*Original Research*

# Roles of Peripheral Blood CD34+ Cell Count and Midpoint Collection CD34+ Cell Yield for Peripheral Blood Stem Cell Collections from Autologous Patients Mobilized by G-CSF and Plerixafor

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**Autologous peripheral blood stem cell transplantation is used to treat multiple hematologic malignancies including multiple myeloma, lymphomas and amyloidosis. G-CSF plus plerixafor has increasingly become a viable first-line PBSC mobilization option in the autologous patients. The aim of our study was to determine whether peripheral blood CD34+ cell count (PB CD34) or midpoint collection CD34+ yield (MPY) is a better predictor of the final collection CD34+ yield (FY) to guide decision-making to ensure collection target achievement and, when possible, to reduce collection sessions for adult autologous PBSC patients mobilized with both G-CSF and plerixafor. Eighty-eight autologous patients who were mobilized by the 2-pronged regimen underwent 171 PBSC collection sessions in 2011. Retrospective data analysis for the PBSC collections showed: (1) Both PB CD34 and MPY correlate strongly with FY; (2) Reduction of apheresis sessions in 24 patients was achieved by decision-making based on FY estimation using PB CD 34 and/ or MPY. Reduction of apheresis sessions decreases the discomfort, inconvenience, cost, and time spent associated with the stem cell collection for the patients, and also decreases the cost and increases the efficiency of our apheresis operation. Based on the prediction value of either PB CD34 or MPY, a guideline is developed for our apheresis facility for autologous PBSC patients, and 1 increased TBV is preferred at most of the time.**

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**Key Words:** *Autologous peripheral blood stem cell collection, Peripheral blood CD34+ cell count, Collection CD34+ yield, Total blood volume, Collection efficiency*

## INTRODUCTION

Since the first attempt at bone marrow transplant in humans in 1957, advances in immunology, chemotherapy and stem cell technology have allowed clinicians to harvest large amounts of stem cells from peripheral blood for transplantation.[1-2] In the mid-1980s, successes with autologous hematopoietic stem cell transplantation (HSCT) were reported.[3] Peripheral blood stem cells (PBSC) can be sourced from allogeneic or autologous donors, with autologous transplantation being used to treat non-Hodgkin's lymphoma (NHL), Hodgkin's lymphoma (HL) and to prolong survival in multiple myeloma (MM).

Autologous patients undergo stem cell mobilizing regimens that consist of chemotherapy and/or granulocyte colony stimulating factor (G-CSF; filgrastim; Neupogen),with or without additional mobilizing agent, in order to produce the highest possible population of circulating stem cells.[4-5] G-CSF plus plerixafor (Mozobil) has become a viable first-line option in autologous patients with MM and as a second line rescue strategy for NHL.[4] The ultimate goal is to collect enough stem cells (enumerated by presence of the surrogate cell-surface marker CD34+) to promote successful engraftment and prompt marrow repopulation after transplantation.

Stem cell count is measured by quantifying CD34+ cells by flow cytometry, which produces accurate results for CD34 cell concentration with a relatively fast turnaround time within hours.[6-7] Maximum PBSC mobilization generally occurs on the fifth day of G-CSF administration, at which point apheresis is initiated and continued daily until target CD34+ cell concentrations are reached.[8]

It has been shown that transplants with stem cell concentrations of $\geq 5 \times 10^6$ CD34+ cells/kg have high engraftment rates, however, a graft can be successful at concentrations as low as $2 \times 10^6$ CD34+ cells/kg.[9-12] Historically, the most sensitive method for determination of mobilization and prediction of adequate apheresis stem cell product is flow cytometric enumeration of PBSC prior to apheresis.[13-15] Pre-collection peripheral blood CD34+ cell concentration (PB CD34) has been used to determine

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necessity of the apheresis procedure, processing volume and number of procedure days.[16-19] Other studies have evaluated the predictive potential of intra-procedural enumeration of CD34+ cell concentration on final stem cell yield (FY) in the setting of fixed-volume apheresis and in the pediatric population.[20-22] To our knowledge, predictive values of both PB CD34 and midpoint collection CD34+ yield (MPY) have not been reported for patients mobilized with both G-CSF and plerixafor.

