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EVALUATING THE ROLE OF MONONUCLEAR CELL COUNT IN PREDICTING THE CELL DOSE OF APHERESIS DERIVED PERIPHERAL BLOOD STEM CELLS BY CORRELATING WITH FLOW CYTOMETRY BASED CD 34 ENUMERATION

Hematology

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ABSTRACT

The high cost associated with the Peripheral Blood Stem Cell (PBSC) transplants still remains a major concern and any possible measure to mitigate the financial burden is always welcome. The engraftment of transplanted PBSCs is predicted by the enumeration of CD34+ cells, which is carried out multiple times before, during and after the PBSC harvest procedure. The study included 107 consecutive apheresis procedures performed on donors who were sent to our centre for PBSC harvest. Correlations between the number of CD34+ cells and MNC in the apheresis products were assessed using Karl Pearson's Coefficient of Correlation. The study group was divided into two categories, comprising of allogenic harvests and autologous harvests. The correlation between the stem cell product yield calculated using MNC count and CD34 count was found to be moderately significant (0.673) in case of allogenic but no statistically significant coefficient of correlation could be observed in case of autologous donors. The findings of this study suggest that obtaining the MNC count can be employed as a useful and cost-effective method of predicting the yield during the mid-cycle of the PBSC harvest procedure in case of an allogenic donor

KEYWORDS

PBSC Harvest, Stem Cell Transplant, CD34 Enumeration, MNC count

INTRODUCTION

Peripheral Blood Stem Cell (PBSC) transplants have increasingly gained importance over the past two decades, especially with advancements in apheresis platforms and improved immunosuppressive drugs(1). PBSC transplantation offers many advantages over bone marrow transplantation, most important being obviating the need of general anaesthesia and more rapid neutrophil as well as platelet engraftment(2) leading to reduction in transfusion requirements in the post-transplant period.

With passage of time, the stem cell transplant procedure is no longer restricted to the developed countries and now it is being performed even in developing countries on a regular basis.(3) The high cost associated with the entire procedure still remains a major concern and any possible measure to mitigate the financial burden is always helpful in a resource constraint set up(4). The rapid engraftment of transplanted PBSCs is predicted by the number and the quality of the hematopoietic stem cells transfused as characterized by the quantity of CD34+ cells. Before the advent of flow cytometry technique, Mononuclear Cell (MNC) count was a useful tool to calculate the quantity of haematopoietic stem cells in the collected product. Though it's predictive value for estimation of stem cell yield has been acknowledged, there is limited literature which substantiates the same(5). The cost of CD 34 enumeration by the flow cytometry technique is much higher compared to the MNC count estimation by conventional slide staining techniques. The CD 34 enumeration process may have to be carried out multiple times before, during (mid procedure) and after the PBSC harvest procedure, which further increases the financial burden on the patient. The MNC count may be employed for estimation of the yield during mid cycle of the process of PBSC harvest and provides a low-cost alternative to CD 34 enumeration in such situations and in resource poor patient category.(6)

We analysed the data from 107 PBSC collections to study the predictive value of the MNC count and CD34+ cell counts in the peripheral blood for the yield of CD34+ cells in the apheresis component. We also examined the robustness of the correlation between CD34+ cell yields and the MNC counts in a variety of clinical situations.

MATERIALS AND METHODS

Patient and donor population

The study included all the patients who were sent to our centre for autologous or allogenic PBSC harvest over a period of three years (August 2015 to July 2018). Patients undergoing allogenic PBSC transplant had an available HLA-matched, related donor. This group consisted mostly of patients having leukaemia, aplastic anaemia and thalassemia. Patients undergoing autologous transplant of PBSCs largely comprised of those with multiple myeloma and lymphomas or those cases of hematological malignancies who did not have any HLA-matched donor available. Patients characteristics are shown in Table 1.

