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A Randomized Clinical Trial Comparing G-CSF Administration Sites for Mobilization of Peripheral Blood Stem Cells for Patients with Hematological Malignancies Undergoing Autologous Stem Cell Transplantation

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Abstract

Background—To investigate whether granulocyte colony stimulating factor (G-CSF) injection in lower adipose-tissue-containing sites (arms and legs) would result in a lower exposure and reduced stem cell collection efficiency compared with injection into abdominal skin.

Study Design and Methods—We completed a prospective randomized study to determine the efficacy and tolerability of different injection sites for patients with multiple myeloma or lymphoma undergoing stem cell mobilization and apheresis. Primary end-points were the number of CD34+ cells collected and the number of days of apheresis. Forty patients were randomized to receive cytokine injections in their abdomen (group A) or extremities (group B). Randomization was stratified based upon diagnosis (myeloma; N=29 vs. lymphoma; N=11), age, and mobilization strategy, and balanced across demographic factors and body mass index.

Results—35 subjects were evaluable for the primary end-point: 18 in group A and 17 in group B. One evaluable subject in each group failed to collect a minimum dose of at least 2.0×10^6 CD34+ cells/kg. The mean numbers of CD34+ cells ($\pm$ SD) collected were not different between groups A and B ($9.15 \pm 4.7 \times 10^6$ /kg versus $9.85 \pm 5 \times 10^6$ /kg, respectively; $p=NS$) following a median of 2 days apheresis. Adverse events were not different between the two groups.

Conclusion—The site of G-CSF administration does not affect the number of CD34+ cells collected by apheresis or the duration of apheresis needed to reach the target cell dose.

Keywords

granulocyte colony stimulating factor; cytokines; stem cell collection

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest relevant to the manuscript submitted to **TRANSFUSION**.

INTRODUCTION

Mobilized peripheral blood stem cells (PBSCs) are used as a source of stem cells for autologous hematopoietic stem cell transplant (HSCT). The mobilization of PBSCs is an important component of the transplantation process; however, the optimum mobilization strategy in preparation for autologous transplant is unknown. Mobilization typically includes hematopoietic growth factors, most often granulocyte colony-stimulating factor (G-CSF), with or without combination with chemotherapy.^{1,2} PBSCs are then collected when peripheral counts increase to a preset white blood cell (WBC) or ANC threshold, using large volume apheresis.³ Target numbers of CD34+ cells for a single autologous transplant are typically $5.0 \times 10^6/\text{kg}$, a cell dose that consistently results in rapid cell engraftment. However, collecting the target number of CD34+ cells can be difficult. Not all patients will collect adequately in 7 days^{4,5} and repeated attempts at mobilization are costly and can delay transplant.^{6,7} In a recent retrospective analysis of 1133 patients with multiple myeloma (MM), Non-Hodgkin lymphoma (NHL) and Hodgkin disease (HD), when G-CSF alone or G-CSF + chemotherapy were used as mobilization regimens, approximately 25% of lymphoma patients and 6% of MM patients failed to collect $>2 \times 10^6$ CD34+ cells/kg for transplantation.⁸ Furthermore, it has been suggested that there is a longer disease free survival and overall survival when the cell dose for transplantation exceeds 5.0×10^6 CD34+ cells.^{9,10} Thus, optimization of every step of the mobilization strategy is important in order to produce and collect the highest yield of CD34+ cells.

