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Determination of mononuclear cell count using peripheral smear and flow cytometry in peripheral blood stem cell products: A retrospective study from an Indian cancer center

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Abstract:

BACKGROUND: Mononuclear cells (MNCs) are considered equivalent to hematopoietic stem cells, and differential count using peripheral smear was routinely practiced to enumerate MNC. Flow cytometry plots used for CD34 enumeration assay can also be used in MNC enumeration as it counts more WBC events than manual methods. The aim was to determine the relationship and degree of agreement between peripheral smear and flow cytometry in MNC enumeration of peripheral blood stem cell (PBSC) products.

METHODS: In 63 patients, 73 PBSC products were collected between January 2017 and September 2019. The differences in MNC count estimated by peripheral smear method and from flow cytometry plots used for CD34 enumeration were analyzed using Mann–Whitney test. Agreement between the two methods for MNC enumeration was determined by regression analysis. Receiver operating characteristic curve was performed to determine MNC threshold in peripheral blood and PBSC product for adequate mobilization and harvest.

RESULTS: There was no difference in enumeration of median MNC count between peripheral smear and flow cytometry (52% vs. 59%, $P = 0.185$) in PBSC product. However, regression analysis indicated a constant and proportional difference between the methods with $r = 0.52$. Cumulative sum test for linearity showed deviation from linearity ($P = 0.04$). MNC counts in peripheral blood failed to achieve discrimination capacity in predicting adequate CD34+ yield/kg body weight in product.

CONCLUSION: Peripheral smear estimated lower MNC counts than flow cytometry with weaker agreements between the two methods. Hence, MNC count derived from flow cytometry plot can substitute peripheral smear method for MNC dose calculations. MNC dose at 3.4×10^8/kg consistently predicted $>2 \times 10^6$/kg CD34+ cells collected.

Keywords:

CD34 enumeration, flow cytometry, mononuclear cell, peripheral blood stem cell

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Introduction

Peripheral blood stem cells (PBSCs) collected through apheresis machine harvest the buffy coat layer to concentrate hematopoietic stem cells. In PBSC products, harvests are not only limited to hematopoietic stem cell population alone but also contain monocytes, lymphocytes, erythrocytes, and platelets.[1] Hematopoietic stem cells are identified by dim CD45 expression/low side

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scatter and CD34+ population using sequential Boolean gating strategy in flow cytometry.[2,3]

Mononuclear cells (MNCs) are considered equivalent to hematopoietic stem cells, and differential count using peripheral smear was routinely practiced in India to enumerate MNC, in addition to CD34 enumeration by flow cytometry for evaluating PBSC harvest. In peripheral smear, the routine practice was to review 100–200 nucleated cells by microscopy and classify leukocytes based on morphology. However, the process of identification of MNC by peripheral smear is a multistep laboratory test which relies on subjective interpretations.[4,5]

Flow cytometry plots used for CD34 enumeration assay can also be used in MNC enumeration as it counts more WBC events than manual methods. By gating MNCs, flow cytometry seems to be a better alternative to peripheral smear for differential count, as it provides an opportunity to detect progenitor cells and subsets of cells in a standardized manner when available.[5]

For decades, absolute MNC count in relation to the recipient body weight had served as useful predictors for engraftment potential of the transplants.[6] As multiple methods are employed to enumerate MNC counts, transplant centers need to assess the agreements between the methods available. The primary aim was to determine the relationship and degree of agreement between peripheral smear and flow cytometry in MNC enumeration of PBSC products. Additional objectives were to determine whether MNC counts in peripheral blood had a role in predicting >2 × 10^6 CD34+ cells/kg body weight in PBSC product and to set a minimum threshold for product MNC count/kg which could correlate with >2 × 10^6 CD34+ cells/kg in the PBSC product.

Methods

Patient population

In total, 73 PBSC product samples were analyzed from 63 patients, between January 2017 and September 2019. Approval from the Institution Review Board was obtained before the start of the study.

Peripheral blood stem cell collection

PBSC collection was performed either using Spectra Optia (Terumo BCT, Lakewood, Colorado, USA) or MCS+ (Haemonetics, Boston, USA) equipment using central venous access. The target blood volume to be processed was fixed at two blood volumes of the patient. The criterion for adequate collection was set at ≥2 × 10^6 CD34+ cells/kg body weight of patient, and collection was performed daily to achieve the target CD34 cell dose.