Here we report our findings for predictive values of both PB CD34 and MPY on FY for adult autologous PBSC patients mobilized with G-CSF and plerixafor.

## METHODS

At our institution, the minimum and optimal stem cell collection targets for autologous patients with diagnosis of lymphoma and leukemia are 2.5 x $10^6$ and 5 x $10^6$ CD34+ cells/kg body weight, respectively. The minimum target and optimal stem cell collection target (OT) for patients with multiple myeloma (MM) are 5 x $10^6$ and 10 x $10^6$ CD34+cells/kg, respectively.[11, 23]

### Hypothesis

The aim of our study was to determine whether PB CD34 or MPY is a better predictor of FY to guide decision-making to ensure collection target achievement and, when possible, to reduce collection sessions.

We hypothesize: (1) MPY is a good predictor for FY; (2) MPY correlates with FY stronger than does PB CD34; (3) Both PB CD34 and MPY can be used to influence the decision to process a larger apheresis volume to decrease the number of days required to achieve stem cell collection target. This would decrease the patient's use of mobilizing agents, decrease the potential side effects associated with the procedure, and lower the overall cost of the PBSC collection.[24]

### Patients

In 2011, a total of 171 autologous PBSC collection sessions were performed for 100 adult patients mobilized by both G-CSF and plerixafor. All 100 patients received 5 doses (1 dose per day) of G-CSF (~8-10 μg/kg) and one dose of plerixafor (~ 0.24 mg/kg) before starting stem cell collection. Both medications were continued prior to each anticipated apheresis session. A patient's first collection leukapheresis session was scheduled on the 5$^{th}$ day of G-CSF administration, which usually correlates with peak circulating PBSC concentration.[25] Plerixafor was administered the evening before each apheresis session.

A retrospective analysis study was conducted for PBSC collection of these autologous patients. Twelve patients were excluded from this study because of incomplete data, late mid-point apheresis product sampling or initial PB CD34 < 10 cells/μL. The remaining 88 patients underwent 143 stem cell collection sessions. Of these, 2.3% (n=2) had a diagnosis of amyloidosis, 5.7% (n=5) had HL, 12.5 % (n=11) had NHL and 79.5% (n=70) had MM.

### Stem Cell Collection Methodology and Design

Mononucleated cell leukapheresis was performed for PBSC collection using Terumo BCT COBE Spectra Apheresis System (Lakewood, CO, USA). Extracorporeal anticoagulation was achieved with acid citrate dextrose A (ACD-A), and citrate-induced hypocalcemia was managed with calcium gluconate. Collection volumes were measured by number of total blood volumes (TBV), computed with the COBE Spectra apparatus. The collections were performed via peripheral intravenous access or centrally placed double-lumen catheters.

Usually no more than 4 collection sessions (i.e. days) are performed during an autologous PBSC collection cycle at our facility, because central venous catheter is usually inserted to the patients on Monday, and the collection starts on the next day. Default 3 TBV was processed for autologous PBSC collection, and PB CD34 historically was the only predictor for FY to determine if the collection session should proceed. If PB CD34 was less than 10 cells/μL, the FY was expected to be less than 1x $10^6$ CD 34+ cells/kg, and the autologous PBSC collection for MM may not proceed, because even the minimal target could not be reached within the 4-day collection cycle.

To test our hypothesis, 3 changes were made for autologous PBSC collections at our institution in 2011: (1) Both PB CD34 and MPY (taken at 1.5 TBV processed volume) were determined for each collection session; (2) Both PB CD34 and MPY were used to predict FY of the collection session; (3) The estimated FY was then used to guide our decision-making on possible adjustment of blood volume to process, in order to reduce the number of collection days, when possible. Processing volume for each collection session was sometimes increased beyond 3 TBV at the apheresis physician's discretion.

The daily maximum volume of TBV to process was mainly limited by the operation hours of the apheresis facility and patient's arrival time. The apheresis facility operates 8 hours daily on weekdays, and thus 4 TBV is the maximal daily processing volume that can be accomplished in the operation time frame. Sessions with less than 3 TBV processed were often due to patient's late arrival, PB CD34 < 10 cells/uL or an early collection stop upon reaching OT.

