

Comparison of Peripheral Blood CD34+ Concentration, Colony-Forming Units Granulocyte-Macrophage and Mononuclear Cells in Leukapheresed Product for the Prediction of Peripheral Blood CD34+ Cell Yield Harvest

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Abstract

The aim of this study was to evaluate reliability of parameters which may be used to guide peripheral stem cell harvests in cancer patients prior to myeloablative chemotherapy. Each leukapheresed product was analysed for CD34-positive (CD34+) cell count, mononuclear cell (MNC) count and the number of colony-forming units granulocyte-macrophage (CFU-GM). Each patient's peripheral blood (PB) taken before leukapheresis was analysed for CD34+ concentration. We evaluated whether the CD34+ yield from leukapheresis correlated with any of the three parameters. A total of 119 procedures were performed in 33 patients. The yield of CD34+ cells by leukapheresis correlated weakly but significantly with the peripheral blood CD34+ cell count ($R = 0.4$, $P < 0.05$), the MNC cells ($R = 0.4$, $P < 0.05$), and CFU-GM ($R = 0.4$, $P < 0.05$). When a PB CD34+ count of $50 \times 10^6/L$ was used as a cut-off value, the accuracy for prediction of adequate leukapheresis ($>1 \times 10^6$ CD34+ cells/kg) was 78%.

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Introduction

High dose chemotherapy with stem cell rescue is increasingly used in treating patients with malignant conditions that are refractory to conventional chemotherapy. Peripheral blood stem cell (PBSC) is replacing bone marrow as a source of stem cells for haematopoietic reconstitution. Haematopoietic progenitor cells are mobilised from the bone marrow into the peripheral blood with growth factors, with or without chemotherapy and are then collected by leukapheresis.¹⁻³ Pluripotent haematopoietic stem cells represent fewer than 0.5% of nucleated precursors. There are no known surface antigens entirely specific for this population although CD34-positive (CD34+) cells appear to include the pluripotent stem cell population. To determine whether adequate numbers of PBSC have been harvested, various endpoints have been used, including the concentration of mononuclear cells (MNCs), colony-forming units granulocyte-macrophage (CFU-GM) and CD34+ cells. It would be beneficial to the

patient if there were predictors for the optimal day for leukapheresis so that sufficient PBSCs could be collected in one or two procedures. We have retrospectively analysed data from 33 patients who had undergone 119 leukapheresis to correlate the CD34+ cell count with CFU-GM, and MNC in the leukapheresed product and PB CD34+ concentration obtained on the same day prior to leukapheresis.

Materials and Method

Patient Selection

From February 1995 to August 1996, 33 patients with malignant conditions underwent mobilisation of PBSCs with daily subcutaneous granulocyte colony-stimulating factor (G-CSF) or granulocyte-macrophage colony-stimulating factor (GM-CSF). Doses were 600 micrograms per day, starting 4 days before leukapheresis. The patient characteristics are listed in Table I. Patients who had relapsed or metastatic disease also received conventional induction chemotherapy as well as growth

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factors for PBSC mobilisation and underwent leukapheresis after recovery from the nadir counts. All patients had an insertion of a Mahurkar pheresable line prior to the leukapheresis. Peripheral blood was taken prior to leukapheresis daily for PB CD34+. The leukapheresed product was analysed for CD34+ and MNC concentration, and cultured for CFU-GM.

Stem Cell Collection and Cryopreservation Procedure

Stem cell collection was performed with either a Fenwal CS 3000 (Baxter) or Multiple Component System 3p (MCS3p) (Hemonetics). PBSC harvesting was started on day 5 of growth factor administration or when a white blood count of $>1 \times 10^9/\text{L}$ had been reached. A total of 9 to 15 litres of blood per leukapheresis was processed at a flow rate of 50 to 60 ml/min on the Fenwal CS3000 and 4 to 6 litres on the MCS3p. The leukapheresis product of 50 to 100 ml cell suspension was mixed with the same volume of autologous plasma containing 20% dimethylsulfoxide (DMSO, Sigma). The final 100 to 200 ml cell suspension was transferred into freezing bags (Gambro Hemafreeze, GmbH, Germany) and frozen to -160°C with a computer-controlled cryopreservation device (Planer Kryo-lo). The frozen cells were transferred into the liquid phase of nitrogen and stored at -196°C .