Product mononuclear cell counts

By peripheral Smear

The total leukocyte count in the PBSC product was determined with an automated hematology analyzer (Coulter LH 750, Beckman Coulter). Manual differential count of 200 cells was performed on a Wright stained smear of PBSC product under oil immersion field by a pathologist. The MNC count was expressed as a percentage of total leukocyte count and was obtained by the sum of the percent of monocytes, lymphocytes, and progenitor cell population. The absolute MNC count was derived from the total leukocyte count.

By flow cytometry

The CD34+ cells of PBSC products were enumerated by flow cytometer (FC500, Beckman Coulter) using the Stem-Kit reagent in blood bank. The CD34+ plots were retrieved and MNC concentration (%) was determined simultaneously by gating the monocytes, lymphocytes, and progenitor populations in CD45 versus side scatter plot [Figure 1].

Peripheral blood counts

On peripheral blood samples, total leukocyte count and CD34 count were performed before PBSC collection using an automated hematology analyzer and flow cytometer, respectively. The CD34 plots of peripheral blood flow cytometry were retrieved and percentage MNC counts were gated as described earlier.

Comparison

MNC concentrations determined from peripheral smear and flow cytometry of PBSC product were compared. Similarly, the MNC dose of the PBSC product was calculated as described earlier.[7]

$$\text{MNC dose} (\times 10^8/\text{kg}) = \frac{\begin{array}{c}\text{MNC}(\%)\quad \text{total leukocyte count}\\ \text{volume of product}(\text{ml})\end{array}}{\text{Weight of recipient}(\text{kg})}$$

Statistical methods

Descriptive statistics were expressed as proportion and continuous variables were expressed as median (interquartile range [IQR]). MNC count (%) and MNC yield were calculated for both peripheral smear and flow cytometry techniques, and Mann–Whitney test was used to study the difference between them. Correlation of MNC counts between the two methods was performed using Spearman correlation. The differences between the two methods were compared through Passing–Bablok regression analysis.

The relationship between the peripheral blood MNC and harvested CD34+ cells/kg in PBSC product was evaluated. The receiver operating characteristic (ROC) curve was used to set the cutoff for MNC dose/kg in PBSC product

when CD34+ cells had >2 × 10^6/kg resulted in adequate collection. Similarly, ROC was used to determine the cutoff to predict MNC count in peripheral blood for adequate CD34+ yield. A significant level of $P < 0.05$ was chosen.

Results

Among the 63 patients for whom PBSC collection performed, 10 were allogenic and the rest autologous. Multiple myeloma (57%) and acute myeloid leukemia (8%) were the common indications for autologous and allogenic PBSC collection, respectively [Table 1]. The adequate CD34+ cells of >2 × 10^6/kg were collected in 52 (82%) patients during the first apheresis and 11 patients required the second collection.

Agreement between peripheral smear and flow cytometry in mononuclear cell enumeration

Peripheral smear, though estimated a relatively low median MNC concentration than flow cytometry (52% vs. 59%, $P = 0.185$) in PBSC product, the difference was not significant. However, regression analysis, as shown in Figures 2 and 3, indicated a constant and proportional difference between the two methods y = −24.33 + 1.33X, with 95% confidence interval for intercept (−49.05, −7.82) and slope (1.09, 1.75) and showing moderate correlation ($r = 0.52$). The cumulative sum test for linearity between the methods shows a significant deviation from linearity ($P = 0.04$).

Peripheral blood counts

The median CD34+ cells in peripheral blood before apheresis were 54 cells/µl [Table 1]. The estimated MNC concentration from the CD34 plot of peripheral blood was 15.5% (IQR 13.5–18.5). Peripheral blood CD34 count had a positive correlation value for the CD34+ cells harvested in PBSC product ($r = 0.483$, $P < 0.01$) as expected. In contrast, peripheral blood MNC count gave no reliable prediction for number of CD34+ cells harvested in the product ($r = -0.002$, $P = 0.989$) and expressed only a weak correlation with MNC count/kg BW ($r = 0.325$, $P = 0.006$) in product.

ROC curve showed that MNC in peripheral blood failed to achieve discrimination capacity in predicting adequate CD34+ yield in product (area under curve 44%)[Figure 4], whereas the cutoff for peripheral blood CD34+ cells for predicting adequate CD34+ yield in PBSC product was 37 cells (area under the curve 80.9%) with 82% sensitivity and 82% specificity.

