A novel hematopoietic progenitor cell mobilization and collection algorithm based on preemptive CD34 enumeration

Emily Storch,[1,2] Tomer Mark,[3] Scott Avecilla[2], Carlos Pagan[2], Tsiporah Shore[3], Koen van Besien[3], Melissa Cushing[2]

[1]New York Blood Center, New York, NY; [2]Department of Pathology, Weill Cornell Medical College, New York, NY; [3]Department of Medicine, Weill Cornell Medical College, New York, NY

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Abstract

BACKGROUND: The collection of autologous peripheral blood stem cells (PBSC) can be challenging in the subgroup of patients deemed “poor mobilizers” with G-CSF. Plerixafor, a CXCR-4 antagonist, is an alternative mobilizing agent, but is costly and the optimal mobilization algorithm has yet to be determined.

STUDY DESIGN AND METHODS: To address the question we developed a protocol measuring peripheral blood CD34 on Day 4 of mobilization. We examined 26 patients before initiating the protocol versus 24 patients after initiation.

RESULTS: Significant differences ($P \leq 0.05$) included fewer days of collection (1.25 vs 2.42 days), lower total blood volume processed (25.9 vs 57.2 L), lower total product volume (324 vs 691 mL), lower RBC content (9 vs 18 mL), and lower granulocyte percentage per collection (35 vs 11%). There were no significant differences between the two groups in demographics, baseline platelet count, total CD34, or CD34/kg harvested.

CONCLUSION: Use of a protocol to assess PBCD34 a day prior to collection allows for preemptive administration of plerixafor to augment mobilization. Subsequently, days of collection and processed blood volume are reduced while there is less RBC and granulocyte contamination in the collected stem cell product.

Key words: apheresis, plerixafor, stem cell, mobilization

INTRODUCTION

Hematopoietic progenitor cells (HPCs) can be mobilized and recruited into the bloodstream by cytokines, including granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), and stem cell factor (SCF). The recent development of the CXCR4 chemokine receptor antagonist, plerixafor (AMD3100), has provided an additional option for patients refractory to G-CSF HPC mobilization alone. [1] The hematopoietic stem cells are tethered to the bone marrow by the chemokine gradient between CXCR4 on hematopoietic stem cells and CXCL12 on stromal cells. Plerixafor disrupts the gradient and allows the stem cells to be released into peripheral blood. Clinical trials have shown that mobilization with G-CSF plus plerixafor has resulted in a superior yield of CD34+ cells compared to G-CSF alone. [2-4]

A minimum of 2.0 x 10^6 CD34 + cells/kg of body weight is considered a sufficient dose for successful autologous stem cell transplant; however, a dose of 5.0 x 10^6 CD34 + cells/kg is considered preferable for early engraftment. [5-8] A significant number of patients eligible for autologous SCT fail to mobilize a sufficient number of stem cells due to various pre-mobilization factors, including

current or prior use of stem cell toxic chemotherapeutic agents, multiple lines of prior myelosuppressive chemotherapy, older age, heavy marrow involvement with malignancy, prior large field radiation therapy, and poor bone marrow reserve (often marked by baseline thrombocytopenia). Five to thirty percent of patients are deemed to be “poor mobilizers”, defined as a failure to generate at least 2 x 10^6 CD34 positive cells per kg, considered the minimum acceptable dose to ensure timely engraftment of neutrophils and platelets. [9] Poor mobilization can lead to repeated apheresis sessions, low yield products, suboptimal grafts, and the need for remobilization, but an ideal algorithm has not been established.

The timing of plerixafor dosing in relation to previous treatment has been hypothesized to affect efficacy of mobilization and HPC harvest yield for patients deemed to be poor mobilizers [10-12] Since FDA approval in 2009,[13] it has been safely used in subsets of difficult to mobilize patients on a preemptive basis to increase collection success.[14] Various mobilization algorithms have been previously published; however, the most efficient and resource conscious means of utilizing plerixafor, a highly effective but expensive agent,[3] has yet to be determined. In this study we evaluate the efficacy of a new mobilization algorithm using the Day 4 PB CD34 count to determine the need for plerixafor compared to a previous mobilization algorithm using the hematopoietic progenitor cell (HPC) value on Day 5 of mobilization to determine the need for plerixafor.

