**Study of Peripheral Mononuclear Cells and CD34 Levels as a Predictive Marker for Initiating Apheresis in Autologous Stem Cell Transplant**

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**ABSTRACT**

**Background**: Autologous HCT in multiple myeloma is done as upfront treatment in newly diagnosed transplant eligible patients after induction chemotherapy. In addition, it is standard for relapsed, aggressive non-Hodgkin lymphoma (NHL) and classical Hodgkin lymphoma (HL), and is curative in ~40% to 45% of patients. Over a decade, many efforts were made to find helpful parameters to predict an optimal time for initiating an efficient peripheral blood stem cell collection so that adequate stem cells are collected. It has been well accepted that CD34+ cell count in peripheral blood before leukapheresis is the best parameter to predict CD34 cell yield. However, white blood cell count, mononuclear cell count, and other easily obtained parameters are still used to guide the clinical practice of peripheral blood stem cell mobilization and collection.

**Materials and Methods**: In the present study, we analyzed the correlation between peripheral blood MNC and Apheresis CD34 levels and also between peripheral blood CD34 by flow cytometry and apheresis CD34 levels.

**Results**: We found that there was a statistically insignificant weak correlation between peripheral MNC and apheresis CD34. There was a statistically significant strong correlation between peripheral CD34 and apheresis CD34.

**Conclusion**: The results show that peripheral blood MNC was analogous indicating that no reliable prediction can be done for CD34 cells collected in apheresis while peripheral CD34 by flow cytometry is the strongest predictor for initiating stem cell collection.

**Keywords**: Peripheral blood mononuclear cells; Peripheral CD34 cells; Apheresis CD34 cells; Predictive marker for apheresis

**INTRODUCTION**

Hematopoietic cell transplantation (HCT) is a critical and potentially curative treatment for a range of malignant and nonmalignant diseases. The sources of multipotent hematopoietic stem cells are bone marrow¹ or peripheral blood² of a related or unrelated donor. Peripheral blood as a stem cell source was introduced in 1981². Hematopoietic stem cells from other sources like Fetal liver stem cell³ and cord blood⁴ are also used for the reconstitution of...
lympho-hematopoietic function after myeloablative treatment. Autologous peripheral blood stem cell (PBSC) transplantation has been an important method of support following the use of high-dose chemotherapy with or without the administration of total body irradiation.\textsuperscript{5} Autologous HCT in multiple myeloma are done as upfront treatment in newly diagnosed transplant eligible patients after induction chemotherapy. Two large randomized trials\textsuperscript{6,7} and several nonrandomized comparisons\textsuperscript{8-10} have demonstrated the improved efficacy of autologous HSCT in prolonging event-free survival (EFS) and overall survival (OS) compared with non-transplant therapies in multiple myeloma patients\textsuperscript{11}. In multiple myeloma, even those that do not receive an upfront autograft may undergo transplant at relapse. Similarly, those who have benefited from an upfront autograft may receive a second autologous HCT after a subsequent relapse.\textsuperscript{10} Autologous HCT is also standard for relapsed, aggressive non-Hodgkin lymphoma (NHL) and classical Hodgkin lymphoma (HL), and is curative in ~40% to 45% of patients\textsuperscript{12-15}. The stem cell transplant process consists of distinct steps like: (1) Administration of mobilization agents, (2) Mobilization, (3) Collection of stem cells, (4) Preparation of product for storage, (5) Cryopreservation, (6) Administration of preparative regimen, (7) Stem cell transplantation, and (8) Engraftment and recovery.\textsuperscript{16-19}

CD34, a transmembrane phosphoglycoprotein (also called hematopoietic progenitor cell antigen) is a cell surface marker expressed on stem cells and is used to determine the extent and efficiency of peripheral blood stem cell collections.\textsuperscript{20} The peripheral white blood cell count has previously been used as an indicator of the optimum day for harvesting.\textsuperscript{21} Quantification of CD34\textsuperscript{+} cells by flow cytometry is widely used in clinical practice as an indicator of HSC. The percentage of CD34\textsuperscript{+} cells among circulating total nucleated cells at steady state in healthy donors is 0.06%; it is 1.1% in the Bone marrow, an 18-fold difference in favor of the latter stem cell source. For mobilizing HSC from the bone marrow into peripheral blood requires the use of mobilizing agents. The standard mobilizing agent was commonly used in healthy donors is recombinant human granulocyte colony-stimulating factor (rhG-CSF). Recombinant human granulocyte colony-stimulating factor (rhG-CSF; 15 μg/kg per day) over 3 days, and another rhG-CSF dose given before the stem cell apheresis procedure increase the mean peripheral blood (PB) CD34\textsuperscript{+} cell concentration from $3.8 \times 10^9/L$ to $61.9 \times 10^9/L$, a 16.3-fold increase over baseline.\textsuperscript{22} A cut-off for CD34+cell count of ≥10 to 20 × 10\textsuperscript{9}/mL is usually considered a reasonable cut-off value for initiating PBSC collection.\textsuperscript{23} The goal of apheresis collection is to collect enough CD34\textsuperscript{+} cells to ensure timely neutrophil and platelet recovery. Over a decade, many efforts were made to find helpful parameters to predict an optimal time for initiating an efficient peripheral blood stem cell collection. Predictive factors, including parameters obtained prior to the beginning of the procedure that influence the efficiency of harvesting CD34\textsuperscript{+} cells for effective harvesting, have been broadly studied.\textsuperscript{23} The total leukocyte count, number of monocytes and lymphocytes, and percentage of circulating immature cells of the granulocytic lineage have all been mentioned as possible predictive factors for apheresis collection.\textsuperscript{24-26}