Table 1: Patient/Donor profile in case of autologous and allogenic harvests

	AUTOLOGOUS HARVEST			ALLOGENIC HARVEST		
Underlying disease	Multiple Myeloma	Lymphoma (HL/ NHL)	Others	Leukaemia (AML/ALL /CML)	Aplastic Anaemia /MDS	Others
Number of patients	31	08	13	15	07	04
Number of apheresis procedures	45	14	22	15	07	04
Average age of donors (in years)	52.5	35.8	47.2	19.4	21.4	26.8
Age of donors (in years)	38-72	06-74	03-62	02-34	04-38	05-43

PBSC Mobilization regimen

The PBSC mobilization technique was decided based on the Institutional protocol and the disease status of the patient. For 42/52 patients who underwent autologous PBSCs were mobilized using a protocol with chemotherapy followed by G-CSF (filgrastim) injections, both to reduce the tumour burden and facilitate PBSC harvesting respectively. Plerixafor was also used in 06 of these cases who were poor mobilizers. The remaining 10 /52 patients of autologous harvest, who did not need cytoreduction and all the 26 allogeneic PBSC donors underwent mobilization following filgrastim injections alone(7). The dose of filgrastim when used in conjunction with a chemotherapy regimen for autologous harvests was 10 µg per kg, once or twice daily while that for those autologous patients and all allogenic donors treated with filgrastim alone was 16 µg per kg once or twice daily(8).

Collection

Leukapheresis was performed with a continuous flow blood cell separator (COM.TEC, Fresenius-Kabi, Germany). Venous access was established by either peripheral vein or central venous catheter. Anticoagulant, consisting of ACD-A, was infused at a ratio of 1 mL of anticoagulant to 09 mL of whole blood. Inlet flow rate was maintained at 50 to 80 mL per minute in the small-volume apheresis procedure and 80 to 100 mL per minute in the large-volume apheresis procedures. The collection rate was maintained at 1 to 2 mL per minute. The blood volume processed was around 3 × total blood volume, for all patients who underwent large volume apheresis. The apheresis kit was primed with cross matched PRBC in case of paediatric donors who weighed less than 15 kgs. The criteria for adequate PBSC collection was a target number of 2 × 10e^6 CD34+ cells per kg weight of the recipient. (9)The procedure was repeated on subsequent days; in case the yield was inadequate during first harvest.

PBSC Product Yield: CD 34 Enumeration

The enumeration of CD34+ cells in the peripheral blood and the PBSC component was performed by flow cytometric analysis using a single platform flowcytomter, FACS Calibur (Becton Dickinson, Palo Alto, USA) using lyse/no wash(LNW) technique(10). The reagents used (BD True-Count Kit) were provided by the same manufacturer.

20 microL of FITC/PE (fluorescein isothiocyanate/ phycoerythrin) and 20 MicroL of AAD (Amino Actinomycin D) dye were added to the BD True count tube by taking the tip of pipettes close to the base of the the tubes. 100 microlitre of diluted Sample was then added to the True Count tube and allowed to incubate at room temperature in dark, for 20 minutes. Aluminum foil was wrapped around the tube to protect from light. Following this, 2 mL of lysing ammonium chloride solution was added to the tube and incubated for 10 minutes in the dark. The sample was then acquired by flow cytometer within 1-hour post incubation and was kept over melting ice in the interim, till such time it was analyzed. List-mode data (LMD) was analysed using software Cell Quest Pro (Becton Dickinson, Palo Alto, USA). The CD 34 was obtained as total no of CD34 +ve cells per microliter, which was further evaluated in terms of total cell dose per kg weight of the recipient as per International Society of hemotherapy and Graft Engineering (ISHAGE) guidelines (11).

PBSC product yield: Mono Nuclear Cell (MNC)Count

Total leucocyte count of each sample was determined in a five-part haematology analyser. Differential counts were done microscopically on a well-prepared Wright stained smear of the PBSC product harvested and observed under oil immersion power (100X magnification) of a light microscope. The MNC count was expressed as a percentage of total leucocyte count in the product and further expressed in terms of MNC per kg weight of the recipient. The MNC count was also performed after taking a product sample in the mid-cycle of the stem cell harvest procedure, to get an approximate estimate of the product yield.

Statistical Analysis

For calculating the correlation between MNC count and CD34 count in the different subsets of patients, the study group was divided into two categories, comprising of allogenic harvests and autologous harvests. The statistical analysis for both the groups was carried out separately. Statistical analysis was carried out using SPSS -24 (IBM, IL, U.S.A.) software. The characteristic parameters of patients as well as apheresis components were described using summary statistics as mean values and ranges. Correlations between the number of CD34+ cells and total MNCs in all the apheresis products sampled, were assessed using Karl Pearson's Coefficient of Correlation. Significance levels (p value) was set at 0.05.