### Measurement of CD 34+ Cell Counts

Flow cytometric measurement of stem cell CD34+ counts are performed by the in house Flow Cytometry Laboratory using the Stem-Kit™ CD34+ HPC Enumeration Kit, (Beckman Coulter, FL, USA) applying a single-platform modified ISAGE protocol.[24] Automated stem cell counts were performed on the Beckman Coulter Gallios Cytometer (Beckman Coulter, FL, USA). Flow cytometry was performed on a 10 mL sample of pre-apheresis heparinized peripheral blood to determine PB CD34, a 0.5-1 mL EDTA anticoagulated sample of the apheresis collection product at the midpoint of the collection (corresponding to processed volume of 1.5 TBV) to determine MPY and another 0.5-1 mL sample of the apheresis product at the end of the collection

session to determine final collection product CD34 yield (FY). Pre-collection and midpoint samples were taken by the apheresis nurses and the final collection product was sampled at our stem cell processing laboratory (Cellular Therapy Laboratory).

**Estimation of FY, Calculation of MPY, FY, Collection Efficiency**

To calculate MPY and FY, the related CD34 count measured by flow cytometry was multiplied by the apheresis product volume and divided by the recipient's body weight (kg). MPY was calculated at the apheresis facility while FY was calculated at the Cellular Therapy Laboratory.

Estimation of daily FY (CD34+ cells x106/kg) at 3 TBV was made by dividing PB CD34 by a factor of 10. After receiving the MPY (CD34+ cells x106/kg) (at 1.5 TBV) result, a new estimation of FY (at 3 TBV) was made by multiplying MPY by a factor of 2.

Calculated MPY = midpoint apheresis product stem cell CD34 count (cells/μL) x midpoint apheresis product volume (μL) ÷ patient's weight (kg)

Calculated FY = final apheresis product stem cell CD34 count (cells/μL) x final apheresis product volume (μL) ÷ patient's weight (kg)

Collection efficiency (CE) is the ratio of number of CD34+ cells collected to the number of CD34+ cells processed. It is calculated through the following 4 steps:

Blood volume processed = (Final inlet flow volume) minus (final anticoagulation volume) displayed at the end of the collection session by the COBE Spectra Apheresis System.
Number of CD34+ cells processed = (PB CD34) x (blood volume processed)

Number of CD34+ cells collected = (CD34 cell concentration in final product) x (final product volume)
CE = (CD34+ cells collected) / (CD34+ cells processed)

**Statistical Analysis**

Retrospective analysis was performed to determine the correlations between PB CD34 and FY vs. MPY and FY. Correlation and linear regression analysis were used for a subset of 53 patients who underwent ~3 TBV (between 2.7-3.4 TBV) processing in a total of 86 sessions. All patients in this subset had MPY sampled at the time when 1.5 TBV was processed.

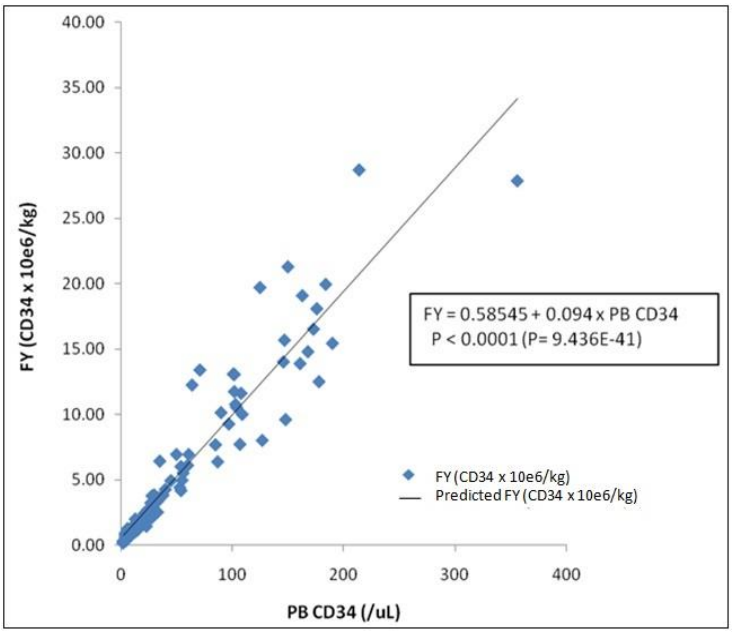


**Figure 1.** Regression analysis with linear fit plot shows a strong correlation and mathematic relationship between PB CD34 and FY for autologous PBSC patients mobilized by G-CSF and plerixafor.