It has been well accepted that CD34+ cell count in peripheral blood before leukapheresis is the best parameter to predict CD34 cell yield. However, white blood cell count, mononuclear cell count, and other easily obtained parameters are still used to guide the clinical practice of peripheral blood stem cell mobilization and collection. Periphera blood mononuclear cells are taken as the sum of lymphocytes and monocytes, which has showed correlation with the final CD34 harvest in some studies. In the present study, we analyzed the correlation between peripheral blood MNC and Apheresis CD34 levels so that whether peripheral blood MNC can be used as a predictable marker of the timing of apheresis. In our hospital, we use flow cytometric analysis of CD34 and peripheral blood mononuclear cells to predict adequate cell yield. The current flow cytometric techniques to measure CD34 cells are technically difficult and expensive. This study aims at finding the correlation between peripheral blood...
mononuclear and apheresis CD34 and also between peripheral blood CD34 and apheresis CD34.

MATERIALS AND METHODS
Our study was done at HCG hospital Bangalore which is a tertiary care oncology center. A total of 49 patients undergoing stem cell transplantation for hematological malignancies during the period June 2016 to May 2018 were analyzed. Considering alpha = 0.05 and 95% statistical power, the correlation between apheresis CD34 + and peripheral blood mononuclear cells was analyzed. Patients of multiple myeloma or relapsed lymphoma undergoing autologous peripheral blood stem cell transplantation and receiving ≤ 3 lines of induction chemotherapy were included in the study. Patients undergoing autologous peripheral blood stem cell transplantation for other indications and of age < 18 years and > 68 years were excluded.

All study patients were started on mobilization by G-CSF at 10-15mcg/kg/day in two divided doses for 3 days. Apheresis was started on 4th day after taking consent once white blood cells were > 10 x10^9 cells/L and peripheral blood CD34 count > 10^5 /ml and G-CSF was continued at the same dose. Before each leukapheresis routine metabolic panel was done including CBC, Serum electrolytes, and calcium. CD34 count was performed by flow cytometry from the peripheral blood daily before apheresis. Peripheral blood mononuclear cells which were the sum of lymphocytes and monocytes were noted along with peripheral blood CD34. Then, apheresis CD34 cell count was done by flow cytometry post-collection/Leukapheresis. Decision regarding the continuation of the collection was based on the adequacy of the CD34 count of apheresis.

The values and results were entered onto an Excel database and analyzed. Statistical analysis was performed on a computer by Statistical Package for Social Sciences [SPSS] for Windows Version 22.0. Relationships among study variables were assessed using Pearson’s correlation. Based on the normality of data, either independent samples t-test or Mann-Whitney U-test was used to evaluate differences between groups. Chi-square test was used to find association between the categorical variables. P < 0.05 was considered significant. The correlation coefficients are denoted by ‘r’. Minus sign denotes a negative correlation. Correlation coefficient ranges are 0.0- No correlation, 0.01 to 0.20 – Very weak correlation, 0.21 to 0.40 – Weak correlation, 0.41 to 0.60 – Moderate correlation, 0.61 to 0.80 – Strong correlation, 0.81 to 1.0 – Very strong correlation.

RESULTS
Forty-nine patients with multiple myeloma or lymphoma who achieved a remission and received autologous peripheral blood stem cell transplant were enrolled into the study, and their data was analyzed. Out of 49 patients, 26 were males and 23 were females. The median age was 50 years with a range of 18-68 years. Twenty-six patients were diagnosed to have multiple myeloma and 23 patients were diagnosed with lymphoma. Among Lymphoma patients, 12 had a diagnosis of diffuse large B-cell lymphoma, 7 Hodgkin’s lymphoma, 3 T-cell lymphoma and 1 Mantle cell lymphoma.