Apheresis nurses at our institution made the clinical observation that patients who received subcutaneous G-CSF injections in their abdomen mobilized a higher yield of CD34+ cells than similar patients receiving the injections in their extremities. There are known differences in the pharmacokinetics of subcutaneously injected drugs based upon site adiposity.^{11,12} Extensive information is available on the subcutaneous absorption of injected insulin. The concentration of serum insulin has been shown to be related to the depth and site of injection; deltoid and abdominal higher than anterior thigh or buttocks.¹¹ Similarly, subcutaneously injected human growth hormone has been found to be better absorbed from the abdominal site than from the thigh.¹² There is little information on the absorption of subcutaneously injected G-CSF and whether there is any difference in G-CSF absorption based upon site adiposity. If a difference in G-CSF absorption based upon site adiposity does exist than this could result in differences in number of CD34+ cells collected. We hypothesized that injection in lower adipose-tissue-containing sites in the extremities would result in a reduced reservoir effect leading to lower exposures of G-CSF and therefore reduced yield of CD34+ stem cells collected by apheresis as compared to injection in higher adipose-tissue-containing sites in the abdomen. The current study represents a prospective, single-institution, randomized open-label trial comparing the site of G-CSF administration in lymphoma and myeloma patients scheduled for autologous stem cell transplantation. The primary objectives of the study were to determine if the total number of CD34+ cells collected and the number of days of apheresis required to collect target numbers of CD34+ cells were different between the two groups, one group receiving injections in the abdomen and the other receiving injections in the extremities.

METHODS

Patient Characteristics

Patients at our institution between the ages of 18 and 70 years old with relapsed/refractory Hodgkin's disease, non-Hodgkin's lymphoma, or multiple myeloma who were scheduled for an autologous HSCT were eligible to participate in the study. However, patients who were pregnant or nursing, had active invasive fungal infection, active CNS malignant disease, life expectancy limited by diseases other than the disease for which the patient is being transplanted, or known hypersensitivity to G-CSF were excluded from the trial. From April 2008 to March 2009, all eligible patients were given the option to participate in the study. Forty patients were enrolled and randomized by the study team following informed consent to an IRB-approved protocol to receive cytokine injections in their abdomen (group A) or extremities (group B). The injection site was not blinded. Randomization was stratified based upon diagnosis (myeloma; N=29 vs. lymphoma; N=11), age (≤ 50 ; N=13 vs. >50 ; N=27), and mobilization strategy (cytokine mobilization; N=27 vs. chemo-mobilization; N=13).

Statistical design

The primary endpoint for this phase II clinical study which was to determine the optimum cytokine injection site. The success of stem cell collection was judged based on two criteria: the CD34+ cells collected and the mean number days of apheresis required to collect an optimal target number of CD34+ cells/kg. Sample size of and the minimum number of $>3 \times 10^6$ CD34+ cells/kg. Based upon enrollment of 20 subjects per arm, the study was designed with a 0.9 power to reject the null hypothesis that there is no difference between the two sites for cytokine injections if the true difference a two-fold difference in the mean number of CD34+ cells collected if the standard deviation of the mean is equal (in both groups) to the lower mean value (i.e., a mean of $5 \pm 5 \times 10^6$ CD34+ cells/kg versus a mean of $10 \pm 5 \times 10^6$ CD34+ cells/kg) and 0.9 power to reject the null hypothesis that there is no difference in the fraction of subjects who achieve the minimum number of CD34+ cells in a single day apheresis if the true difference between the groups is 0.5 (i.e., 0.25 of subjects are collected in a single day in Arm A versus 0.75 subjects collected in a single day in Arm B).

Of those enrolled, 90% were evaluable with 18 subjects in group A and 17 in group B. Five subjects were deemed non-evaluable due to one of the following reasons: failure to proceed to the planned mobilization procedure (1 in group A and 2 in group B), lack of consistent injection site (1 in group A), or received a non-protocol specified mobilization strategy (1 in group B). The single subject who received a non-protocol specified mobilization strategy received an injection of plerixafor (MozobilTM) due to poor mobilization and collected a total of 13.6×10^6 CD34+ cells/kg in 2 days of apheresis. The characteristics of the 35 evaluable patients included in the study are summarized in Table I.

Mobilization Regimen

For the chemo-mobilized patients, the mobilization regimen was left to the discretion of the primary treating physician and included chemotherapy alone for 9 patients and rituximab with chemotherapy for 4 patients.