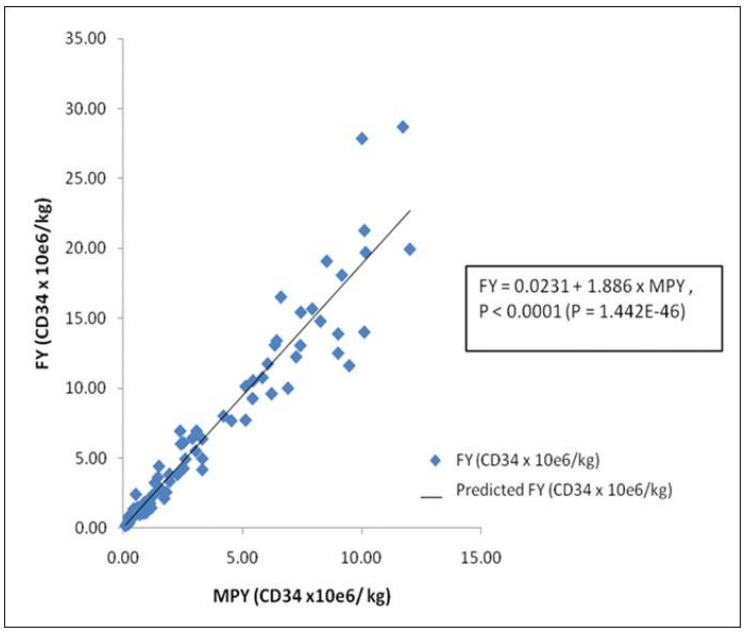


**Figure 2.** Regression analysis with linear fit plot showed a strong correlation between MPY and FY for autologous PBSC patients mobilized by G-CSF and plerixafor.

## RESULTS

**Determination of PB CD34, MPY, FY and Collection Efficiency (CE)**

A total of 143 sessions of apheresis collection were performed on 88 patients with volumes processed ranging from 1.4 TBV to 5.0 TBV (median=3.1 TBV). Only one of the sessions processed 5.0 TBV, the rest processed 4.4 TBV or less. PB CD34 ranged from 2 - 377 cells/μL (median 44 CD34+ cells/μL), MPY ranged from 0.09 - 21.47 $x10^6$ CD34+ cells/kg (median=2.41 $x10^6$ CD34+ cells/kg). Daily FY ranged from 0.18 - 40.07 $x10^6$ CD34+ cells/kg (median=4.91 $x10^6$ CD34+ cells/kg). CE ranged from 21.92% - 123.42%

(median 56%). All patients included in our study achieved at least minimal target stem cell concentrations during their collection cycle.

**Effects of Estimated FY based on PB CD34 and MPY for Decision-making**

A sample of representative patient data (11 patients with 13 collection sessions) is shown in **Table 1**. During each collection session, PB CD34 and MPY were reviewed in order to estimate FY at 3 TBV as described above. Based on this estimation, the apheresis physician made the decision whether or not to increase processed volume beyond 3 TBV in order to reach OT in fewer apheresis sessions. As shown in the representative patient examples in **Table 1**, processed TBV was increased beyond 3.4 TBV for patients # 4, 5, 9, 10 and 11; consequently, the number of collection sessions to reach OT for each of these patients was reduced by one session.

