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PERIPHERAL BLOOD STEM CELL COLLECTION: ESTABLISHING PRE-PROCEDURE CBC BENCHMARKS TO PREDICT CD34+ YIELD

Transfusion Medicine

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ABSTRACT

Introduction: Peripheral blood stem cells (PBSCs) have become the preferred source of hematopoietic stem cells in many transplantation settings due to their ease of collection and faster engraftment. Ensuring an adequate CD34+ cell yield is essential for successful transplantation outcomes. Although granulocyte colony-stimulating factor (G-CSF) is routinely used to mobilize PBSCs, predicting the CD34+ yield prior to apheresis remains challenging. This study aims to explore whether pre-apheresis complete blood count (CBC) parameters—particularly total leukocyte count (TLC), monocyte count, and other commonly measured variables—can be used to predict CD34+ yield and improve PBSC collection efficiency. **Methods:** A retrospective analysis was conducted to evaluate the associations between pre-apheresis peripheral blood parameters (including monocyte count), mid-procedure mononuclear cell (MNC) counts, and final apheresis CD34+ cell yields. **Results:** A statistically significant moderate correlation was found between peripheral blood monocyte count and CD34+ yield in the final product. In contrast, mid-apheresis MNC levels showed a statistically insignificant moderate correlation with end-apheresis CD34+ counts. **Conclusion:** A strong relationship was observed between pre-apheresis mono-lymphocyte count and final CD34+ cell yield, indicating its potential utility as a predictive marker. Mid-procedure lymphocyte-monocyte cellularity was also moderately associated with CD34+ outcomes. However, pre-apheresis TLC and monocyte infusion showed only weak correlations with final yield and engraftment timing. Although not definitive alone, mid-apheresis MNC counts may help guide collection adjustments—such as increasing processed volume—to enhance stem cell yield. These findings support the use of pre-apheresis lympho-monocyte count as a practical and accessible indicator to optimize apheresis outcomes.

KEYWORDS

Peripheral blood mononuclear cells, CD34+ stem cells, apheresis, predictive markers, stem cell mobilization

INTRODUCTION:

Hematopoietic stem cell transplantation (HSCT) is a well-established therapeutic approach for a range of hematologic malignancies and non-malignant disorders. Among the available sources, peripheral blood stem cells (PBSCs) are increasingly preferred due to their advantages, which include faster neutrophil and platelet engraftment, elimination of the need for general anesthesia during collection, and potentially improved graft survival in certain settings. [1,2] A minimum CD34+ cell dose of approximately 2×10^6 cells/kg body weight is generally recognized as essential for successful engraftment. [3]

The collection of PBSCs involves a multi-step process: (1) administration of mobilization agents, (2) mobilization of stem cells into peripheral blood, (3) harvesting of stem cells, (4) product preparation, (5) cryopreservation, (6) delivery of a conditioning regimen, (7) stem cell infusion, and (8) engraftment and patient recovery. CD34, a surface glycoprotein, is a key identifier of hematopoietic stem and progenitor cells, making the CD34+ cell count a vital metric in evaluating collection efficacy. [4-7]

Peripheral white blood cell counts have previously been used to predict the ideal timing for stem cell collection. [9] Flow cytometry is the standard technique used to measure CD34+ cells in clinical settings. Under normal conditions, CD34+ cells make up only about 0.06% of total nucleated cells in the peripheral blood of healthy individuals, though their concentration is significantly higher—up to 18 times—in the bone marrow. To enhance their presence in peripheral blood, mobilizing agents are administered, typically recombinant human granulocyte colony-stimulating factor (rhG-CSF) at 5 µg/kg daily for five days, to increase circulating CD34+ cell levels.

The main objective of the apheresis process is to collect an adequate number of CD34+ cells to ensure prompt recovery of neutrophils and platelets. Therefore, identifying simple and reliable markers to guide the optimal timing of stem cell collection is essential. [10] Several variables, such as patient characteristics and granulocytic lineage activity, have been considered as predictors for successful harvesting. Parameters like total leukocyte count, lymphocyte count, and monocyte count have also been proposed, though the predictive value of lymphocytes and monocytes remains uncertain due to inconsistent findings in various studies. [14]

This study explores the relationship between peripheral blood mononuclear cell (MNC) count and CD34+ levels in apheresis, including their association with lymphocyte-monocyte counts and total leukocyte count. It also evaluates whether MNC count can serve as a predictive marker for timing apheresis. In our institution, flow cytometry is employed to assess both CD34+ and MNC levels to estimate cell yield. However, flow cytometry can be technically complex and costly. Thus, the study seeks to clarify whether there is a correlation between pre-apheresis peripheral blood MNC counts, intra-procedure apheresis MNC levels, and final CD34+ yield, and also aims to assess engraftment timelines under varying clinical conditions.

