AN ATTEMPT TO ESTABLISH A CUTOFF VALUE FOR PERIPHERAL BLOOD ABSOLUTE MONONUCLEAR CELL COUNT TO PREDICT THE VIABLE CD34 COUNT IN MULTIPLE MYELOMA PATIENTS UNDERGOING AUTOLOGOUS PERIPHERAL BLOOD STEM CELL TRANSPLANTATION

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Background: Autologous Peripheral Blood Stem Cell Transplantation (PBSCT) has emerged as a vital therapeutic intervention for multiple myeloma (MM) patients. However, the success of PBSCT relies on various factors, including the composition of peripheral blood cells. Specifically, the absolute mononuclear cell count in peripheral blood (AB_MNC_PB) has been identified as a potential predictor of the viable CD34 cell count (V_CD34) in MM patients undergoing autologous PBSCT. Determining this cut-off value improves autologous PBSCT effectiveness in multiple myeloma.

Objective: The aim of this study was to establish a cutoff value for AB_MNC in peripheral blood to predict V_CD34 in the peripheral blood of MM patients undergoing PBSCT. This enables decentralization of the process of PBSCT by replacing the flow cytometer (FC) with the automated hematology analyzer (AHA) or manual method.

Method: MM patients at the age of 40-65 years, and admitted to the Bone Marrow Transplant Unit in Apeksha Hospital, Maharagama were selected for the study (n=45). The results of AB_MNC_PB from AHA and V_CD34_PB from FC were obtained and, AB_MNC_PB was enumerated manually after performing differential counts (DC) counts on the day of harvesting. Statistical analysis was performed using IBM SPSS v26. First, the data were separately tested for normalization, followed by correlation bivariate analysis and, receiver operating characteristic (ROC) curve analysis to establish relationships and cutoff values for AB_MNC_PB.

Results: V_CD34_PB_FC and AB_MNC_PB enumerated from both manual and AHA showed normal distributions ($p>0.05$). Only AB_MNC_PB_AHA represented a weak positive significant ($p=0.038$) correlation with V_CD34_PB. As there was no direct strong correlation between them, a cutoff value was obtained for AB_MNC_PB using the ROC curve. Accordingly, the cutoff value of AB_MNC_PB is 7000/µL at V_CD34_PB of 92.3/µL with a sensitivity (86.7%), specificity (56.2%), and area under the curve (AUC) of 0.696 ($p=0.000$). However, AB_MNC_PB_Manual did not provide reliable results.

Conclusion: AB_MNC_PB_AHA can be used as a predictive marker to determine V_CD34_PB_FC as an alternative for FC. Furthermore, these initial findings should be validated by performing the same process with a large cohort of MM patients.

Keywords: *Multiple Myeloma; Autologous Peripheral Blood Stem Cell Transplantation; Mononuclear Cells; Flow Cytometer*
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INTRODUCTION

Autologous Peripheral Blood Stem Cell Transplantation (PBSCT) has emerged as a vital therapeutic intervention for multiple myeloma (MM) patients. However, the success of PBSCT relies on various factors, including the composition of peripheral blood cells. Specifically, the absolute mononuclear cell count in peripheral blood (AB_MNC_PB) has been identified as a potential predictor of the viable CD34 cell count (V_CD34) in MM patients undergoing autologous PBSCT (Copelan, 2009).

MNCs in peripheral blood, resembling hematopoietic stem cells, have traditionally been estimated using methods such as the hemocytometer, which is less accurate (Krishnan et al.,2021). Advanced technology now employs automated hematological analyzers (AHA) and flow cytometers (FC) for MNC enumeration. However, some underdeveloped countries still rely on peripheral blood smears for estimating mononuclear cell counts (Ahmed et al., 2020; Rundgren et al., 2019). In the absence of flow cytometers, the high cost of current flow cytometric techniques for measuring CD34 cells and the lack of technical expertise pose challenges in determining the optimal time for efficient peripheral blood stem cell collection. Therefore, using AB_MNC in peripheral blood (PB) without assessing V_CD34 can minimize unnecessary blood product wastage in stem cell transplantation, even without a flow cytometer.

Numerous studies highlight the value of MNCs and CD34 as predictive markers for initiating apheresis in stem cell transplantation (Krishnamurthy et al.,2021; Bhat et al., 2019; Yu et al.,2016). To address this need, attempts have been made to establish a cutoff value for AB_MNC in relation to V_CD34 cells in PB, providing accurate predictions for the optimal day of harvesting in autologous peripheral blood stem cell transplantation.

Therefore, by determining this cut-off value, clinicians can make informed decisions regarding patient selection and personalize the treatment approach, ultimately enhancing the efficacy and success of autologous PBSCT in multiple myeloma management.Additionally, this enables decentralization of the process of PBSCT by replacing the flow cytometer with an automated hematology analyzer or manual method. This facilitates a better yield in the harvest as well as optimal engraftment by analyzing only peripheral blood parameters (AB_MNC_PB) directly.