MATERIALS AND METHODS

Patients

HPC collection data were retrospectively reviewed for 26 autologous stem cell transplant patients at “X” Medical Center/“Y” Hospital between December 2010 and April 2011 and for 24 patients treated between December 2012 and April 2013.

Protocol Design

In 2010 an algorithm for mobilization was developed based on pre-harvest HPC values and preemptive use of plerixafor in predicted poor mobilizers. Progenitor cell quantification was performed using HPC counts enumerated by the Sysmex XE5000 hematology analyzer. Sysmex HPC quantitation is a rapid and inexpensive test that is available as part of a complete blood count (CBC) and identifies a population of immature hematopoietic precursor cells (HPCs) according to size, density, and differential lysis resistance. [15] Risk factors for mobilization failure were defined as prior treatment with lenalidomide or patients with delayed count recovery from previous chemotherapy. All patients deemed to be at risk of mobilization failure were preemptively given plerixafor on Day 4 prior to Day 5 collection. If patients were not at risk of mobilization failure, treatment followed a Day 5 preharvest HPC algorithm, stratified according to collection goals. Table 1 delineates the protocol employed in 2010.

The algorithm was revised in 2012 to include all autologous donors without an a priori definition of a poor mobilizer. Patients were collected based on the Day 4 determination of circulating CD34 positive cells in peripheral blood (PB CD34+). PB CD34+ cells were enumerated with single platform four-parameter flow cytometry using the ISHAGE protocol. If Day 4 morning PB CD34+ counts were <10/uL, Day 4 late afternoon plerixafor was administered, and a subsequent Day 5 morning PB CD34+ count was checked. If the results of the Day 5 PB CD34+ were <8/uL, no collection was performed, G-CSF and plerixafor were given, and PB CD34+ was rechecked on Day 6. For Day 5 PB CD34+ counts ranging from 8–20/uL, 24 L of donor blood was processed via apheresis. For Day 5 PB CD34+ counts >20/uL, the volume processed was 20L. For patients achieving a Day 4 PB CD34+ count ranging from 10–40/uL, Day 4 late afternoon plerixafor was administered, however the Day 5 am PB CD34+ count was not rechecked. Day 5 Collection proceeded at a volume processed of 20L. Patients with a Day 4 am PB CD34+ count >40/uL did not receive plerixafor, and collection (20L processed) proceeded without further PB CD34 values. Plerixafor was dosed according to estimated glomerular filtration rate (eGFR): for eGFR >60, a full 24 mg dose of plerixafor was administered. If eGFR was ≤60 but weight was >50 kg, likewise a full 24 mg plerixafor dose was given. The dose was reduced to 12 mg if eGFR was ≤60 and weight was ≤50 kg.

Patients with multiple myeloma were mobilized using 1) G-CSF alone; 2) G-CSF + cyclophosphamide; or 3) G-CSF + DCEP (4-day continuous IV infusion of dexamethasone 40 mg / day, cyclophosphomaide 400mg/m2/day, etoposide 40 mg/m2/day, and cisplatin 10 mg/m2/day). [16,17]

Apheresis

Collections were performed using the COBE Spectra cell separator (Terumo BCT, Lakewood, CO). 20L or 24L were processed on each day of collection using the manufacturer's mononuclear cell procedure. Anticoagulant citrate dextrose solution (ACD-A) was used. Calcium was replaced as required. The target (optimal) dose for stem cell collection was 5 x 10^6 CD34/kg for patients with NHL, MDS, AML, ALL, HL or Germ Cell Tumor and 10 x 10^6 CD34/kg for patients with MM. The minimal acceptable dose to proceed with transplant was 2 x 10^6 CD34/kg for NHL, MDS, AML, ALL, HL or Germ Cell Tumor and 2 X 10^6 CD34/kg or 4 x 10^6 CD34/kg for single and double MM transplants respectively.