MATERIALS AND METHODS

This retrospective study analyzed data from a total of 64 patients who underwent stem cell transplantation for malignancies between January 2017 and December 2024. The study population included patients of all age groups, genders, and diagnoses who underwent peripheral blood stem cell transplantation. All study patients received G-CSF at a dosage of 10-15 µg/kg/day, administered in two divided doses for 5 days, to mobilize peripheral blood stem cells. Apheresis commenced after the completion of the 5th day of G-CSF administration. Prior to each leukapheresis procedure, routine metabolic panel testing, including CBC, serum electrolytes, and calcium levels, was performed. Leukapheresis was conducted using a continuous-flow cell separator (COM.TEC Fresenius Kabi) at a planned flow rate of 30 to 60 mL/minute, with the rate adjusted based on patient condition and tolerance. Vascular access was typically achieved via an internal jugular vein using a hemodialysis catheter. A CD34+ cell count of ≥ 2 x 10^6/kg was considered an adequate yield in our institution's practice. Peripheral blood mononuclear cells (defined as the sum of absolute lymphocytes and monocytes) and absolute neutrophil counts were recorded. The product lymphocyte-mononuclear cell count was determined using a complete blood count, and the CD34+ cell count of the final product was assessed by flow cytometry post-collection/leukapheresis. The decision to continue the collection was based on the adequacy of the CD34+ cell count obtained during apheresis. All collected data were maintained in an Excel spreadsheet and subsequently analyzed. Relationships among the studied variables were evaluated using Pearson's correlation coefficient. The chi-square test was employed to identify associations between categorical variables where a p-value of < 0.05 was considered statistically significant. Correlation coefficients are denoted by 'r'. A minus sign indicates a negative correlation. The following scale was used to interpret the strength of the correlation coefficients: 0.0 - 0.01 (No correlation), 0.01 - 0.20 (Very weak correlation), 0.21 - 0.40 (Weak correlation), 0.41 - 0.60 (Moderate correlation), 0.61 - 0.80 (Strong correlation), and 0.81 - 1.0 (Very strong correlation).

RESULTS

From January 2017 to December 2024, we performed 87 collections in 64 patients, who were diagnosed with leukemia, lymphoma or solid

malignancies. Distribution of diagnosis and the characteristics of these patients are summarized in **Table 1**. The median age and weight of the patients were 10.8 (range 1-53) years and 25.7 Kg (8- 94). In these 18 females and 46 were males. Majority, 36 (57%) of the patients were children 1 years to 9 years, rest 28 (43%) were adults age ranging, 24 years to 53 years. 41 of the harvests were autologous and rest 23 were allogenic. Patient were mobilized with G-CSF and Plerixafor. Each harvest consisted of one apheresis in 52 patients and multiple (2-5) apheresis collections in 12 patients. This depended on the yield of CD34 in the apheresis product. However, the yield on Day 1 was more as compared to the subsequent days in most of the patients. **Table 4**

These patients were given infusion post the chemotherapy session. Most of the patients showed rapid engraftment and hematological recovery (ANC greater than 0.5 × 10/L for three consecutive days after PBSC infusion). Prompt engraftment occurred in all the patients. In patients in which the yield was not sufficient after one cycle and the procedure had to be repeated had somewhat delayed engraftment, Day 16 (10-28) as compared to the patients with single cycle of apheresis, Day 10 (7-14). Parameters for patients requiring single collection cycle is given in **Table 2** and those requiring multiple cycle are given in **Table 3**.

All patients tolerated the apheresis well except for 22 patients (34%) who developed tingling and numbness over limbs and mouth, that improved after receiving additional calcium gluconate infusion. No vascular, ischemic, or infection complications occurred with vascular cannulation. Patients having muscle cramping and weakness were 8 patients (12.5%). Other complications, including nausea/vomiting in 4 patients (6%).

