Hematopoietic Progenitor Cells (HPC) and Immature Reticulocytes Evaluations in Mobilization Process: New Parameters Measured by Conventional Blood Cell Counter

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Monitoring the timing of leukapheresis in peripheral blood stem cells (PBSC) mobilization is an important clinical decision that requires an accurate analytical tool. The present study assessed hematopoietic progenitor cells (HPC) and immature reticulocyte fraction (IRF) counts provided by a routine automated blood counter as potential parameters for predicting the appropriate time for harvesting. The HPC and IRF values were compared with white blood cell (WBC) and CD34⁺ cell counts obtained by flow cytometry in 30 adult patients with hematological malignancies undergoing PBSC mobilization. It was observed that there was a significant correlation between HPC counts and CD34⁺ cells in peripheral blood counts ($r = 0.61$, $P = 0.0003$) and

between the number of HPC and CD34⁺ cells collected by leukapheresis ($r = 0.5733$, $P = 0.0009$). Comparing HPC, IRF, WBC, and CD34⁺ cells parameters as a sign of hematological recovery showed that the raise in immature reticulocytes counts preceded the increase of WBC ($P = 0.0002$), HPC ($P = 0.0001$), and CD34⁺ ($P = 0.0001$) cells in peripheral blood counts. According to our results, HPC and IRF parameters may be integrated into clinical protocols to evaluate the timing of leukapheresis. IRF, as previously demonstrated in bone marrow transplantation, is the earliest sign of hematopoietic recovery in mobilization process. *J. Clin. Lab. Anal.* 20:149–153, 2006.

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INTRODUCTION

The development of successful hematopoietic stem cell transplantation as a therapeutic and research modality has revolutionized patient care in hematology and oncology, and has provided a curative method for some diseases including cancer, genetic and autoimmune disorders. Hematopoietic stem cells are collected by leukapheresis after mobilization with high-dose chemotherapy and/or hematopoietic growth factors; this process increases the number of circulating hematopoietic stem cells (1,2). The aim of peripheral blood stem cell harvesting is to collect the target number of stem cells (usually $>2 \times 10^6$ CD34⁺ cells/kg) in a minimum number of apheresis procedures.

Some studies have been performed in order to define the exact time to commence apheresis. Many reports advocate starting collections on a fixed day after the

onset of growth factor application in the steady-state mobilization. Peripheral blood mononuclear cell count, white blood cell (WBC) count, and immature reticulocytes count have been tested for determining their ability to predict the outcome of the stem cell harvest (3–5). The immunological analysis of CD34⁺ cells is currently considered the best method for identifying hematopoietic and progenitor cells and also the most precise marker to guide timing peripheral blood stem cells (PBSC) apheresis. However, it is an expensive technique and it

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requires technical equipment and well-trained technicians (6).

A new method has been introduced recently: hematopoietic progenitor cells (HPC) have been identified by an automated hematology analyzer, by analyzing the immature myeloid information channel (IMI) (3,7).

The aim of this study was to investigate the value of HPC counting obtained by an automated whole blood counter in predicting the optimal harvesting time of PBSC. In addition, the immature reticulocyte fraction (IRF) counts were evaluated following the mobilization process.

MATERIAL AND METHODS

Patients

The study involved a prospective analysis over a 12-month period of 33 adult patients seen at the Haematology and Blood Transfusion Center of the University of Campinas, Brazil. Patients were affected by hematological malignancies and underwent PBSC mobilization in order to perform autologous PBSC transplantation. Three patients (two with Hodgkin's disease and one with non-Hodgkin's lymphoma) died during the mobilization process. Consequently, 30 patients were studied (Table 1).

PBSC Mobilization

Patients were mobilized by chemotherapy and growth factors treatment. Patients received cyclophosphamide (CY) at a dose of 4–7 g/m², and one day after starting chemotherapy, they received a subcutaneous administration of growth factors of granulocytes and monocytes (GM-CSF) at a dosage of 5 µg/kg per day until the last leukapheresis PBSC collection.

TABLE 1. Patient characteristics and data of mobilization process

Diagnosis	Patients (n = 30)
HD	4
NHL	10
MM	16
Blood cell counts	Median (range)
IRF (%)	20.3 (2.1–42.4)
HPC (µL)	53.53 (1.0–233.0)
CD34 ⁺ cells in PB (µL)	121.56 (0.79–625.81)
CD34 ⁺ cells collected (10 ⁶ /kg)	12.15 (0.08–78.06)

HD, Hodgkin's disease; NHL, non-Hodgkin's lymphoma; MM, multiple myeloma; PB, peripheral blood; IRF (%), quantity of immature reticulocytes on the day of harvest; HPC (µL), quantity of hematopoietic progenitor cells determined by XE-2100 on the day of harvest; CD34⁺ cells in PB (µL), quantity of stem cells in peripheral blood determined by flow cytometry on the day of harvest; CD34⁺ cells collected (10⁶/kg), total of stem cells collected by leukapheresis.