Peripheral blood stem cell product counts

The counts of PBSC product harvested are shown in Table 1. The median CD34 dose harvested was 4.4 × 10^6/kg. Table 2 shows that there was a significant difference between the MNC yield estimated using peripheral

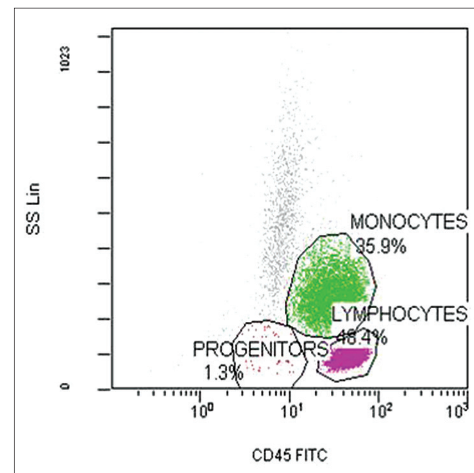

Figure 1: CD45 versus side scatter plot obtained by flow cytometry. Gating strategy for mononuclear cell count with CD45 on X-axis and side scatter on Y-axis to include lymphocytes, monocytes, and progenitor population

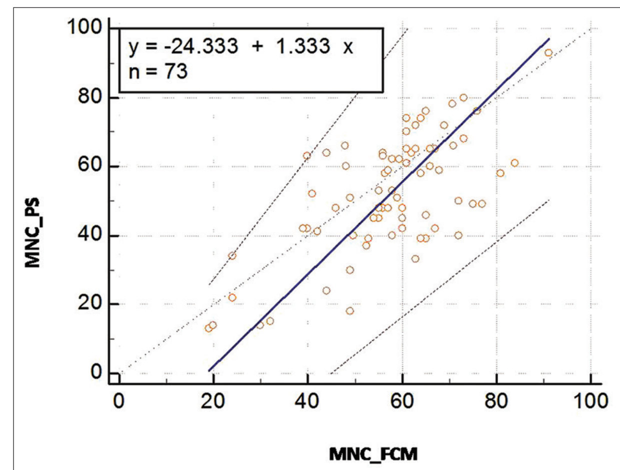

Figure 2: Scatter diagram of mononuclear cell counts with regression line between hypothetical measurements done by peripheral smear and flow cytometry with confidence bands for regression line. Continuous line represents the regression line (y = −24.333 + 1.333x) and 95% confidence interval limits are presented as dotted line intercept (−49.05, −7.82) and slope (1.09, 1.75)

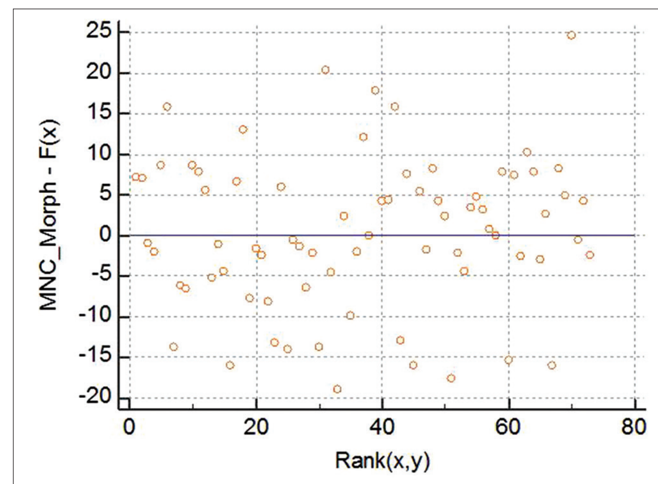

Figure 3: The residual plots of mononuclear cell enumeration presenting distribution of difference around fitted regression line between measurements done by peripheral smear and flow cytometry methods

Table 1: Patient diagnosis with blood counts in peripheral blood and dose in peripheral blood stem cell products

Diagnosis	*n* (%)	Median (IQR)		
		PB CD34 (cells/μl)	CD34 dose (×10⁶/kg)	TNC dose (×10⁸/kg)
Autologous (*n*=53)				
Multiple myeloma	36 (57)	48 (22-62)	3.9 (2.5-5.4)	7.9 (5.8-9.4)
Non-Hodgkin's lymphoma	9 (14)	82 (27-140)	3.2 (1.9-6.4)	8.5 (7.5-11.6)
Hodgkin's disease	8 (13)	78 (58-148)	8.8 (4.0-12.9)	9.1 (8.5-11.1)
Allogenic (*n*=10)				
Acute myeloid leukemia	5 (8)	101 (30-218)	5.9 (5.1-5.9)	6.2 (3.5-6.2)
Acute lymphoblastic leukemia	3 (5)	101 (96-101)	5.5 (5.4-5.5)	4.5 (4.2-4.5)
Aplastic anemia	2 (3)	57 (53-57.5)	4.7 (3.5-4.7)	5.4 (3.5-5.3)
Overall	63	54 (30-85)	4.4 (3.0-6.1)	8.0 (5.8-9.5)