G-CSF administration

G-CSF was administered per our institution's standard practice. Patients undergoing chemo-mobilization received G-CSF 5 µg/kg once daily for five days following the completion of mobilization chemotherapy. (G-CSF was initiated at least 24 hours after completion of the last chemotherapy dose). On the sixth day of G-CSF administration, patients initiated G-CSF 5 µg/kg twice daily (10 µg/kg/day) until stem cell collection was complete. Patients mobilized with cytokines alone received G-CSF 7.5 µg/kg twice daily (15 µg/kg/day) for five days before the planned first day of stem cell collection. Actual mean G-CSF doses were 11.9 µg/kg/day using chemo-mobilization during BID dosing days and 13 µg/kg/day using cytokines alone due to rounding to nearest vial size. Patients maintained a consistent injection site (extremities vs. abdomen) depending on which study arm they were randomized. Patients recorded the injection site for G-CSF and symptoms daily in a patient diary supplied to the patient.

Apheresis

Among chemo-mobilized patients, collection of PBSCs was typically scheduled to occur following the second cycle of salvage chemotherapy at the time of hematological recovery from chemotherapy-induced cytopenia. Among patients mobilized with cytokines only, collection of PBSCs was typically scheduled to occur on the fifth day of cytokine administration. Daily large volume apheresis (20–24 L) was performed using a COBE Spectra™ Apheresis System. Apheresis was initiated when the content of CD34+ cells in the peripheral blood reached $10 \text{ CD34+ cells}/\mu\text{L}$. Emory Medical Laboratories determined the CD34+ cell yield using the ISHAGE protocol and flow cytometry from a 0.5 mL sample of PBSC collected after the apheresis procedure was completed. The remainder of the PBSC product was cryo-preserved. The administration of twice-daily G-CSF and daily apheresis was continued until a target sample of $5 \times 10^6 \text{ CD34+ cells/kg}$ had been collected for patients with lymphoma or $10 \times 10^6 \text{ CD34+ cells/kg}$ for patients with myeloma, or for a maximum of 4 days apheresis. The minimum number of CD34+ cells that was considered adequate to proceed with autologous transplantation was $2.0 \times 10^6 \text{ CD34+ cells/kg}$.

RESULTS

Patient Characteristics

Group A and B were balanced for demographics, mobilization strategy, disease type, number of prior therapy cycles, and prior radiotherapy (Table I).

Toxicities

Both groups reported similar frequencies adverse events including bone pain and redness at the injection site. There were no serious adverse events observed in either cohort.

Statistical Analysis of Number of CD34+ Cells Collected and Duration of Apheresis

Among the 35 evaluable subjects, 1 subject in each group failed collection with a total of $< 2.0 \times 10^6 \text{ CD34+ cells/kg}$ collected. Mean numbers of CD34+ cells/kg ($\pm$ SD) collected were not significantly different between groups A and B (9.15 ± 4.7 versus $9.85 \pm 5 \times 10^6/\text{kg}$,

respectively; $p=NS$). The proportion of subjects in whom the target number of CD34+ cells was collected did not significantly differ between the two groups (55.6% of patients in group A and 52.9% in group B, $p=NS$). The mean duration of apheresis was not significantly different between groups A and B (2.18 ± 1 days versus 2.00 ± 1 days, respectively; $p=NS$), with 2 days as the mode and median duration of apheresis for both groups. Data are presented in Table II.

Of the subjects who did achieve the target yield of CD34+ cells/kg, the total number of days of apheresis needed to achieve the target yield was not significantly different between the two groups. See Figure I.

Hematologic Recovery Post-Transplant

The Center for International Blood and Marrow Transplant Research (CIBMTR) guidelines were used to define hematologic recovery. Neutrophil engraftment was defined as the first of three consecutive days of an absolute neutrophil count of greater than 500 per μL . Time to platelet engraftment was defined as the first of three consecutive days of a platelet count greater than 20,000 per μL without platelet transfusions within the previous seven days.

Among the 35 evaluable subjects who collected stem cells, 23 went on to receive an autologous HSCT, with 13 in group A and 10 in group B. There was no statistical relationship between the probability of neutrophil or platelet engraftment and G-CSF administration site. The mean number of days to neutrophil engraftment was 12 ± 1.58 for group A and 12 ± 1.57 for group B, $p=NS$. And the mean number of days to platelet engraftment following PBSCT was 19 ± 5.15 for group A and 17 ± 3.09 for group B, $p=NS$.