**Table 1.** Product Yield and CD34 Efficiency Data of Representative Patients.

| Patient # | Diagnosis | Optimal Target (CD34x 10e6/kg) | Collection Day # | PB CD34 (/ul) | MPY (CD34x10e6/ kg) | # of TBV Processed | FY (CD34x10e 6/kg) | # of Expected Collection Days (if 3 TBV/day) | Actual # of Collection Days | # of Saved Days | CE (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | NHL | 5 | Day 1 | 54 | 2.42 | 3.0 | 6.03 | 1 | 1 | 0 | 55 |
| 2 | MM | 10 | Day 1 | 125 | 10.17 | 3.0 | 19.72 | 1 | 1 | 0 | 83 |
| 3 | MM | 10 | Day 1 | 104 | 5.44 | 3.0 | 10.55 | 1 | 1 | 0 | 47 |
| 4 | MM | 10 | Day 1 | 87 | 5.68 | 3.6 | 11.44 | 2 | 1 | 1 | 52 |
| 5 | MM | 10 | Day 1 | 80 | 6.32 | 3.5 | 9.31 | 2 | 1 | 1 | 55 |
| 6 | MM | 10 | Day 1 | 102 | 6.06 | 3.0 | 11.76 | 1 | 1 | 0 | 69 |
| 7 | MM | 10 | Day 1 | 150 | 10.11 | 3.0 | 21.3 | 1 | 1 | 0 | 95 |
| 8 | MM | 10 | Day 1 | 148 | 6.22 | 3.0 | 9.62 | 1 | 1 | 0 | 42 |
| 9 | Amyloidosis | 10 | Day 1 | 310 | 4.95 | 3.6 | 11.7 | 2 | 1 | 1 | 25 |
| 10 | MM | 10 | Day 1 | 32 | 2.67 | 4.0 | 6.02 | 3 | 2 | 1 | 72 |
| 10 | MM | 10 | Day 2 | 17 | 2.52 | 4.1 | 4.91 | 3 | 2 | 1 | 123 |
| 11 | MM | 10 | Day 1 | 50 | 3.30 | 4.1 | 5.56 | 3 | 2 | 1 | 49 |
| 11 | MM | 10 | Day 2 | 48 | 3.60 | 4.4 | 5.81 | 3 | 2 | 1 | 49 |

PBCD34 = Pre-collection peripheral blood CD34 (cells/uL)
MPY = Midpoint collection yield CD34 ($x10^6$ CD34+ cells/kg)
TBV = Total blood volume
FY = Final collection product yield CD34 ($x10^6$ CD34+ cells/kg)
CE = Collection Efficiency (%)

**Table 2.** R-square values of regression linear fit analysis (n = 86).

| | CE | PB CD34 | MPY |
|---|---|---|---|
| PB CD34 | 0.035293 | | |
| MPY | -0.008657 | 0.823305 | |
| *FY* | -0.011811 | 0.880656 | 0.913202 |

PBCD34 = Pre-collection peripheral blood CD34 (cells/uL)
MPY = Midpoint collection yield CD34 (x106 CD34+ cells/kg)
FY = Final collection product yield CD34 (x106 CD34+ cells/kg)
CE = Collection Efficiency (%)