Statistical Analysis

Patient demographics and outcomes between the two mobilization cohorts were compared using the Student's t-test and Fisher-exact test. Analysis was performed using Stata version 13 (StataCorp, College Station, Texas). A p value of 0.05 or less was considered statistically significant.

RESULTS

Patient Characteristics/Demographics

We reviewed the records of 26 patients with stem cell collection using the 2010 protocol and 24 patients using the 2012 protocol. There were no significant differences in gender, weight, age, or underlying disease diagnosis between the 2010 and 2012 patient populations (Table 3). The mean pre-collection platelet count was slightly higher for patients in 2012, but the count was within the expected reference range for the majority of patients in both groups. The difference approached statistical significance at p=0.08 (Table 3).

Patient Characteristics/Clinical

There were no significant differences in the previous treatment regimens between the two cohorts of patients. There were a similar number of patients with refractory disease in each group. The lines of previous chemotherapy were similar between the groups. This was the first mobilization attempt for all patients, except for one patient in the 2012-13 cohort who was being remobilized. More patients received chemotherapy mobilization in 2010 (23%) than 2012 (8.3%). In addition, more patients received plerixafor as part of their mobilization regimen in 2010 (58%) than in 2012 (46%). Neither the difference for chemotherapy mobilization nor the difference for plerixafor mobilization was statistically significant. Overall 92% of patients achieved minimum collection goals (4.0 x 10^6 CD34 + cells/kg of body weight for multiple myeloma, 2.0 x 10^6 CD34 +

cells/kg of body weight for other conditions), and 60% of patients achieved the collection goals (10.0 x 10^6 CD34 + cells/kg and 5.0 x 10^6 CD34 + cells/kg for multiple myeloma and other conditions respectively.

Collection results

There were significantly less days of collection in 2012 versus 2010 (1.25 versus 2.42 days) ($p = 0.001$). In addition, total processed blood volume was markedly reduced at 25.9 L versus 57.2 L in this cohort ($p = 0.0002$). Platelet attrition during the apheresis procedure was similar for both cohorts (2010 cohort 129 $x10^3$/ul, 2012 cohort 120 $x10^3$/ul) (Table 5).

Product results

The product volume was significantly reduced for patients in the 2012 protocol ($p=0.009$). In addition, the volume of RBCs per product and the percentage of granulocytes decreased significantly for patients treated under the 2012 protocol (Table 6). There was no significant change in either percentage CD34 positive of CD45 positive cells or total CD34 positive cells in the products collected.

Plerixafor toxicity

Plerixafor was well tolerated by all patients. No adverse events were identified, however this information was obtained retrospectively. It can be asserted that no CTCAEv4.0 grade ¾ non-hematologic toxicity was seen with plerixafor use.

DISCUSSION

This study presents an effective algorithm for the use of plerixafor in stem cell mobilization based on the Day 4 peripheral blood CD34 counts. While the benefits of administering plerixafor in addition to G-CSF for stem cell mobilization have been shown, [3,4] a standard algorithm has not heretofore been adopted. Use of plerixafor has varied depending on institution, often limited by its prohibitively high cost. While factors including prior chemotherapy, disease treatment and baseline thrombocytopenia, reflecting poor bone marrow reserve, have been observed to affect CD34 mobilization, [18,19] these parameters do not provide a robust measure for prediction of a successful stem cell mobilization and collection. Determination of Day 4 peripheral blood CD34 can assess a patient's early response to G-CSF mobilization and help determine the necessity of administering plerixafor, thereby avoiding the cost of an unnecessary drug when mobilization is effective with G-CSF alone.