METHODOLOGY

MM patients at the age of 40-65 years, and admitted to the Bone Marrow Transplant Unit in Apeksha Hospital, Maharagama were selected for the study (n=45). The results of AB_MNC_PB from AHA (7-part Sysmex XN 1000) and V_CD34_PB from FC (BDFACS Lytic ™) were obtained and, AB_MNC_PB was enumerated manually after performing differential counts on the day of harvesting (D9). The manual DC count of 100 cells from the peripheral blood was performed using a freshly prepared modified Giemsa stain and observed

under a 40× in light microscope. Mainly focus on the cells; neutrophils, stem cells, MNCs including lymphocytes, monocytes, and myeloid precursor cells. Then from the AHA the total WBC count and MNCs including monocytes, lymphocytes, and immature granulocytes in peripheral blood (IMG) were obtained. Statistical analysis was performed using IBM SPSS v26. First, the data were separately tested for normalization, followed by correlation bivariate analysis and, receiver operating characteristic (ROC) curve analysis to establish relationships and cutoff values for AB_MNC_PB.

RESULTS

Table 1: Data normalization using SPSS (Descriptive Statistics Explore).

Shapiro-Wilk	
Parameter	**Sig.**
V_CD34_PB_AHA	0.061
AB_MNC_PB_AHA	0.079
AB._MNC_PB_Manual	0.135

The Shapiro-Wilk model test was used when the data were n<50. V_CD34_PB_FC and AB_MNC_PB enumerated from both manual and AHA showed normal distributions (p = 0.061, p =0.079, p =0.135 > 0.05 respectively). Therefore parametric analysis was performed. In the next step, Pearson correlation bivariate analysis was performed to establish correlations between V_CD34_FC and AB_MNC_PB that were obtained in manually and using an analyzer (AHA).The following table shows the correlations among parameters.

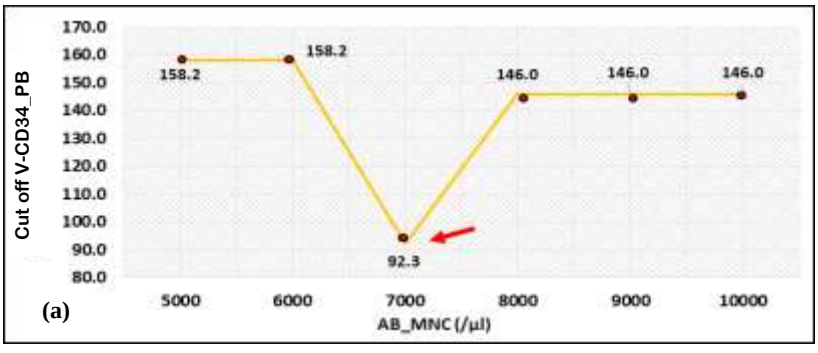
Table 2: Pearson correlation bivariate analysis of V_CD34_FC and AB_MNC_PB.

Parameter	**AB_MNC_PB_AHA**		**AB._MNC_PB_Manual**	
	r (Correlation coefficient)	Sig. (2-tailed)	r (Correlation coefficient)	Sig. (2-tailed)
V_CD34_PB_FC	0.333	0.038	0.035	0.901

The results in Table 2 revealed that AB_MNC_PB_AHA represented a significant (p<0.05) weak positive correlation with V_CD34_PB (r = 0.333). However, AB_MNC_PB_Manual did not show a reliable correlation with V_CD34_PB.

As there was no direct strong correlation between them, a cutoff value was obtained for AB_MNC_PB using the ROC curve. The cutoff values for V_CD34_PB were established above and below the series of AB_MNC_PB values; 5000 cells/µL, 6000 cells/µL, 7000 cells/µL, 8000 cells/µL, 9000 cells/µL and 10,000 cells/µL. The two group variables were set up as, variable 0 < AB_MNC_PB value and, variable 1 ≥ AB_MNC_PB value.

The cutoff V_CD34_PB obtained by the analysis along with the corresponding sensitivity and (1-specificity) is shown in Figure 1.



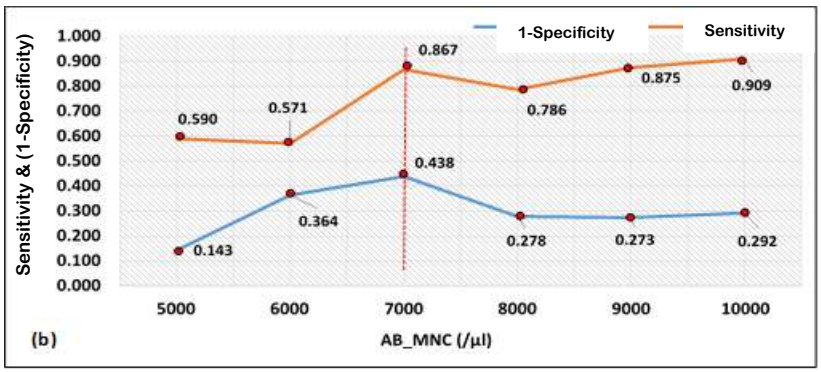


Figure 1: Receiver Operating Characteristic (ROC) curve analysis over the series of AB_MNC_PB values; (a) Cutoff V_CD34_PB values obtained for the different groups of AB_MNC. (b) Sensitivity and 1-Specify related to each group.