Clonogenic Assay for Haematopoietic Progenitor Cells

The concentration of haematopoietic progenitor cells in each leukapheresis product was assessed using a semisolid clonogenic culture assay with normal leukocytes at $1 \times 10^6/\text{ml}$ as feeder layer. The culture medium used was McCoy 5A Modified (Gibco, BRL) with 20% fetal calf serum. $2 \times 10^5/\text{ml}$ mononuclear cells were plated as overlay in triplicate and incubated at 37°C and 5% CO_2 in humidified atmosphere. After 14 days, colonies were scored using an inverted microscope.

Immunofluorescence Staining and Flow Cytometry

For immunofluorescence analysis, 1×10^6 mononuclear cells were incubated for 30 minutes at 4°C with the phycoerythrin (PE)-conjugated monoclonal antibody (MAb) HPCA2 (CD34) (Becton Dickinson, Heidelberg) or IgG1-isotypic antibody control. Red blood cells were lysed using coulter lysis reagent (Q-prep system). The cells were analysed with a Coulter EPICS-Profile II, with a 2 W argon ion laser as a light source. Excitation was allowed at 488 nm and fluorescence measured at 575 nm. A forward scatter versus log side scatter was used to discriminate between the smallest lymphohaematopoietic cell population and erythrocytes or debris. The CD34+ cell population was analysed in a log FL-2 versus log side scatter. Only cells with lymphoid or lymphoblastoid characteristics (low side scatter) were counted as CD34+ cells. The percentage of CD4+ cells

was read off from the Coulter.

Statistical Methods

The CD34+ concentration of the leukapheresis product was correlated with the MNC concentration, CFU-GM and the PB CD34+ concentration using Spearman correlation coefficient. The linear relationship was shown by the best fit linear graphs (Figs. 1 to 3). The data were analysed using the SPSS computer program. The accuracy (percentage of correct classification), positive predictive value and negative predictive value of the PB CD34+ concentration to predict a target of greater than $1 \times 10^6 \text{ CD34+}/\text{kg}$ with each leukapheresis was calculated by the following equations:

$$\text{Accuracy} = [(b + c)/(a + b + c + d)] \times 100\%$$

$$\text{Positive predictive value (PPV)} = [c/(c + d)] \times 100\%$$

$$\text{Negative predictive value (NPV)} = [b/(a + b)] \times 100\%$$

where

a = Number of collections below specified PB CD34+ and above target

b = Number of collections below specified PB CD34+ and below target

c = Number of collections above specified PB CD34+ and above target

d = Number of collections above specified PB CD34+ and below target

Results

Since February 1995, the Department of Medical Oncology, Singapore General Hospital has treated patients with myeloablative chemotherapy with autologous peripheral stem cell rescue harvested by leukapheresis. A total of 33 patients underwent 34 mobilisation procedures and 119 leukapheresis procedures. The patients' characteristics are listed in Table I. One patient had a second mobilisation one year after the first for relapsed disease. The median number of leukapheresis procedures per patient was 3 (range 2 to

TABLE I: PATIENT CHARACTERISTICS

Total number of patients	33
Sex	
Male	15
Female	18
Age (y)	
Median	42
Range	16 to 62
Diagnosis	
Malignant lymphoma	12
Breast cancer	12
Multiple myeloma	3
Sarcoma	1
Peripheral neuroectodermal tumour	1
Germ cell tumour	1
Acute lymphoblastic leukaemia	1

TABLE II: RESULTS OF LEUKAPHERESIS PROCEDURES AND PERIPHERAL BLOOD CD34+ CONCENTRATION

Leukapheresis procedures	119
Number of leukapheresis per patient	
Median	3
Range	2 to 5
CD34+ concentration per procedure ($\times 10^6/\text{kg}$)	
Total number analysed	113
Median	0.92
Range	0.1 to 5.76
MNC concentration per procedure ($\times 10^8/\text{kg}$)	
Total number analysed	113
Median	1.96
Range	0.52 to 5.52
Number of CFU-GM per procedure ($\times 10^4/\text{kg}$)	
Total number analysed	111
Median	3.11
Range	0.01 to 129.10
Peripheral blood CD34+ concentration ($\times 10^6/\text{L}$)	
Total number analysed	79
Median	27.15
Range	3.00 to 214.00

