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HEMATOPOIETIC PROGENITOR CELLS AS A PREDICTIVE OF CD34$^+$ ENUMERATION PRIOR TO PERIPHERAL BLOOD STEM CELLS HARVESTING

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Background: To date, the CD34$^+$ cell enumeration has relied predominantly on flow cytometry technique. However, flow cytometry is time consuming and operator dependent. The application of the hematopoietic progenitor cells (HPCs) channel in Sysmex XE-2100, a fully automated hematology analyzer offers an alternative approach, which is with minimal sample manipulation and less operator dependent. This study evaluates the utility of HPC counts as a predictive of CD34+ enumeration prior to peripheral blood stem cells harvesting. **Materials and methods:** HPC, CD34+, white blood cell (WBC), reticulocytes (retic), immature platelet fraction (IPF) and immature reticulocyte fraction (IRF) were determined in 61 samples from 19 patients with hematological malignancies (15 lymphoma and 4 multiple myeloma patients) at Hospital Universiti Sains Malaysia (Hospital USM) who had received granulocyte-colony stimulating factor (G-CSF) and planned for autologous transplantation. **Results:** CD34$^+$ count showed strong and significant correlation with HPC. The receiver operating characteristics (ROC) curve analysis revealed that HPC count > 21.5 x 106 / L can predicts a pre harvest CD34+ count of >20 x 106 / L with sensitivity of 77%, specificity of 64% and area under the curve (AUC) of 0.802. **Conclusion:** We concluded that HPC count can be a useful potential parameter in optimizing timing for CD34$^+$ enumeration prior to leukapheresis.

Keywords: CD34$^+$ count; hematopoietic progenitor cell; flow cytometry; automated hematology analyzer; peripheral blood stem cell mobilization.

INTRODUCTION

Hematopoietic progenitor and stem cells express high levels of the cell surface glycoprotein CD34. As these cells mature and differentiate, the levels of CD34 expression will be decreased. Therefore, expression of CD34$^+$ is a defining hallmark for hematopoietic stem cells (HSCs) and progenitor cells (HPCs) in bone marrow (BM), peripheral blood (PB) and cord blood. As a result, this marker is widely used as a tag for the enumeration of these cells.[2] Peripheral blood stem cells (PBSCs) are harvested to be used in autologous and allogeneic hematopoietic stem cells transplantation.[5] For PBSCs collection, HSCs and HPCs will be mobilised from BM into the PB and further collected by leukapheresis [5]. HPC then were cryopreserved before transplantation. A crucial point during this process is to determine precisely the suitable time for HSCs to be collected.[2] Obtaining sufficient amount of PBSCs with a dose of at least 2.5-5.0 X 106/ kg CD34+ cells is recommended for reliably, rapid and successful hematological recovery.[10] Therefore, an accurate quantification of circulating CD34$^+$ stem cells is important for the stem cell laboratories to decide the optimal time for collection.

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Materials and Methods

Peripheral blood collection

A number of 3 ml of blood was collected into anticoagulant tripotassium ethylenediamine tetraacetic acid (EDTA) tubes (Vacutainer; Becton Dickinson Systems, San Jose, CA, USA). 68 samples were collected from 19 adult patients with hematological malignancies and were planned for autologous stem cell transplantation. The blood samples were stored at 4∘c until analysis. Samples were collected daily from each patient starting from the time they received granulocyte growth stimulating factor (G-CSF) for mobilization till PBSC harvesting. CD34$^+$ enumeration and hematological parameters were performed by standard flow cytometry and Sysmex XE-2100

hematology analyzer, respectively. This study was approved by the research ethics committee (Human), Universiti Sains Malaysia.

Sysmex XE-2100

Sysmex XE-2100 provides immature myeloid information (IMI) channel in which HPCs can be quantitated in selected samples. All samples were analysed using the (IMI) channel by Sysmex XE-2100. In the IMI channel, all matured white blood cells (WBC) are lysed except immature WBC. Immature WBC is resistance to the action of surfactants on the lipid components of the cell membrane. The reagent used in the IMI channel contains polyoxyethylene, a nonionic surfactant, and sulphur-containing amino acids to the cell membrane [3, 8]. Cells in the IMI channel are analysed using radio frequency (RF) and direct current (DC). The RF/DC method detects impedance changes as pulses when blood cells pass through the sensor. The RF signal conveys information about cell contents such as nuclear size and presence of granules; the DC signal reflects overall cell size (volume). This type of analysis provides detailed morphological information about each cell. When the blood sample was pre-treated with IMI reagent, immature myeloid cells can be distinguished from other cells 3. The more immature the cells, the lower the RF signal strength. Consequently, they will appear lower on the IMI scattergram.[8]