PBSC Collection

In order to collect more than 5.0×10^6 CD34⁺ cells/kg, leukapheresis began when the WBC count reached 1.0×10^9 /L and CD34⁺ cells count was ≥ 10 cells/µL after the nadir. Based on the number of CD34⁺ cells collected, patients were considered as "good" mobilizers when more than 5×10^6 cells/kg were collected, and "medium and poor" mobilizers when only $2\text{--}5 \times 10^6$ cells/kg and under 2×10^6 cells/kg could be collected, respectively (8).

WBC, HPC, and IRF Determinations

A hematological automated analyzer, Sysmex XE-2100 (Sysmex Corporation, Kobe, Japan), was used to determine WBC, HPC, and IRF. Counting peripheral blood cells was performed daily from the first day after starting chemotherapy until the last leukapheresis collection. Blood samples were collected in Vacutainer tubes, using K₃EDTA as an anticoagulant (BD Vacutainer, Sao Paulo, Brazil), and all samples were processed within 4 hr of venesection.

The automated analyzer uses flow cytometry with red semiconductor laser technologies to qualify WBC. Side scatter intensity measures cell volume and forward light scatter reflects inner cell complexity. On the other hand, the technology used to quantify HPC consists of using a reagent capable of separating the immature cells from the ripe ones through the difference of cell membrane composition in agreement with the degree of maturity. Subsequently, cells are detected by radio frequency (RF), which supplies information on the cellular content and by direct current (DC), which classifies the cells by size.

Reticulocyte analysis is based on fluoro-flow cytometry and red semiconductor laser technologies. RNA content is analyzed by a patented fluorescent dye with polymethine, a membrane permeable basic dye that binds to RNA. The distribution cytogram separates red blood cells and three different reticulocytes maturity classes: low fluorescence rate (LFR), medium fluorescence rate (MFR), and high fluorescence rate (HFR) according to RNA content. IRF is obtained based on the calculation $\text{MFR} + \text{HFR}$.

PBSC Enumeration

PBSC were determined by flow cytometry as previously described by Marques et al. (8). Monoclonal antibodies were obtained from Becton Dickinson Immunocytometry Systems (San Jose, CA, USA). PBSC quantification was performed using the antibody anti-CD14 conjugated with isothiocyanate (FITC) to exclude myeloid/monocytic cells in order to prevent the

contamination of anti-CD34/phicoeritrin (PE)-positive population, defined as CD14 negative and presenting a low side scatter. All data were collected and analyzed in the FACScalibur flow cytometry equipment (Becton Dickinson), using Cell Quest software. CD34⁺ cells were measured in peripheral blood samples and in leukapheresis yield.

Hematological Recovery

Peripheral blood cell counts, including WBC, HPC, CD34⁺ cells, and reticulocytes counts were performed daily during the whole mobilization process. Hematological recovery was defined as the first day with an HPC count ≥ 10 cells/ μ L, CD34⁺ cells count ≥ 10 cells/ μ L, and WBC count $\geq 1.0 \times 10^9$ /L. An abrupt increase in the IRF, as previously studied in bone marrow transplantation recovery, was also tested as a possible predictor for the increase of circulating hematopoietic stem cells during the mobilization process (9).

Statistical Analysis

Variables (HPC, CD34⁺ cells, and IRF) were transformed in $\log_{10}$. Student's *t*-test for independent samples was used for comparing HPC values obtained by two different software versions. Correlations were tested by Pearson's correlation coefficient. In the description of the relationship between CD34⁺ cells and HPC, a model of linear regression was adjusted and the equation was obtained by the method of least squares. The Student's *t*-test for paired samples was used to compare the recovery day between different parameters. The level of statistical significance was set at 5% (P -value < 0.05) for all analyses.

All calculations were made using the Statistical Analysis System (SAS) System for Windows, version 6.1 2 (SAS Institute Inc, 1989–1996, Cary, NC, USA).

RESULTS

For whole analyzed samples ($n = 30$), HPC was positively correlated with the number of CD34⁺ cells measured in peripheral blood sample ($r = 0.61$, $P = 0.0003$) and leukapheresis yield ($r = 0.5733$, $P = 0.0009$) (Fig. 1).

As a predictor of the hematological recovery, the increase in the number of immature reticulocytes evidenced by IRF value preceded the increase of WBC counts ($P = 0.0002$), HPC counts ($P = 0.0001$), and CD34⁺ cells in peripheral blood counts ($P = 0.0001$) (Table 2; Fig. 2). However, there was no correlation between the day of recovery of IRF with the total CD34⁺ cells collected by leukapheresis ($r = -0.028$, $p = 0.885$).

Fig. 1. Analysis of regression line between HPC and CD34⁺ cells in peripheral blood (μ L)/CD34⁺ cells (10^6 /kg) show: (a) CV = 41.4%, $R^2 = 37.4\%$, SD = 0.55, $P_{\text{Intercept}} = 0.9375$, $P_{\text{Slope}} = 0.0003$; (b) CV = 42.9%, $R^2 = 32.9\%$, SD = 0.57, $P_{\text{Intercept}} = 0.0001$, $P_{\text{Slope}} = 0.0009$. HPC, hematopoietic progenitor cells; CD34⁺ cells in PB, quantity of stem cells in peripheral blood; CD34⁺ cells (10^6 /kg), total of stem cells collected by leukapheresis.