PB=Peripheral blood, TNC=Total nucleated cell, IQR=Interquartile range

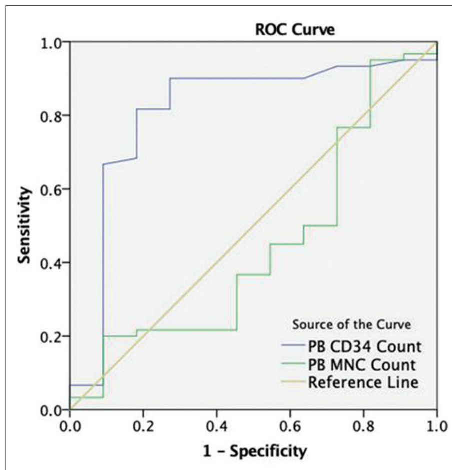

Figure 4: Receiver operating characteristic curve analysis comparing the peripheral blood CD34+ cell count (area under the curve = 0.809) versus mononuclear cell count (area under the curve = 0.444) as criteria for adequate CD34+ cell yield ≥2 × 10^6/kg collected

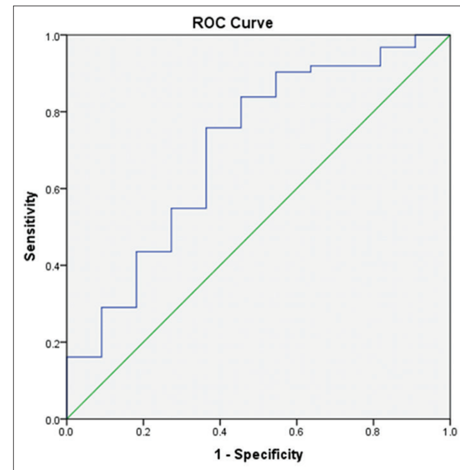

Figure 5: Receiver operating characteristic curve analysis showing mononuclear cell dose estimated using flow cytometry (area under the curve = 0.704) for predicting adequate CD34+ cell yield ≥2 × 10^6/kg collected

smear and flow cytometry methods (3.8 vs. 4.8 × 10^8/kg BW, $P = 0.047$). The ROC analysis selected a MNC yield with cutoff of above 3.4 × 10^8/kg for flow cytometry data to achieve >2 × 10^6 CD34+ cells/kg collected with 75% sensitivity and 64% specificity [Figure 5].

Discussion

The present study observed a significant difference in linearity for MNC counts estimated by peripheral smear and flow cytometry methods. Estimating an accurate count of hematopoietic stem cells is considered the most important step, as it helps in quantifying the dose given for transplantation purpose. The PBSC product is not only limited to the progenitor population alone but also contains monocytes, lymphocytes, erythrocytes, and platelets. Most centers enumerate CD34+ cells and MNC counts for assessing PBSC dose adequacy. Nucleated cell counts though performed are not as informative, as they count entire nucleated cells including nucleated red cells and granulocytes with variable levels of maturation, which do not contribute to hematopoietic engraftment within the nucleated cell populations.[1]

It is well established that flow cytometric enumeration of CD34+ assays in PBSC products is most preferred and it could produce results within few hours.[6] Most centers concomitantly perform MNC count along with CD34+ cell enumeration during PBSC collection. As MNC count includes all monocytic cells, lymphocytic cells, and precursors of myeloid series, centers derive these MNC fractions either from peripheral smear or hematology analyzer or flow cytometer.[1] Nevertheless, there is no uniformity in enumeration of MNC counts in these methods.

As correlation methods study only the relationship between one variable over another and not their differences, regression analysis was preferred to study the data distribution and to look for constant or proportional difference between two methods.[8] The peripheral smear and flow cytometry methods had a moderate positive correlation level ($r = 0.52$), but with a significant deviation from the linearity in regression methods, as shown in

Figure 3. There was a significant systematic difference, that flow cytometry method constantly under- or overestimates MNC count enumeration compared to peripheral smear method ($P = 0.04$) in the study.

Flow cytometry provides results in a consistent manner unlike manual differentials which are time consuming and rely on subjective interpretations.[4] The differences in our study may be due to the reasons stated above and in peripheral smear method; a 200-cell count was performed by one pathologist. The Clinical and Laboratory Standards Institute had recommended a mean of 200-cell counts by two observers to generate a reference differential count in method for comparisons or validations.[5] As manual differential counting requires standardization and flow cytometry counts thousands of cells with high degree of accuracy and precision, MNC enumeration by flow cytometry method when available seems to be an attractive alternative to peripheral smear method.