All study patients underwent mobilization by G-CSF. Leukapheresis was started once the total leukocyte count was >1x10^9/L and a peripheral CD34 count of >10^5/ml. Of the 49 patients, only one patient had single leukapheresis, 20 required second collection, 19 required a third collection, and 9 underwent a fourth collection. A total of 134 leukapheresis was done with an average of 2.73 leukapheresis per person (Table 1). A median of 7.75x10^6 cells/kg patient body weight (range 3.99 to 18.66) of stem cells were collected per harvest.

MNC was calculated on each day of harvest before leukapheresis. Total MNC was calculated by the sum of MNC of all days. The median Day 1 MNC was 3.55 with the range of 1.00 to 10.58. The median total MNC was 10.82 with the range of 2.41 to 24.28. Pre-leukapheresis MNC of each day of harvest was correlated with the CD34 content of apheresis product of corresponding days. The correlation coefficient (r-value) and p-value is summarized in Table 3. There was a very weak correlation on Day 1, a weak correlation on Day 2, negative correlation on Day 3, and moderate correlation on Day 4. But, the correlation was not statistically significant (Table 2). The results suggest that MNC was non-predictive of final CD34 cell content of harvest in our study. In the present study, statistically insignificant weak
correlation was found between peripheral blood MNC and CD34 content of apheresis. The correlation between peripheral blood (PB) CD34 cells in pre-leukapheresis sample and CD34 cells in apheresis sample or harvest (as expressed per kg of the patient’s body weight) of corresponding days was also analyzed. The correlation coefficient between D1 PB CD34 and D1 harvest CD34 was r = 0.59 with p-value < 0.001 (Fig 1), D2 PB CD34 and D2 harvest CD34 was r = 0.41 with p-value < 0.005 (Fig 2), D3 PB CD34 and D3 harvest CD34 was r = 0.64 with p-value < 0.001 (Fig 3) and D4 PB CD34 and D4 harvest CD34 was r = 0.44 with p-value 0.38. There was a good correlation (moderate to strong) between the above variables, which was statistically significant (Table 3). The results suggest that peripheral blood CD34 cells are a strong predictor of apheresis CD34 stem cell yield.

### Table 1. Average Leukapheresis / Person

| Variables | Mean & SD | Mean | SD |
|-----------|-----------|------|----|
| Range     | 01 – 04   |      |    |

### Table 2. Correlation of D1, D2, D3 and D4 MNC with D1, D2, D3 and D4 Total CD34 using Pearson Correlation Test

| Variables | Values | Total CD34_D1 | Total CD34_D2 | Total CD34_D3 | Total CD34_D4 |
|-----------|--------|---------------|---------------|---------------|---------------|
| MNC_D1    | r      | 0.12          | -0.06         | -0.08         | 0.45          |
|           | P-Value| 0.40          | 0.68          | 0.68          | 0.22          |
| MNC_D2    | r      | 0.15          | 0.25          | 0.19          | 0.45          |
|           | P-Value| 0.31          | 0.08          | 0.33          | 0.23          |
| MNC_D3    | r      | 0.07          | -0.20         | -0.19         | 0.07          |
|           | P-Value| 0.71          | 0.32          | 0.33          | 0.85          |
| MNC_D4    | r      | -0.95         | 0.10          | 0.50          | 0.62          |
|           | P-Value| <0.001*       | 0.80          | 0.17          | 0.08          |

### Table 3. Correlation of D1, D2, D3 and D4 CD34 (peripheral blood) with D1, D2, D3 and D4 Total CD34 using Pearson Correlation Test

| Variables | Values | Total CD34_D1 | Total CD34_D2 | Total CD34_D3 | Total CD34_D4 |
|-----------|--------|---------------|---------------|---------------|---------------|
| PB_CD34_D1| r      | 0.59          | -0.10         | -0.01         | 0.31          |
|           | P-Value| <0.001*       | 0.54          | 0.98          | 0.42          |
| PB_CD34_D2| r      | -0.03         | 0.41          | 0.33          | -0.30         |
|           | P-Value| 0.85          | 0.005*        | 0.10          | 0.44          |
| PB_CD34_D3| r      | -0.23         | 0.61          | 0.64          | 0.10          |
|           | P-Value| 0.29          | 0.001*        | 0.001*        | 0.81          |
| PB_CD34_D4| r      | 0.613         | -0.75         | 0.56          | 0.44          |
|           | P-Value| 0.196         | 0.08          | 0.25          | 0.38          |

Figure 1. Scatterplot depicting the correlation between peripheral blood CD34 and total CD34 on Day 1
DISCUSSION