Among the pre-procedure values, i.e.,

Pre-Lympho-mono nuclear cell count and end product CD34 showed a **strong correlation**, (r=0.64), p-value was 0.002 **(Significant).**

Product End Lympho-mono nuclear cell count was compared with the final product CD34 count, which was found to be **moderately correlated** (r= 0.43), p-value was 0.05 **(Significant).**

Pre TLC and end product CD34 count, there was a very weak correlation (r=0.1361), p-value was 0.5 (Not significant)

Pre-Absolute neutrophil count and the end product CD34 count were negatively correlated, (r= -0.18), p-value was 0.4 (Not significant)

The product yield and the day of engraftment post infusion showed a very weak correlation (r=0.02) p -value, 0.9 (Not significant).

Table 1- Patient characteristics and peripheral blood stem cell harvest results

Characteristics (n=64)	No. (%)
Gender	
Male	46
Female	18
Autologous	41
Allogenic	23
Median age, year	10.8 (1–53)
Median weight, kg	25.7 (8- 94)
Diagnosis (%)	
Neuroblastoma	22 (35%)
Multiple Myeloma	09 (14%)
HL	09 (14%)
AML	08 (13%%)
Aplastic Anemia	05 (7.8%)
ALL	03 (4.6%)
MDS	03 (4.6%)
NHL	02 (3%)
B Cell Lymphoma	02 (3%)
GCT	01 (1.5%)
Previous radiotherapy exposure (%)	64 (100%)
Hemogram prior to apheresis	
White blood cells (k/µL)	48.4 (18.2–89.3)
Hemoglobin (g/dL)	11.6 (9.0–13.3)
Platelets (k/µL)	104 (17–487)
PBSC harvest	
Apheresis (time)	240 (150-290)
Access line (%)	
Central venous catheter	64 (100%)
Arterial	None
Peripheral venous access	None
Volume (mL) per apheresis	174 (55–346)
Cell component in each apheresis product (n=64)	
Lympho- Mononuclear cells ($\times 10^8$/kg)	12.8 x 10^8 (1–22.9)
$CD34^+$ cells ($\times 10^6$/kg) (FINAL PRODUCT COUNT)	8.9x10^6 (0.2-35)
Engraftment Time (Mean)	Day 11

Table 2- Parameters in patients with single cycle collection- (n=52)

Pre-Procedure Peripheral	Mid Procedure Apheresis Lympho-mononuclear cell count	End Apheresis CD34 Count
Total Leucocyte Count **48k/cumm (18.2–83.3)**	**14.1 x 10^8 (2–22.9)/kg Body weight**	**7.3x10^6 (2.3-35)/Kg Body weight**
Lympho-mononuclear cell count **10.4 x 10^9/L (3.6–38.18)**		
ANC **50x10^9/L**		

Table 3- Parameters in patients where the harvesting had to be repeated- (n=12)

Pre-Procedure peripheral	Mid Procedure Apheresis Lympho-mononuclear cell count	End Apheresis CD34 Count
Total Leucocyte Count 27.2**k/cumm (19-45)**	**1.9 x 10^8 (1–2.9)/kg Body weight**	1.6x10^6 (0.2-4.4)/**Kg Body weight**
Lympho-mononuclear cell count **4.2 x 10^9/L (0.5–7)**		
ANC **32x10^9/L**		

Table 4- Repeated yield on days (n=12)

	D1	D2	D3	D4	D5	Engraftment day
	1.8	0.7				15
	2.5	4.4				10
	2.1	3.8				10
	0.2	0.9				-
	2	1.8				15
	2.83	0.41	2.2			28
	1.2	1.6				20
	0.7	1	1.1	0.71	1.95	18
	1.5	1.4	0.86	0.55		12
	1.97	2.3				14
	2.9	1				21
	1.2	1.4				-
Mean	**1.7**	**1.4**				**16.3**

DISCUSSION

Autologous peripheral blood stem cell (PBSC) transplantation has become a routine therapeutic approach, particularly for supporting patients undergoing intensive chemotherapy, with or without total body irradiation. [15] PBSCs are often preferred over bone marrow-derived stem cells due to their more rapid and consistent engraftment profiles. [16] The CD34+ antigen, a well-established surface marker, is widely used in clinical settings to evaluate the adequacy and effectiveness of stem cell collections. Typically, a minimum threshold of 2×10^6 CD34+ cells per kilogram of body weight is recommended for a successful transplant outcome. [17]

In recent years, considerable research has focused on identifying simple and reliable predictors to optimize the timing of PBSC collection. Among various indicators studied, peripheral blood mononuclear cells (MNCs)—comprising lymphocytes and monocytes—have been extensively evaluated. [11] Flow cytometry-based measurement of peripheral blood CD34+ cells remains the most

dependable tool to anticipate collection outcomes. [17-19] However, other routinely available and less complex parameters, such as MNC counts, continue to be used in clinical practice for guidance.