Previous studies had shown that circulating CD34+ cells significantly correlated with the number of CD34+ cells harvested and neither circulating MNC or total WBC counts had consistent predictive value for the PBSC collection.[9] Few studies had tried circulating hematopoietic progenitor cells (HPCs), an estimate by counting immature cells using Sysmex automated hematology analyzer as an alternate surrogate for PBSC harvest prediction.[8] Yu JT *et al*. predicted that circulating 60 HPCs/µl and 35 CD34+ cells/µl were estimated to be the probably cutoff cell counts for predicting optimal (CD34 $\geq 5 \times 10^6$/kg) hematopoietic stem cell collection.[8] From our results, it was clear that only CD34+ cell counts and not MNC counts in peripheral blood reliably predict the number of CD34+ cells in the harvest [Table 3]. Peripheral blood CD34 cell count of 37 cells/µl best correlated with CD34 yield of $\geq 2 \times 10^6$ in PBSC product and was comparable with other published literature.[8]

Table 2: Comparison of mononuclear count and dose in peripheral blood stem cell product by peripheral smear and flow cytometry

	Median (IQR)		*P*
	Peripheral smear	Flow cytometry	
MNC (%)	52 (41.5-64.5)	59 (49-65.5)	0.185
MNC dose (×10⁸/kg)	3.8 (2.7-5.2)	4.8 (3.1-5.9)	0.047

MNC=Mononuclear cell, IQR=Interquartile range

As single-platform assays have the potential to be more effective in reducing interlaboratory variation than dual-platform techniques in CD34 enumeration, determining MNC count through CD34 enumeration plot by flow cytometry shall provide instant value with ease of procedure.[3,6] As absolute MNC count in harvest was an easily accessible and alternative variable to assess the adequacy of hematopoietic stem cell collection, it gains importance in centers where CD34+ cell enumeration was outsourced. The study observed a significant difference between MNC doses estimated between these two methods ($P = 0.047$) [Table 2].

We applied a ROC model using MNC dose estimated by both methods to predict the optimal CD34 dose of $\geq 2 \times 10^6$. The area under curve was relatively higher for MNC dose predicted by peripheral smear than flow cytometry. Hence, standardizing MNC estimating method by flow cytometry along with CD34 counts should minimize intra- and interlaboratory difference and shall be preferred for PBSC dosage calculations. We were able to set a limit of MNC dose at 3.4×10^8/kg that correlated well with CD34+ cell count of more than 2×10^6/kg in 70% instances, and

Table 3: Studies comparing correlation of predictors with CD34 yield (≥2×10⁶/kg) in peripheral blood stem cell product

Study	Target	Type of sample	Predictors	Correlation (*r*)	*P*
Park *et al.*[10]	CD34≥2 (×10⁶/kg)	Peripheral blood	WBC	0.02	0.81
			Monocyte count	0.21	0.01
		PBSC product	MNC yield	0.36	<0.01
			IMI channel	0.68	<0.01
Yu *et al.*[8]	CD34 (≥2×10⁶/kg)	Peripheral blood	HPC (≥20 cells/µl)		<0.01
			CD34 (≥10 cells/µl)		<0.01
Sawant and Rajadhyaksha[11]	CD34 (≥2×10⁶/kg)	Peripheral blood	CD 34 (≥50 cells/µl)	0.67	
			WBC	0.23	
			MNC	0.32	
Bhat *et al.*[7]	CD34 (≥2×10⁶/kg)	PBSC product	MNC yield (≥4×10⁸/kg)		
Present study	CD34 (≥2×10⁶/kg)	Peripheral blood	CD 34 (≥37 cells/µl)	0.483	<0.01
			MNC	−0.002	0.98
		PBSC product	MNC yield (≥3.4×10⁸/kg)	75% sensitivity; 64% specificity	

WBC=White blood cells, IMI=Immature information channel to detect immature WBC, HPC=Hematopoietic progenitor cell, PBSC=Peripheral blood stem cell, MNC=Mononuclear cell

the threshold was similar to previous results from Bhat *et al*. [Figure 5].[7]

Conclusion

Our findings confirm that there was a significant systematic difference, that flow cytometry method constantly under- or overestimates MNC count to peripheral smear method. Hence, MNC count derived from flow cytometry plot can substitute peripheral smear method for accurate MNC dose calculations in transplantation. MNC dose at 3.4 × 10^8/kg consistently predicted >2 × 10^6/kg CD34+ cells collected. We believe that our findings should help improve MNC dose estimation methods and also provide reliable information on the cutoff point for MNC dose that yields adequate CD34 dose for transplantation purposes.

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Conflicts of interest

There are no conflicts of interest.

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