**Table 3.** Effect of Processed TBV on Number of Collection Sessions for OT ≥ $10x10^6$ CD34+ cells/kg.

| PB CD34 based Evidence | | | | MPY based Evidence | | | |
|---|---|---|---|---|---|---|---|
| PB CD34 | Number of TBV processed Per Session* | Number of Collection Days to reach OT | Evidence | MPY | Number of TBV processed Per Session* | Number of Collection Days to reach OT | Evidence |
| ≥108 | 3 | 1 | 19 out of 20 (19/20) patients reached OT | ≥ 5.5 | 3 | 1 | 26 out of 28 (26/28) patients |
| 81 - 108 | 4 | 1 | 8 out of 8 patients (8/8) | 4.1 - 5.5 | 4 | 1 | 5 / 6 patients |
| 81 - 108 | 3 | 1 - 2 | 4 /6 patients reached OT within 1 day | 4.1 - 5.5 | 3 | 1 - 2 | 2/ 4 reached OT within 1 day. |
| 61 - 80 | 4 | 1 - 2 | 4 /6 patients reached OT within 1 day. | 3.1 - 4.0 | 4 | 1 - 2 | 1 / 3 reached OT within a day. |
| 61 - 80 | 3 | 1- 2 | 2/4 reached OT within 1 day. | 3.1 - 4.0 | 3 | 2 - 3 | 3/4 sessions reached >$5x10^6$/ kg a day; none reached OT in 1 day. |
| 41 - 60 | 3 - 4 | 2 -3 | 5/8 sessions with 4BV and 6 /11 with 3TBV reached >$5x10^6$/kg a day. Others did not. | 2.1 - 3.0 | 3-4 | 2 – 3 | 9/14 with 4TBV/ session and 5/6 with 3TBV reached >5 $x10^6$/kg. |
| 21- 40 | 4 | 3 – 4 | 5/7: >4.0 $x10^6$/kg; | 1.1 - 2.0 | 4 | 3 - 4 | 3/9 >3.5 $x10^6$/kg. |
| 21- 40 | 3 | 3-4, mostly 4 | 1/14: >4.0 $x10^6$/kg; | 1.1 - 2.0 | 3 | 3-4, mostly 4 | 10/21: >2.5 $x10^6$/kg, among them, 4/21:>3.5 $x10^6$/kg |
| 10 - 20 | 4 | 3 or more, (mostly 4) | 2/5: >3.5 $x10^6$/kg; | 0.5 - 1.0 | 4 | no data | no data |
| 10 - 20 | 3 | 4 or more | 1/14: >3.0 $x10^6$/kg, | 0.5 - 1.0 | 3 | 4 or more | 2 out of 4 patients needed 4 collection sessions to reach OT, the other 2 could not reach OT. |
| <10 | ND | N/A | | <0.5 | ND | N/A | |

* Number of TBV processed Per Session: TBV processed per day, except the last day of the collection cycle.
OT: Optimal Collection CD34 Target
PBCD34 = Pre-collection peripheral blood CD34 (cells/uL)
MPY = Midpoint collection yield CD34 ($x10^6$ CD34+ cells/kg)
TBV = Total blood volume
ND, not determined
N/A, not applicable

**Table 4.** Proposed Guideline Based on PB CD34 or MPY for TBV to Process.

| OT | PB CD34 | Number of TBV to process Per Session | Estimated Number of Collection Days to reach OT |
|---|---|---|---|
| CD34 ≥ $10x10^6$ CD34+ cells /kg | > 120 | 3 | 1 |
| | 81 -120 | 4 | 1 |
| | 61 - 80 | 4 | 1 - 2 |
| | 41 - 60 | 4 | 2 - 3 |
| | 21 - 40 | 4 | 3 - 4 |
| | 10 - 20 | 4 | 4 or more |

| OT | MPY | Number of TBV to process Per Session | Estimated Number of Collection Days to reach OT |
|---|---|---|---|
| CD34 ≥ $10x10^6$ CD34+ cells /kg | > 6.0 | 3 | 1 |
| | 4.1 - 6.0 | 4 | 1 |
| | 3.1 - 4.0 | 4 | 1 - 2 |
| | 2.1 - 3.0 | 4 | 2 - 3 |
| | 1.1 - 2.0 | 4 | 3 -4 |
| | 0.5 -1.0 | 4 | 4 or more |

OT: Optimal Collection CD34 Target
PBCD34 = Pre-collection peripheral blood CD34 (cells/uL)
MPY = Midpoint collection yield CD34 ($x10^6$ CD34+ cells/kg)
TBV = Total blood volume

Among the 143 PBSC collection sessions for 88 patients, 43 (30%) of the procedures were extended to process 3.4 – 5.0 TBV for 28 (32%) of the patients. As a result, 24 (27%) of the patients achieved target stem cell collections in fewer sessions than originally predicted if only 3 TBV were processed. Six patients in this group missed the opportunity to have increased TBV processed due to late arrival, early departure, or waiting for late G-CSF administration.

