would have a fatal course if not transplanted. Randomized clinical trials are required to draw more definitive conclusions and luckily a prospective randomized phase III study in Germany has been initiated by Dr. Huebel, Colonge, and Dr. Sachs Gieben. Furthermore, the NHLBI transfusion medicine/hemostasis clinical trials network has also launched a randomized study. We hope that the results of these two studies can turn out to be a guide in means of efficiency of this treatment modality.

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