In this study, we explored the relationships between peripheral blood MNC count, total leukocyte count (TLC), absolute neutrophil count, and the final CD34+ cell yield obtained through apheresis. Additionally, we compared lymphocyte-monocyte cellular content in the apheresis product and analyzed the timing of engraftment in relation to the stem cell dose administered. A strong correlation (r = 0.67) was observed between lympho-mononuclear cell count in the product and the final CD34+ cell yield. A moderate correlation (r = 0.47) was found between product lympho-mononuclear cells and CD34+ yield, while a very weak correlation (r = 0.13) was observed between pre-collection TLC and CD34+ count. There was a negative correlation (r = -0.13) between pre-apheresis absolute neutrophil count and product CD34+ levels, and only a negligible correlation (r = 0.02) was found between total cell yield and the time to engraftment post-infusion.

Among these variables, only the correlation between pre-apheresis lympho-mononuclear cell count and CD34+ yield reached statistical significance. A weak association (r = 0.38) was found between the pre-collection lympho-mononuclear cell count and apheresis yield. Notably, a strong correlation (r = 0.68) was identified between mid-apheresis MNC count and the final CD34+ yield, suggesting that intra-procedure monitoring and adjusting collection volume may influence outcomes.

Our findings are consistent with those reported by Yang et al., [20] who found that circulating lympho-mononuclear cell levels post-mobilization were useful in predicting successful PBSC collection. In their retrospective analysis of 60 patients undergoing first-time autologous transplantation, they found positive correlations between pre-collection monocyte and MNC counts with CD34+ yield, though total WBC count did not show a statistically significant relationship.

Similarly, our study supports the idea that MNC counts on the first day of collection can serve as a helpful predictor for achieving CD34+ cell targets. Nevertheless, it is evident that absolute peripheral blood CD34+ counts remain the strongest predictor of both CD34+ and CFU-GM yields in collected products. Other researchers, such as Holleringworth et al., [21] have also observed strong correlations between peripheral CD34+ levels and final yield, whereas WBC and MNC counts showed weaker associations.

Krishnamurthy et al. [22] also reported weak correlations between MNC counts and CD34+ yields, although their studies noted that higher initial MNC and CD34+ counts produced cell populations composed largely of these cell types. Despite this, the predictive power of MNC counts for moderate or high CD34+ yields was limited in these studies. In some clinical settings, rapid flow cytometric analysis of CD34+ cells is employed to guide apheresis timing and improve stem cell collection efficiency. Our results suggest that while MNC counts may be useful to some extent in predicting collection success, they do not replace CD34+ cell enumeration as the most reliable indicator. Ultimately, peripheral CD34+ cell levels remain the key parameter for determining the optimal initiation of PBSC collection.

CONCLUSION

A strong correlation was observed between pre-apheresis lymphocyte-monocyte counts and the final CD34+ cell yield. When the lymphocyte-monocyte composition of the apheresis product was analyzed in relation to the final CD34+ count, a moderate association was noted. In contrast, pre-apheresis total leukocyte count (TLC) and the resulting CD34+ cell yield showed only a very weak correlation. Similarly, the absolute neutrophil count before collection demonstrated a negative correlation with final CD34+ levels. Additionally, the relationship between total CD34+ cell yield and the time to engraftment following infusion was found to be minimal.

The peripheral blood mononuclear cell (MNC) count, which is readily measurable in most clinical laboratories, appears to be a practical surrogate marker for assessing mobilization success, determining optimal timing for apheresis, and predicting sufficient CD34+ cell collection. During the apheresis process, product MNC counts were found to correlate moderately with the final CD34+ cell yield, suggesting that this parameter may be useful in modifying collection strategies—such as adjusting cycle or total processed volume—to optimize outcomes.

Limitations :

This study is constrained by a relatively small sample size, a retrospective and uncontrolled design, and its implementation at a single center.

Conflict Of Interest Statement:

The authors report no conflicts of interest related to this work.

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