We further divided these 24 saved sessions into 3 situations: (1) Using estimated FY at 3.0 TBV (both by PB CD34 and MPY), 17 (71%) of these patients were predicted to fall just short of reaching OT on a given collection day. Increasing TBV beyond 3.4 TBV allowed these patients to finish their apheresis cycle one day early. (2) Six (25%) of these patients had relatively low PB CD34 throughout their apheresis cycle (all of PB CD34 < 35 cells/μL). If these patients received a 3.0 TBV volume processing, they would have suboptimal stem cell collection overall and never achieve OT within 4 sessions. (3) The remaining patient was notable for a relatively high PB CD34 (310 cells/μL) who was expected to achieve OT (estimated FY was > 30 x $10^6$ CD34+ cells/kg) within one standard 3.0 TBV processing session. However, the MPY (4.95 x $10^6$ CD34+ cells/kg) was significantly lower than expected (new estimated FY was < 10 x $10^6$ CD34+ cells/kg). Based on this value, the processing volume was increased to 3.6 TBV. In this case, the MPY value was critical in allowing this patient to have an optimal collection within 1 apheresis session.

*Statistical Analysis*

In the subset of 53 patients who had 3 TBV processed (86 collection sessions), our results show a strong correlation between PB CD34 and FY ($r = 0.94$; $P < 0.001$). The correlation between MPY and FY was slightly stronger ($r = 0.96$, $P < 0.001$). A strong correlation also exists between PB CD34 and MPY ($r = 0.91$, $P < 0.001$). None of PB CD34, MPY or FY showed a correlation with CE ($r = -0.22$, $-0.06$ and $-0.01$, respectively).

Linear regression analysis (**Table 2**, **Figures 1** and **Figure 2**) showed similar relationships: strong correlations between any pairing of PB CD34, MPY and FY; with a slightly stronger correlation between MPY and FY than between PB CD34 and FY. There was no correlation between CE and PB CD34, MPY or FY.

The mathematic relationships revealed by the linear regression analysis model between PB CD34 and FY, and between MPY and FY are consistent with the calculation methods for estimated FY as mentioned above. In addition, linear regression analysis revealed that values of PB CD34 > 200 cells/ul, were mostly outliers of the linearity for the mathematic prediction of FY (**Figure 1**). It implies that PB CD34 > 200 cells/uL is not reliable to predict FY. Similarly, values of MPY > 10 x $10^6$ CD34+ cells/ kg are more likely to be outliers for estimation of FY and are not as accurate in estimation of FY (**Figure 2**).

*Evidence-based Guideline*

Further detailed analysis (**Table 3**) was conducted for the autologous collections (consisting of mostly MM patients) with OT of 10 x $10^6$ CD34+ cells/kg. The analysis revealed that for most ranges of PB CD34 or MPY, processing 4 TBV increased the likelihood of earlier OT achievement with a 1-collection day reduction. There were 2 exceptions: For the sessions with PB CD34 of 41-60 cells/μL or for the sessions with MPY of 2.1-3.0 x $10^6$ CD34+ cells/kg, above.

We propose a guideline based on initial PB CD34 and MPY (**Table 4**) to allow for consistent decision-making for blood volumes to process for autologous patients with OT of 10 x $10^6$ CD34+ cells/kg at our facility. This evidence-based guideline demonstrates that either or both PB CD34 and MPY can be used to guide decision-making for possible reduction of collection days.

Although our data analysis showed processing 3 or 4 TBV did not make a difference in collection session reduction for the sessions with intial PB CD34 of 41-60 cells/μL or with MPY of 2.1-3.0 x $10^6$ CD34+ cells/kg, we propose to increase TBV processing from 3 to 4 TBV for these patients because of the possibility of decreased PB CD34 and/ or MPY on the next collection day, and because of the limited sample size of our study. This guideline recommends to increase blood volume processing from 3 to 4 TBV in most situations, except with initial PB CD34 >120 cells/uL and/ or MPY > 6.0 x $10^6$ CD34+ cells/kg.

## DISCUSSION

Leukapheresis is a safe and efficient way to harvest stem cells from the peripheral blood. Processing volumes based on individual patient variables (height, weight, gender, age, tolerance) can range from 1-25 TBV.[23-24] The goal of this procedure is to collect a sufficient CD34+ stem cell yield allowing for a successful marrow engraftment with fewest transplant-associated adverse effects, with stem cell dose as one of the most important factors in HSCT success.[15,25-26]