In this study we showed that assessing a patient's response to G-CSF mobilization one day prior to collection using peripheral blood CD34 count allows for preemptive administration of plerixafor to augment mobilization in a rational manner. Implementing this protocol resulted in several benefits, including decreased days of collection, decreased blood volume processed and consequently less time for apheresis, and less RBC and granulocyte

contamination in the collected stem cell product.

Granulocytes and red blood cells survive cryopreservation poorly and increased concentrations in infused products can cause toxicity from infusion of damaged mature blood cells.[20] Recipient morbidities due to red blood cell contamination of stem cell products include hemolytic transfusion reactions in the case of ABO-incompatible transplants, or in extreme cases renal failure resulting from red blood cells lysing upon thawing and infusion. [21] Granulocytes in increased concentrations may aggregate, impeding stem cell processing. Recent reports have additionally implicated granulocyte contamination in recipient infusion reactions. [22] Adverse technical aspects of increased cell numbers include the requirement of larger freezing volumes, which can lead to volume overload in recipients and cryoprotectant-related toxicity due to the greater concentration infused. [20,21] Therefore reduced product concentration of mature blood cells is important for decreasing patient complications, improving patient survival, and maximizing resource utilization. [23]

In this study it is likely that the key factor contributing to the improved efficiency realized by the 2012 protocol was the use of the Day 4 peripheral blood CD34 count to assess patient response before initiating the use of plerixafor. This probably reflects the fact that the peripheral blood CD34 is a better predictor of peak mobilization [24-28] than the Sysmex HPC value and the fact that the measurement on Day 4 allows us sufficient time to alter the mobilization

effectively before collection.

Several previous studies have shown the benefit of timing the dose of plerixafor in order to predict and improve mobilization [29-31]. Notably, Li et al recently published a study examining 148 patients treated before the FDA approval of plerixafor compared with 188 mobilized patients of whom 64 received plerixafor. [32] These 64 patients included 41 poor mobilizers, defined as patients with fewer than 15 CD34 cells/uL blood after at least 5 days of G-CSF administration and 23 “high-risk” patients who had failed prior G-CSF mobilization. Their study showed that targeted use of plerixafor increased success rate of mobilizing CD34 cells from 93% to 98%. The present study further refines the results of the study by Li et al by modifying the day of assessment of peripheral blood CD34 from day 5 to day 4.

Limitations of our study include the relatively small number of patients, the design as a retrospective review, and uncertainty in adherence to both protocols. While the algorithms were established and instituted in the apheresis units, clinical circumstances dictated slight variation from the protocol at times. Additionally, replacing the Sysmex HPC with the PB CD34 could contribute to the improvement with the algorithm seen in 2012, as there remains some question to the efficacy of the Sysmex HPC. [33]

This study has several important implications. Decreased days of collection and processing volume have an important impact on donor safety. In turn, decreased product volume has beneficial effects on decreasing the amount of storage space required in the lab and DMSO required for cryopreservation. Correspondingly, this results in enhanced efficiency and productivity of both apheresis and cellular therapy resources, in addition to improved patient safety. Decreased RBC and granulocyte contamination result in improved product quality. The study strongly suggests that measurable improvements in collection efficiency can be achieved by monitoring patients using the PB CD34 count at day 4 versus day 5 to assess response to mobilization and need for plerixafor.

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Hematopoietic Stem Cells Using Automated Hematology Analyzer, Sysmex-XN: A Multicenter Prospective Study 2013.

Table 1. 2010 Protocol – HPC Algorithm

Diagnosis	Sysmex HPC Day 5 level	Mobilization regimen
NHL, MDS, AML, ALL, HL, Germ Cell Tumor	<5 cells/uL	No Collection, G-CSF/ PLEX
	5-10 cells/uL	Collect, but give G-CSF/PLEX that evening, and G-CSF next morning (day 6)
	>10 cells/uL	Proceed with collection and evaluate yield
Multiple Myeloma	<5 cells/uL	No Collection, G-CSF/ PLEX
	5-20 cells/uL	Collect, but give G-CSF/PLEX that evening, and G-CSF next morning (day 6)
	>20 cells/uL	Proceed with collection and evaluate yield
NHL = Non Hodgkin Lymphoma, MDS = Myelodysplastic Syndrome, AML = Acute Myelogenous Leukemia, ALL = Acute Lymphocytic Leukemia, HL = Hodgkin Lymphoma, PLEX=Plerixafor		