Autologous peripheral blood stem cell (PBSC) transplantation has been an important method of support, following the use of high-dose chemotherapy with or without the administration of total body irradiation. Peripheral blood stem cells as a source are better than bone marrow in transplantation as recovery after myeloablative therapy is faster and is associated with less morbidity. Pluripotent stem cells express the cell surface marker antigen CD34. This marker is the indicator most frequently used in clinical practice to determine the extent and efficiency of peripheral blood stem cell collections. In general, a collection of CD34 target level of $2 \times 10^6$ cells/kg is desired for successful engraftment. Over a decade, many efforts were made to find helpful parameters to predict an optimal time for initiating an efficient peripheral blood stem cell collection so that adequate stem cells are collected. Predictive factors for effective harvesting have been broadly studied. Peripheral blood mononuclear cells and peripheral CD34 cell count are among factors analyzed in most of the studies. Peripheral blood mononuclear cells, which include lymphocytes and monocytes, have shown the correlation with the final CD34 harvest in some studies. Among these factors, the monitoring of PB CD34$^+$ cell concentrations by flow cytometry has emerged as a reliable method to predict the success/failure rate of collections. Although it has been accepted that
the CD34+ cell count in the peripheral blood before leukapheresis is the best parameter for predicting CD34+ cell yield, other more easily measurable parameters like peripheral blood mononuclear cells (MNC) are still used to guide the clinical practice of PBSC collection.

In our study, we analyzed the correlation between peripheral blood MNC with apheresis CD34 and also between peripheral CD34 count and apheresis CD34. The correlation coefficient (r) between peripheral blood MNC and apheresis CD34 of the corresponding days was weak. The correlation was not statistically significant. The above results show that peripheral blood MNC was analogous, indicating that no reliable prediction can be done for CD34 cells collected in apheresis. Then, we correlated peripheral blood CD34 and apheresis CD34 on the corresponding days of collection. There was a good correlation (moderate to strong) between the above variables, which was statistically significant.

Our study was in comparison with Yang et al32 who reported that circulating monocyte count after mobilization is a helpful parameter to determine adequate stem cell collection. 60 consecutive first mobilization attempts and 145 leukapheresis procedures for patients with hematological malignancies were analyzed to investigate potential predictive parameters for the successful collection of autologous peripheral blood stem cells. Correlation analysis of pre-leukapheresis circulating white blood cells(WBC), monocytes(MO) and mononuclear cells (MNC), and the final CD34 cell yield showed that WBC(rho = 0.385, p = 0.003), MO(rho = 0.415, p = 0.001) counts had positive correlation other than MNC ( p = 0.227) which showed that MNC is not a good predictive marker of CD34 apheresis yield. Ishii et al. showed that only peripheral blood MNC on the first day of harvest predicts successful apheresis33. In Armitage et al, the white cell count on the day of harvest showed only a weak correlation with the total number of CD34 cells in the collection (r = 0.30) which was similar to our results of correlation between MNC and apheresis CD34. In contrast, the absolute number of circulating CD34+ cells strongly correlated with the CD34 cell and CFU-GM yield of the corresponding apheresis product34, which was in comparison with our study.

A study by Hollingsworth et al. analyzed the relationship between CD34+ cell count in the peripheral blood (PB) and the leukapheresis product CD34+ cell yield. Other correlations like WBC and mononuclear cell (MNC) concentration to leukapheresis product CD34+ cell yield/kg were also analyzed. The best predictor of product yield of CD34+ was the PB CD34+ cell concentration with r =0.9335. MR Boulassel et al. also showed a weak correlation between peripheral blood MNC and CD34 stem cell yield36. Schots et al.37 reported similar results with fewer patients. Haas et al. 38 also demonstrated a clear correlation between circulating CD34 cells and their numbers in the resulting harvest, but in a population that consisted entirely of lymphoma patients. Hence, our study showed that there was a statistically insignificant very weak to weak correlation between peripheral MNC and apheresis CD34, but there was a statistically significant strong correlation between peripheral CD34 and apheresis CD34. In some transplant setups where flow cytometry is not available, MNC is still used as a predictor for timing of apheresis and stem cell yield. But, our study results show that MNC is not a good parameter to assess the timing of apheresis and it cannot indicate the adequacy of stem cell yield. Hence, peripheral CD34 cells by flow cytometry should be considered during autologous HSCT which is the strong predictor for initiating stem cell collection.

CONCLUSION

Peripheral blood MNC correlates poorly with yield CD34 as it gave analogous results. Even though peripheral blood MNC is an easily available parameter, which can be done in all centers, it is not a reliable parameter for successful mobilization. Peripheral blood CD34 is a very useful predictor of timing of apheresis and adequate CD34 collection. Limitations of our study are a small sample size, and an uncontrolled retrospective single center study.
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CONFLICT OF INTEREST

Authors declare that they do not have any conflict of interest.

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