Standard flow cytometry

Samples from peripheral blood were quantitated for CD34 + cells by flow cytometric. The International Society of Hematotherapy and Graft Engineering (ISHAGE) gating strategy was used for CD34 + cell detection 9. A reagent kit containing a PE-labelled anti- CD34 monoclonal antibody was used for immunostaining CD34 + cells. Control samples were stained with a PE-labelled IgG-1 isotype. Samples were analysed in duplicate, by a single operator, using a flow cytometer. CD34 + cells were identifed and counted.[8]

Statistical analysis

Data was analyzed using Statistical Package for Social Sciences (SPSS) version 12.0. For numerical variables, means and standard deviations were calculated. For categorical variables, Frequency and percentages were calculated. For the statistical analysis, paired T-test, Pearson correlation and receiver operating characteristics (ROC) curve were used. ROC curve was used to calculate area under the curve (AUC), sensitivity and specificity of HPC count that best can predicts CD34+ count of > 20 X 10^6 / L. AUC is a test of accuracy. (1-0.9 = excellent, 0.9-0.8=good, 0.8-0.7=fair, 0.7-0.6=poor and 0.6-0.5= fail).[1,6] P value of <0.05 is considered as significant.

Results

Patient details are shown in Table 1. There were 68 samples taken from 19 adult patients with age range from 15 to 64 years old (mean = 42.8 years old).

Table 1
Patient characteristics

Variables	Frequency	Percentage
Gender		
Male	11	57.90
Female	8	42.10
Disease		
Lymphoma	15	78.90
Multiple myeloma	4	21.10
Race		
Malay	17	89.40
Chinese	1	5.30
Siamese	1	5.30

The correlation between CD34+ counts with other hematological parameters was analysed and are shown in Table 2. By Pearson correlation, $CD34^+$ count showed a strong and significant correlation with HPC and significant correlation with other parameters WBC, retic and IPF but not with IRF.

Table 2
Correlation between $CD34^+$ count and other hematological parameters using Pearson correlation (n=68)

	r	*p*
WBC	0.482	0.0001
IRF	- 0.073	0.552
IPF	0.453	0.0001
Retic	0.498	0.0001
HPCs	0.703	0.0001

Remarks: WBC = white blood cells, IRF = immature reticulocyte fraction, IPF = immature platelet fraction, RETIC = reticulocyte, and HPC = hematopoietic progenitor cells.

Receiver operating characteristics (ROC) curve analysis were generated for the samples to determine the optimal cut-off point of HPC that can predicts CD34+ count of > 20 X 10^6 / L. Sensitivity, specificity and AUC are shown in Table 3.

Table 3
Receiver operating characteristics (ROC) analysis

HPC($x10^6$L)	Sensitivity	Specificity	AUC
21.5	77%	64%	0.802

Discussion

Monitoring the timing of leukapheresis in peripheral blood stem cells (PBSC) mobilization is an important decision that requires an accurate analytical tool.[7] The enumeration of peripheral blood stem cell is important for the evaluation of stem cell mobilization. The timing of apheresis and accurate quantification of $CD34^+$ is also important for an efficient and cost effective transplantation process.[7] The prediction of adequate HPC mobilization prior to leukapheresis also plays an important role in maximizing PBSC collection while minimizing the number of leukapheresis [1]. Detection and enumeration of HPC by Sysmex system could possibly provide a standard alternative for $CD34^+$ count in comparison with flow cytometry technique. Thus, expensive and time-consuming $CD34^+$ enumerations can perhaps be minimized. HPC can be detected effectively by Sysmex XE-2100. Our result showed that HPC threshold of > 21.5 X 10^6/L can predicts a pre harvest CD34+ count of > 20 X10^6/ L with sensitivity (77%) and specificity (64%) which are slightly lower compare to those reported in previous study, HPC> 23.0 X 10^6/L with (sensitivity and specificity of 83% and 90% respectively)[1]. As such, the HPC measurements can be used to monitor the optimal point for leukapheresis.[4] Similarly, our data have shown and supported that the HPC enumeration using the Sysmex technology can be used as a guide to determine when is the best time to initiate apheresis after chemotherapy-based mobilization regimens. This can definitely reduce resource utilization.

Conclusion

Detection and enumeration of HPC in the peripheral blood could possibly provide a standard and rapid alternative for predicting the yield of stem cells collected by apheresis. We concluded that HPC count can be a useful potential parameter in optimizing timing for CD34+ enumeration prior to leukapheresis.

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