RESULTS

A total of 107 apheresis procedures for collection of PBSC product were performed during the 3-year study period at our centre. Out of the 78 donors, 26 were allogenic and 52 were autologous donors.

Amongst the allogenic donors, the mean pre-apheresis peripheral blood WBC count was 5.05 × 10e^10/ microL (range: 2.1-8.1 X 10e^10/ microL), while average donor age was 22.4 years (range: 4 - 60 years). The mean total CD 34 + cell dose in the PBSC product in terms of cells was 7.6 X 10e^6/Kg recipient body weight and in terms of MNC count was 6.14 X 10e^8 MNCs/Kg recipient body weight.

Similarly, in the category of autologous donors, the average donor age was 46.3 years (range: 9- 70 years), much older than the allogenic group. The mean pre-apheresis WBC count was 4.4 × 10e^10/ microL (range: 1.9-8.7 X 10e^10/ microL) in this category., The average cell dose of PBSC product in terms of CD 34 + cells was 2.3 X 10e^6/Kg recipient body weight and in terms of MNC count was 3.9 X 10e^8 MNCs/Kg recipient body weight.

The Coefficient of correlation between the MNC count and CD34 count was evaluated for autologous (Table 2) and allogenic (Table 3) harvests:

Table 2: Pearson's Coefficient of Correlation between MNC and CD 34 count

Type of Harvest (Sample size)	MNC Count	CD 34 count	Level of Significance (2Tailed)
Autologous (N=81)	1	0.158	0.310
Allogenic (N=81)	1	0.673	0.001*

* P value < 0.05 = Significant

Table 3: Correlation between MNC and CD34 count in case of allogenic PBSC harvest

MNC and CD34 Correlation(Allogenic)			
		MNC count	CD34 count
MNC count (Allogenic)	Pearson Correlation	1	.673
	Sig. (2-tailed)		.001
	N	26	26
CD34 count (Allogenic)	Pearson Correlation	.673	1
	Sig. (2-tailed)	.001	
	N	26	26

DISCUSSION AND CONCLUSION:

The average donor age was significantly higher in case of autologous harvests (mean: 46.3 years) compared to that of allogenic harvests (mean: 22.4 years). The higher age of donors in case of autologous harvest can be attributed to the underlying disease profile, which tend to occur more with the advancing age(9). In case of allogenic harvests, the donors were mostly healthy young adults and few children. This was also reflected in the average cell dose obtained after each procedure, which was far more in case of healthy allogenic donors (7.6 X 10e^6/Kg) compared to that of autologous harvests (2.3 X 10e^6/Kg).

The correlation between the stem cell product yield calculated using MNC count and CD34 count was found to be moderately significant (0.673) in case of allogenic donors with p value = 0.001, but no statistically significant coefficient of correlation could be observed in case of autologous donors. The difference in the correlation between these two groups can be attributed to the fact that most of the times, the bone marrow of an autologous donor has already been exposed to various chemotherapy/radiotherapy cycles before the stem cell transplant is planned and various other atypical cell populations maybe present in the product harvested from a patient of leukaemia or lymphoma. On the other hand, the product harvested from a healthy donor lacked these important confounding factors and hence the stem cell yield (CD34 count) correlated better with the MNC count of the product. The results obtained in case of autologous harvest was similar to that obtained in the study conducted by Yu et al(12) however, their study population consisted mostly of autologous donors and did not evaluate allogenic PBSC harvests separately.

The findings of this study suggest that obtaining the MNC count can be employed as a useful and cost-effective method of predicting the yield during the mid-cycle of the PBSC harvest procedure in case of an allogenic donor. This can be a valuable aid when the flow cytometry laboratory is not co-located near the apheresis centre and a considerable time is use wasted to get the results of the CD 34 enumeration. However, as the correlation between the two methods is only moderate, the MNC count maybe used just as a guiding tool during the procedure regarding any alterations in the numbers of

apheresis cycles or a re-harvest on the successive day. and only the CD34 enumeration, as specified by ISHAGE guidelines should be employed for calculating the pre-apheresis donor counts and final PBSC yield in the product.

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