TABLE 2. Hematological recovery data of mobilized patients

Parameters	Media	SD	Days of recovery		
			Median	Minimum	Maximum
WBC	13.30	2.79	13.0	9.0	20.0
IRF	11.70	3.15	12.0	7.0	18.0
HPC	13.97	3.06	14.0	9.0	22.0
CD34 ⁺ cells	14.67	2.92	14.5	10.0	22.0

WBC, white blood cells; HPC, hematopoietic progenitor cells; IRF, immature reticulocyte fraction; CD34⁺ cells, peripheral blood stem cells; SD, regression standard deviation.

Figure 3 shows the evolution of the mobilization process during the studied parameters of a patient with multiple myeloma.

DISCUSSION

Monitoring the timing of PBSC apheresis is an important clinical decision which requires an accurate analytical tool. The optimal timing of the apheresis

Fig. 2. Box-plots related to days of hematological recovery of the parameters used in the determination of the timing of leukapheresis. WBC, white blood cells; HPC, hematopoietic progenitor cells; IRF, immature reticulocyte fraction; CD34⁺ cells, peripheral blood stem cells.

Fig. 3. An MM patient's evolution during the mobilization process from the second day after starting chemotherapy to the last leukapheresis collection. MM, multiple myeloma; IRF (%), quantity of immature reticulocytes on the day of harvest; WBC, white blood cells; HPC (μL), quantity of hematopoietic progenitor cells determined by XE- 2100 on the day of harvest; CD34⁺ cells in PB (μL), quantity of stem cells in peripheral blood determined by flow cytometry.

procedure can improve the mobilization process by reducing drug therapy and occurrences of side effects. It can also cut the cost. In addition, nonresponders to cytokines can be identified and they may avoid unsuccessful apheresis with consequent material and labor savings (3,6). Currently, flow cytometry represents the standard laboratory test to determine the timing of apheresis as well as the quality of stem cells products. However, it is an expensive technique and requires technical equipment and well-trained technicians (6,10,11). In this study, we evaluated a new approach for monitoring the optimal timing of leukapheresis: the measurement of HPC in patients with different hematological malignancies using an automated hematology analyzer.

The first HPC evaluation was conducted in 1995, and the results showed a significant correlation between the percentage of CD34⁺ cells identified by conventional

flow cytometry and HPC detected by hematology equipment (12). Peng et al. (13) studied HPC and CD34⁺ cells in mobilization process and observed a correlation between those parameters that would indicate the timing for apheresis (13). The possibility of other cells different from CD34⁺ being included in the IMI channel was reported by Pollard et al. (14), and the HPC counting was suggested as a screening test, prior to conventional CD34⁺ cells determination (14). The precise nature of the progenitors measured in the HPC channel is unclear. The dual fluorescence analysis of different maturation stages of CD34⁺ cells (CD34⁺/CD38⁺, CD34⁺/HLADR⁺, CD34⁺/CD14⁺, and CD34⁺/CD33⁺) could not identify a CD34⁺ subpopulation that could explain differences between HPC and CD34⁺ counts (15). However, the same authors demonstrated a good correlation between HPC counts and CD34⁺ cells in the group of patients with lymphoma and multiple myeloma and concluded that HPC measurement could be used for timing of apheresis in these patients. Our results are in concordance with published data, because a significant correlation between HPC counts, CD34⁺ cells in peripheral blood counts, and the number of CD34⁺ cells collected by leukapheresis was observed (15,16).

Several parameters have been tested for monitoring PBSC during mobilization process. The absolute neutrophil, WBC and the total mononuclear cell counts, or the recovery of platelets for determining the timing of leukapheresis have not proved to be totally reliable (5). In the present study, for the first time, the percentage of immature reticulocytes was tested for determining the optimal timing of leukapheresis and predicting the outcome of a stem cell harvest. As in bone marrow transplantation recovery, IRF early response was the best predictor of the hematological recovery when compared with the WBC, HPC, and CD34⁺ cells counts in peripheral blood (9). Our results show that the immature reticulocytes count may be used for determining the timing of leukapheresis, as the increase of the IRF preceded the CD34⁺ cells counting by two days in most of the patients. However, the day of the recovery of IRF was not a determinant of the total CD34⁺ cells collected by leukapheresis.

We conclude that HPC count and IRF can be applied for monitoring mobilization process. Furthermore, the results showed, for the first time, that an abrupt increase in the IRF value is the earliest indicator of the hematological recovery and of the success of the mobilization (more than 5×10^6 PBSC/kg collected). Quantification of immature reticulocytes may be integrated into clinical protocols as a good predictor of the optimum time for stem cell harvesting.

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