* G-CSF 10 mcg/kg/day; PLEX: 240 mcg/kg/treatment both by subcutaneous injection

† Patients received 5 days of G-CSF then proceeded to follow collection algorithm

‡ All collections were performed by processing of 24L of donor blood

Table 2. 2012 Protocol

Day 4 AM CD34 count	<10/ul			10-40/ul	>40/ul
Day 4 Late PM plerixafor administered	Yes			Yes	No
D5 AM CD34 measured	Yes			No	No
D5 AM CD34 measured	<8/ul	8-20/ul	>20/ul	N/A	N/A
Donor blood volume processed	No collection, continue G-CSF/PLEX and recheck PB CD34 on D6	24L	20L	20L	20L

* eGFR >60, 24 mg PLEX (plerixafor)

Table 3. Demographic data

	2010 (12/10-4/11)	2012 (12/12-4/13)	p value
Patients (n)	26	24	
Gender F/M	8/18	12/12	p=0.43
Weight (kg) Mean (STD)	84 (21)	78 (13)	p=0.26
Age Mean (Min, Max)	57 (22, 74)	57 (29, 73)	p=0.90
Diagnosis			
Multiple myeloma	17 (65%)	14 (58.3%)	
NHL	8 (30.8%)	4 (16.6%)	
HD	0	3 (12.5%)	
Testicular cell cancer	1 (4.2%)	0	
ALL	0	1 (4.2%)	
AML	0	1 (4.2%)	
MDS	0	1 (4.2%)	
Baseline pre-collection platelet count Mean (STD)	169 (14)	203 (12)	P=0.08

Table 4. Previous treatment and mobilization received

	2010	2012	p-value
Previous radiotherapy	6/26 (23%)	3/24 (13%)	p=0.33
Refractory disease	0/26	2/24 (8.3%)	p=0.13
Previous lines of chemotherapy, mean (STD)	1.85 (.97)	2 (1.10)	p=0.6
Chemotherapy mobilization	5/26 (20%)	1/24 (4.2%)	p=0.16
Plerixafor mobilization	15/26 (58%)	13/24 (54%)	p=0.4

Table 5. Apheresis parameters for patients treated under the 2010 and 2012 protocols.

	2010	2012	p value
Days of Collection	2.42 (0.3)	1.25 (0.11)	p=0.001
Total Processed Volume (L)	57.2 (35)	25.9 (13)	p=0.0002
Platelet attrition $x10^3$/ul	129 (64)	120 (65)	p=0.67 (NS)

Table 6. Properties of stem cell collection product

	2010	2012	p value
Mean total product volume collected (mL)	691 (461)	324 (158)	0.0006
Mean CD34 percentage per product (SD)	0.48 (0.3)	0.62 (0.4)	0.3398 (NS)
Mean total CD34 x 10^9 collected (SD)	989 (743)	756 (435)	0.1858 (NS)
Mean RBC per product (mL)	18(11)	9(3)	<0.001
Mean Percentage granulocytes (SD) per product	35(2.8)	11(3.1)	<0.001

Figure 1. CD34/Liter in 2010 vs. 2012. CD34 per liter was higher in 2012 compared to 2010 (mean CD 34/L in 2012 = 2416.5; mean CD 34/L in 2010 = 1474.9, p = .046). Although there was no change in either percentage of CD34/CD45 positive cells or total CD34 positive cells in the collected product, the processed volume was reduced by almost half in 2012 (25.9L in 2012 vs 57.2L in 2010). Therefore CD34/L was greater in 2012, correlating to improved collection efficiency.

Figure 1. CD34/L in 2010 vs 2012