G-CSF and plerixafor are potent stem cell mobilizers, but have significant adverse effect profiles. G-CSF is associated with bone pain in more than 80% of patients, while plerixafor has been associated with injection site reactions, nausea and diarrhea.[27-28] Furthermore, both of these agents cause leukocytosis, and plerixafor is associated with thrombocytopenia, parameters that need to be carefully monitored during administration.[29] Rare adverse effects of G-CSF include splenic rupture, acute respiratory distress syndrome and precipitation of sickle cell crisis.[30]

Besides the side effects of the mobilization agents noted above, another source of patient discomfort that could be reduced with fewer apheresis sessions is catheter-associated discomfort. Many patients do not live within easy commuting distance from apheresis centers. Each apheresis day incurs costs of traveling or hotel expenses to patients and families. Importantly, autologous donor patients are often status-post chemotherapy and sometimes immunocompromised at baseline, therefore saving an apheresis session reduces exposure to potential infection. Performing fewer apheresis

sessions also decreases the cost to the apheresis center, allowing for more efficient use of resources including the leukapheresis kits used for stem cell collection and apheresis staff time.

Other studies have confirmed that the MPY shows a stronger correlation to FY than does the PB CD34 in settings of fixed-volume apheresis, pediatric patients and different mobilization regimens.[20-22] Our study analyzing adult autologous stem cell donors mobilized with G-CSF and plerixafor shows that using estimated FY to increase processing volumes for some patients can allow them to achieve OT with fewer apheresis sessions.

Collection efficiency (CE) is one of the quality indicators for PBSC collection. Our correlation and linear regression analyses showed no correlation between CE and any of the measured values (PB CD34, MPY, or FY). Thus these values cannot be used to estimate and improve CE.

The major limitations of using MPY in regular clinical practice are: (1) the inherent late timing (midpoint sampling) of MPY determination, whereas PB CD34 is available earlier in the session than MPY to guide TBV decision-making. (2) Pre-procedure PB CD34 measurement is required by the agency Foundation of Accreditation for Cellular Therapy (FACT). Thus, PB CD34 is a not a replaceable parameter, but MPY is. MPY becomes an additional measured parameter. Its additional workload and cost to the apheresis facility, and late timing of MPY determination outweigh its usefulness. (3) PB CD34 alone is a good predictor for FY TBV decision-making. Although MPY statistically shows a slightly stronger correlation to FY than does PB CD34, it adds minimal practical value in determining processing when used routinely.

We recommend using MPY selectively in cases when PB CD34 alone is not sufficient to guide TBV decision-making. For example, MPY can be used to predict FY when initial PB CD34 > 200 cells/uL, because such high PB CD34 is often not reliable for FY prediction. MPY can also be used for FY prediction when attempting to shorten the collection procedure for stem cell donors who have severe physical discomfort with clinical conditions (severe neuropathy, back pain, myeloma nephropathy, history of congestive heart failure, etc.) or who are sensitive to fluid overload and therefore are intolerant of the entire procedure. For example, MPY was used to shorten our collection procedure to 2 TBV in a patient with an initial PB CD34 of 410 cells/uL and a history of a myocardial infarction and poor cardiac function. MPY was used to ascertain that OT would be reached with a lower processed blood volume, thereby reducing collection time.

Based on our data analysis from stem cell collections at our facility (Table 3), we developed a guideline (Table 4) for PBSC collection for autologous stem cell donors with OT of $10 \times 10^6 > 6.0 \times 10^6$ CD34+ cells/kg. The sample size was limited for our data analysis, however such a guideline may help our apheresis facility to consistently maximize the opportunity for reduction of collection sessions while achieving OT. The limitation of 8 hours for our apheresis facility prevents routine processing of blood volumes greater than 4 TBV and prediction of FY (using either/or PB 34 or MPY) becomes important for decision-making on whether or not additional collection days are required in order to reach OT.

Our study presents data and obvservations in a unique situation comprising of: (1) a set of patients who underwent mobilization regiments comprised of G-CSF and plerixafor; (2) estimation of FY using both PB CD34 and MPY; (3) using PB CD34 and MPY to guide our apheresis decisions regarding processing volume and (4) developing a guideline based on data analysis for consistent decision-making geared towards maximizing the opportunity of collection session reduction.

**CONFLICT OF INTEREST**

The authors have no conflict of interest to disclose.

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Not applicable.

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