The optimum cut-off value of AB_MNC_PB could be considered as 7000/µL at V_CD34_PB of 92.3 cells/µL, since it shows the maximum sensitivity (86.7%) and specificity (56.2%) among all with an area under the curve (AUC) of 0.696.
Figure 2, represents the above finding more clearly when comparing AB_MNC_PB with V_CD34_ PB.

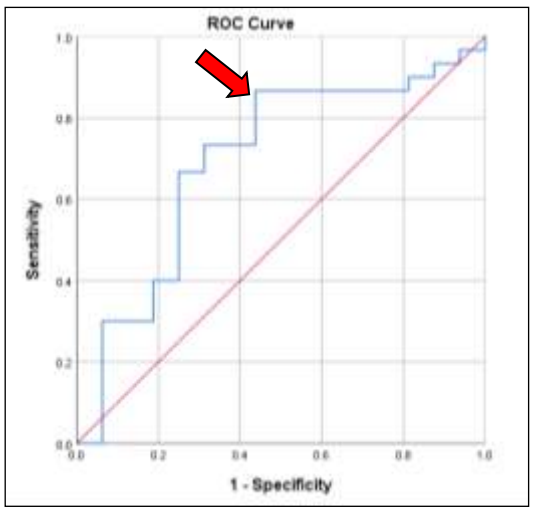


Figure 2: Receiver operating curve analysis for V_CD34_ PB (AUC = 0.696) with as, AB_MNC_PB variable 0 < 7000/µL and, variable 1 ≥ 7000/µL.

DISCUSSION

The primary objective of this study was to determine a cutoff value for AB_MNC_PB using either the AHA or manual method in relation to V_CD34_PB_FC to ensure successful peripheral blood stem cell (PBSC) collection. We focused on multiple myeloma (MM) patients who were undergoing autologous PBSCT. We selected 45 patients, aged 40-65, with a slightly higher number of males than females.
During autologous PBSCT, patients receive Cyclophosphamide and growth factors (G-CSF) to suppress the immune system and promote the mobilization of stem cells into the peripheral blood (Lisenko et al., 2017; Palumbo et al.,2014). The 9th day after mobilization is generally considered the optimal time for stem cell harvesting in routine practice (Bhat et al.,2019; Wuchter et al., 2010; Wuchter et al.,2013).
Since all parameters exhibited a normal distribution, we conducted a parametric analysis. In

routine practice, V_CD34_PB is determined using flow cytometry (FC), and a cutoff value is used to determine the optimal harvesting time. As there were only weak significant correlations between AB_MNC_PB_AHA and V_CD34_PB, we obtained a cutoff value for AB_MNC_PB_AHA using the ROC curve. The optimum level was determined to be 7000/µL for AB_MNC_PB from AHA at V_CD34_PB of 92.3/µl, as it exhibited the highest sensitivity and specificity in the acceptable range among all cutoff values.

Previous studies have suggested different cutoff values for effective PBSC collection, ranging from 10-40/µL of V_CD34 in routine practice (Lemos et al.,2018;Yu et al.,2016). In our data, with a V_CD34_PB count of approximately 90 cells/µL, we achieved a more precise prediction for maximum stem cell yield compared to the routinely used minimum cutoff values. Therefore, we supposed that we could achieve the maximum yield of harvested stem cells more precisely than the limited to minimum cut-off for V_CD34_PB that is routinely used in laboratories. Furthermore, according to Krishnan et al., (2021) the aV_CD34_PB count of 37 cells/µl correlated well with a CD34+ yield in harvested products of $\geq 2\times10^6$/kg, which is the minimum amount of V_CD34 in the harvested product responsible for optimum engraftment. Therefore, we conclude that a minimum AB_MNC_PB_AHA count of 7000 cells/µL with a V_CD34_PB count of 90 cells/µL should be sufficient for achieving effective PBSC collection ($\geq 2\times10^6$/kg). However, we were unable to derive a reliable cutoff value for AB_MNC_PB from the manual method using V_CD34_PB which may be due to an insufficient number of data points in our study.

CONCLUSIONS

AB_MNC_PB_AHA can be used as a predictive marker to determine the V_CD34_PB_FC as an alternative for FC. Accordingly, the optimum cut-off value of AB_MNC_PB could be considered to be 7000 cells/µL at a V_CD34_PB of 92.3 cells/µL. Furthermore, we anticipate that our findings will contribute to the decentralization of the autologous PBSCT process by substituting the flow cytometer with automated hematology analyzers in the long run. However, it is essential to validate these initial findings by conducting the same analysis with a larger number of multiple myeloma patients or by considering different chemotherapy drugs or mobilizing agents. It would be worthwhile to explore whether this cut-off value is applicable to post-mobilization days 8 or 7 as well, as this could help determine the feasibility of selecting day 9 as the most suitable day for stem cell harvesting.

RECOMMENDATIONS

These initial findings should be validated by performing the same process with a large cohort of MM patients. It enables improvement with the other peripheral blood parameters too and could also be used to validate the initial findings.

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