MNC: mononuclear cell; CFU-GM: colony-forming units granulocyte-macrophage

TABLE III: PERIPHERAL BLOOD CD34+ CONCENTRATIONS AND ACHIEVEMENT OF TARGET CD34+ YIELD OF $1 \times 10^6/\text{kg}$ FROM A SINGLE LEUKAPHERESIS

PB CD34+ concentration ($\times 10^6/\text{L}$)	Number of leukapheresis		Accuracy (%)	PPV (%)	NPV (%)
	Above target*	Below target*			
<10	1	2	34	33	67
>10	25	51			
<20	3	19	53	40	86
>20	23	34			
<30	6	38	73	57	86
>30	20	15			
<40	12	45	75	64	79
>40	14	8			
<50	13	49	78	76	79
>50	13	4			

* Target of greater or equal to $1 \times 10^6 \text{ CD34+}/\text{kg}$

PB: peripheral blood; PPV: positive predictive value; NPV: negative predictive value

5). Peripheral blood CD34+ concentration was taken only prior to 79 procedures.

The median CD34+, MNC and CFU-GM concentrations are listed in Table II. The harvested CD34+ concentration showed a moderate but significant correlation with the MNC concentration ($R = 0.4$, $P < 0.001$) and the number of CFU-GM ($R = 0.4$, $P < 0.001$) in the leukapheretic product (Figs. 1 & 2). The moderate and positive relationship between harvested CD34+ concentration with that of the PB CD34+ was demonstrated by

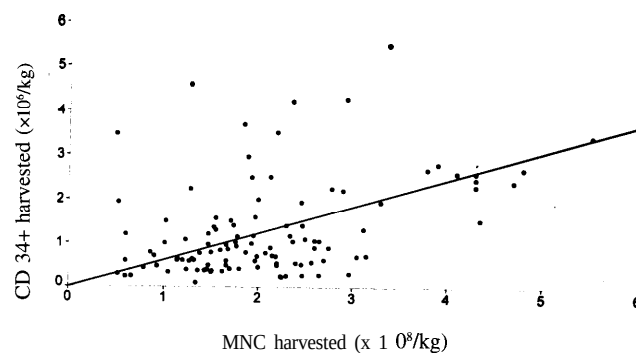


Fig. 1. Correlation analysis by linear regression between the harvested CD34+ cells and mononuclear cell (MNC) in the leukapheretic product ($R = 0.4$, $P < 0.001$).

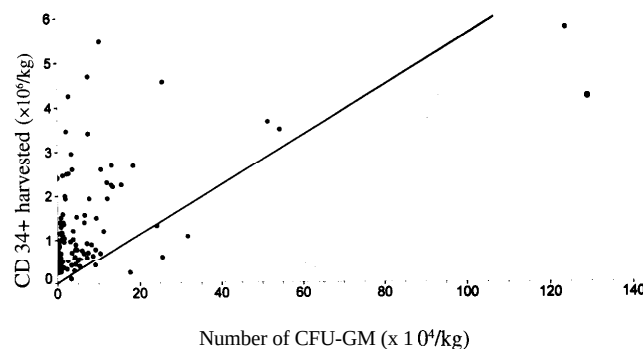


Fig. 2. Correlation analysis by linear regression between the harvested CD34+ cells and colony-forming units granulocyte-macrophage (CFU-GM) in the leukapheretic product ($R = 0.4$, $P < 0.001$).

