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# Large-volume leukapheresis in pediatric patients: pre-apheresis peripheral blood CD34+ cell count predicts progenitor cell yield

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Abstract

*Background and Objective.* In children it is very important to optimize PBPC harvesting and to reduce the number of leukaphereses per patient. The value of pre-apheresis peripheral blood CD34+ cell concentration as a predictor of PBPC yield was studied in 23 pediatric patients with hematologic and non-hematologic malignancies in order to optimize duration of PBPC collection.

*Design and Methods.* The patients underwent 25 stem-cell mobilization episodes with G-CSF alone and 40 large-volume leukapheresis procedures. Peripheral blood and harvested CD34+ cell concentrations were analyzed by means of flow cytometry.

*Results.* Using linear regression analysis, a highly significant correlation was found between the peripheral blood CD34+ cell count and the CD34+ cells/kg patient body weight collected on the apheresis day ($r=0.826$, $p=0.0001$). The results indicate that at least $1\times10^6$/kg CD34+ cells can be harvested during one leukapheresis procedure in all patients if the pre-apheresis blood CD34+ cell count is ≥ 30/μL and a CD34+ cell target of ≥ $5\times10^6$/kg is achieved in at least 80% of patients if this value is ≥50 CD34+ cells/μL processing a median blood volume of 438.7 mL/kg (range, 207-560) over a median time of 232.5 minutes (range, 182-376).

*Interpretation and Conclusions.* Our results suggest that the number of CD34+ cells harvested in a single large-volume leukapheresis can be predicted from the measurement of peripheral blood CD34+ cell concentration on the collection day.


Key words: large volume leukapheresis, CD34+ cells, children

Large-volume leukapheresis (LVL), which involves the processing of at least three blood volumes in a single session,[1,2] has been safely and easily performed in pediatric patients.[3] However, optimization of peripheral blood progenitor cell (PBPC) collection remains a problem to be solved especially in pediatric patients undergoing PBPC mobilization by recombinant human granulocyte colony-stimulating factor (G-CSF) alone, mainly due to the high inter-patient PBPC mobilization variability. Most studies suggest that PBPC yield can be predicted from preapheresis peripheral blood CD34+ cell counts,[4-7] but a recent report has found a relatively poor correlation between pre-apheresis peripheral blood CD34+ cell counts and total CD34+ cells harvested.[8] The goal of this study was to ascertain whether the PBPC yield can be predicted from pre-apheresis peripheral blood CD34+ cell counts in order to optimize the duration of each apheresis procedure in children undergoing PBPC mobilization by G-CSF alone.

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## Design and Methods

### *Patients*

Between July 1997 and December 1997, 23 consecutive patients with a variety of malignancies underwent progenitor cell collection for autologous transplantation (Table 1). The patients underwent 40 aphereses after 25 mobilizations. Patients were subjected to PBPC mobilization and collection after obtaining parental consent.

### *PBPC mobilization*

PBPC were mobilized by G-CSF alone (Filgrastim®; Amgen, Thousand Oaks, CA, USA) at a dose of 12 μg/kg/day given subcutaneously for 4-5 days before starting PBPC apheresis.

### *Access*

Access was obtained via a central venous catheter (Arrow-Howes, Reading, PA, USA) with offset lumens. An attempt was made to use the same catheter for blood sampling, harvesting, conditioning and the post-transplant period.

### *Anticoagulation*

A solution of 500 mL ACD-A and 5,000 IU of preservative free heparin was infused at a whole blood to anticoagulant ratio of 30:1 for anticoagulation. Platelet clumping was prevented by adding ACD to the collection bag.