DISCUSSION

The yield of CD34+ cells collected during apheresis varies widely among patients with hematologic malignancies due to the timing of collecting circulating PBSCs, the variety of mobilization protocols, and other factors such as past treatment history.¹³ The optimal mobilization strategy remains unknown. One factor that could potentially affect the yield of CD34+ cells is the adiposity of G-CSF injection site. Differences in the pharmacokinetics of subcutaneously injected drugs based upon site adiposity have been demonstrated for other agents.^{11,12} Higher concentrations of subcutaneously injected human growth hormone have been seen following injection into the abdomen as opposed to into the thigh.¹¹ However, it is unknown whether there is any difference in G-CSF absorption based on site adiposity or whether this could result in differences in yield of CD34+ cells collected. Based upon the differences in the pharmacokinetics of other subcutaneously injected drugs we would expect higher numbers of CD34+ cells among those who administer G-CSF in the abdomen. However, this study demonstrated that G-CSF administration site (extremities versus abdomen) does not affect the number of CD34+ cells collected by apheresis or the duration of apheresis needed to reach the target cell dose. Though we do not know if this can be attributed to no difference in G-CSF absorption between injection sites, we can conclude that adiposity of administration site is not a significant factor affecting yield of CD34+ cells.

It is not clear if these results are generalizable to PBSC collections from healthy related and unrelated allogeneic donors. However, these findings can be utilized in apheresis practice and patient care for autologous donors. Because administration site does not affect the number of CD34+ cells collected, the site for G-CSF administration should be determined according to other factors such as comfort and convenience.

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Figure I.
Cumulative Number of Patients Reaching the Target Yield of CD34⁺ cells/kg According to Day of Apheresis

Table I

Patient Demographics and Baseline Characteristics

	Group		P-value
	A (abdomen)	B (extremities)	
	(N=18)	(N=17)	
Age¹	53 (13.76)	52 (17.02)	0.85
Gender²			
Female	10 (55.6%)	7 (41.2%)	0.39
Male	8 (44.4%)	10 (58.8%)	
Weight¹ (kg)	77.9 (13.32)	85.3(16.67)	0.16
Body Mass Index (kg/m²)¹	27.25 (4.67)	29.39 (5.67)	0.23
Mobilization Strategy²			
Cytokines Alone	13 (72.2%)	10 (58.8%)	0.42
Chemotherapy + Cytokines	5 (27.8%)	7 (41.2%)	
Disease Type²			
Lymphoma	4 (22.2%)	6 (35.3%)	0.39
Myeloma	14 (77.8%)	11 (64.7%)	
Prior Cycles of Chemotherapy¹	5 (3.22)	6 (3.17)	0.62
No. of Patients Receiving Prior Radiotherapy²	2 (11.1%)	6 (35.3%)	0.09

¹ Age, Weight (kg), Body Mass Index (kg/m²), and Prior Cycles of Chemotherapy are described by mean and SD in parenthesis. Comparison was done by two sample t-test.

² Gender, Mobilization Strategy, Disease Type, and Prior Radiotherapy are described by total number and percentage (%) of each group. Comparison was done by Chi-square test.

Table II

Apheresis Results

	Group		
	A (abdomen)	B (extremities)	
	N=18	N=17	P-value
Total # CD34+ cells Collected ¹	9.15 (4.73)	9.85 (4.97)	0.67
Collected Target # CD34+ cells ²	10 (55.6%)	9 (52.9%)	0.88
Total # of Days of Apheresis ¹	2.18 (1.01)	2.00 (0.97)	0.61

¹Total # CD34+ cells and Total # of Days of Apheresis are described by mean and SD in parenthesis. Comparison was done by two sample T-test.

²Collected Target # CD34+ cells is described by total number and percentage (%) of each group. Comparison was done by Chi-square test.