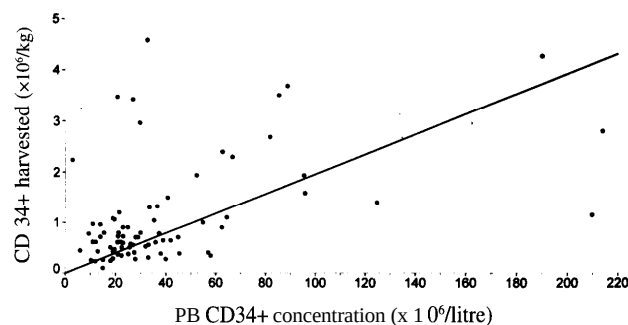


Fig. 3. Correlation analysis by linear regression between the harvested CD34+ cells and peripheral blood CD34+ concentration ($R = 0.4$, $P < 0.001$).

coefficient of $R = 0.4$ ($P < 0.001$) (Fig. 3). The accuracy, positive predictive value (PPV) and negative predictive value (NPV) of PB CD34+ for predicting a yield of $1 \times 10^6 \text{ CD34+}/\text{kg}$ are listed in Table III for different peripheral blood CD34+ concentrations. Using a cut-off value of $50 \times 10^6 \text{ PB CD34+}/\text{L}$, the accuracy was 78%, PPV was 76% and NPV was 79%.

Discussion

Using peripheral stem cell rescue (PSCR) alone following high dose chemotherapy has been shown to be superior to marrow with a shorter engraftment period and equivalent to marrow in long-term haematopoietic

reconstitution.^{4,5} There is conflicting data on the value of CD34+ cells, MNC and CFU-GM as surrogate markers for engraftment and correlation with each other. This may be due to variation in technique of analysis, different methods of mobilisation of PBSC, the myeloablative regimen used, and the use of post-infusion growth factors. Studies that have shown harvested CD34+ cells to be the best predictor of rapid engraftment found that a minimum dose with a range of 1 to 5×10^6 CD34+ cells/kg was required.^{5,6} CFU-GM has been shown to correlate with CD34+ cells/kg, harvested and engraftment in some studies⁵ and not others.⁶ Although MNC and CFU-GM showed a significant correlation with harvested CD34+ cell concentration in our study, this was a moderate relationship only.

Because of limited availability of leukapheresis facilities and to optimise usage, there is a need for a method to predict the probable CD34+ cell/kg yield on a given day since it would enable identification of the most appropriate day for maximal harvest. This would also minimise the number of procedures for the patient to reach an adequate collection of CD34+ cells. Peripheral white blood cell (WBC) has been used in previous studies to determine when leukapheresis should be commenced.^{7,8} However in our study, there was no linear relationship between the peripheral WBC and the CD34+ cell yield. CFU-GM concentration would not be a useful predictor because of the period required to culture these cells. Measuring bone marrow CD34+ cell content in a steady state has been shown to identify patients with a low or high probability of collecting a large number of PBSC.⁹ This was not confirmed in another study, instead PB CD34+ cells in the steady state were shown to identify patients at risk of poor collection.

We have demonstrated that there is a correlation between PB CD34+ on the day of harvest and the CD34+ cell yield. The likelihood of achieving 1×10^6 CD34+ cells/kg was greatest for PB CD34+ concentration greater or equal to $50 \times 10^9/L$. When the PB CD34+ was below this threshold, 79% of the leukapheresis procedures did not achieve a harvest of 1×10^6 CD34+ cells/kg. Our results from 33 patients suggest that PB CD34+ taken prior to leukapheresis may be helpful in predicting the yield of CD34+ and a cut-off value of $50 \times 10^9/L$ may be the optimal value for prediction of outcome of the leukapheresis.

The results of our study are in agreement with 2 previous studies that have shown that mobilised PB CD34+ concentration either preceding or on the day of leukapheresis had highly predictive value for CD34+

cell yield.^{10,11} However, the optimal cut-off value was different. Both showed that 20×10^6 PB CD34+ cells/L was associated with equal or greater than 2×10^6 CD34+ cells/kg per leukapheresis. The difference in cut-off values may be due to interlaboratory variation, mobilisation therapy and patients' diagnosis and prior therapy. It would therefore be prudent for respective centres to evaluate the correlation of PB CD34+ and CD34+ cell yield from leukapheresis as well as the cut-off value of PB CD34+ that would give a minimum of 1×10^6 CD34+ cells/kg.

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