*Table 1. Patients' characteristics (no.=23).*

| *Characteristics* | | *No. of patients* |
|---|---|---|
| Age (years) | | |
| Median | 11 | |
| Range | 1-16 | |
| Sex (male/female) | | 13/10 |
| Weight (kg) | | |
| Median | 37.5 | |
| Range | 11-89 | |
| Prior chemotherapy duration (months) | | |
| > 6 | | 11 |
| < 6 | | 12 |
| Prior radiotherapy | | |
| Yes | | 10 |
| No | | 13 |
| Diagnosis | | |
| Acute lymphoblastic leukemia | | 3 |
| Acute myeloblastic leukemia | | 3 |
| Non-Hodgkin's lymphoma | | 2 |
| Hodgkin's disease | | 1 |
| Ewing's sarcoma | | 5 |
| Neuroblastoma | | 2 |
| Central nervous system tumor | | 6 |
| Wilms' tumor | | 1 |

### *PBPC collection*

All collections were performed using a COBE Spectra (COBE BCT Inc., Lakewood, CO, USA) blood cell separator under manual control, 12 h after the G-CSF dose. For patients who weighed less than 25 kg, the extracorporeal line was primed with a unit of packed red blood cells before the procedure. The packed red blood cells were crossmatched and compatible with the patient, leukocyte-depleted and irradiated. The collection rate was adjusted to 0.9 mL/min, with a manual maintenance of the collection line to obtain a product hematocrit of 2%. The optimum target number of CD34+ cells to collect was $5\times10^6$/kg, although a minimum of $1\times10^6$/kg was considered sufficient for transplantation as we have previously reported.[9] Additional leukaphereses were performed in 4 patients to obtain more PBPC for tandem transplants. The final product containing 10% DMSO was frozen using a computer controlled freezer and stored in liquid nitrogen at –196° C.

### *Sampling*

Pre-apheresis blood samples were obtained, using EDTA as the anticoagulant, for white blood cell (WBC), mononuclear cell (MNC) and CD34+ cell counts. Each apheresis product was analyzed for CD34+ cell, and MNC content.

### *MNC count*

MNC counts in apheresis products were performed by a Technicon H2 blood cell analyzer.

### *CD34+ cell analysis*

The CD34+ cell content in both the peripheral blood and the apheresis products were assessed by means of flow cytometric analysis using an Epics Elite flow cytometer (Coulter Corporation, FO, USA). For the estimation of CD34+ cells, $1\times10^6$ nucleated cells were incubated for 20 minutes at 4°C with anti-human CD34 class III epitope PE-conjugated monoclonal antibody (Immunotech, Coulter Corporation, FO, USA). For CD34+ characterization, an amorphous gate was drawn in a dual parameter cytogram of CD34-PE and orthogonal light scatter. Only cells with low forward and side scattering properties (lymphoid blast region) were accepted as CD34+ cells. A minimum of 200,000 events were analyzed. Absolute numbers of CD34+ cells were calculated by multiplication of the total amount of nucleated cells in leukapheresis products with the percentage of CD34+ cells.

### *Statistical methods*

A software program (Statview 4.0, Abacus Concept, Berkeley, CA, USA) was used for statistical analysis. Correlations were determined using linear regression. Results were considered significant if the *p* value was < 0.05 for a two-tailed t-test.

## Results

The pre-apheresis peripheral blood values of CD34+ cells on the day of harvest ranged from 2.0/µL to 170 /µL (median 28/µL). In all cases the peripheral blood CD34+ cell concentration on the collection day was related to the CD34+ cell content in the leukapheresis products (r=.825, p=.0001). Significant correlations were found between peripheral blood CD34+ cell concentration on the day of collection and circulating mononuclear cells on the same day (r=.659, p=.0001) and duration of previous chemotherapy (r=.605, p=.0001). No significant correlation was observed between peripheral blood CD34+ cell concentration on the day of collection and patient age (r= 0.315), body weight (r=0.267), gender (r=0.350) or diagnosis: hematologic malignancies vs non-hematologic malignancies (median 20/µL vs 31.5/µL, p=ns).

The characteristics of the aphereses are given in Table 2. A median number of $1.99\times10^6$ CD34+ cells /kg patient body weight (range, 0.15-18.5) was collected per apheresis. There was a highly significant correlation between CD34+ cells $\times10^6$/kg collected and the pre-leukapheresis peripheral blood CD34+ cell count as shown in Figure 1. A correlation was also found between pre-leukapheresis MNC and CD34+ cell yield (r=0.645, p= 0.0001).

To obtain more than $1\times10^6$ CD34+ cells /kg (median 4.69; range,1.45-18.5) with a single apheresis in 100% of patients, an absolute number of circulating

*Table 2. Apheresis characteristics.*

| *Characteristics* | |
|---|---|
| Number of aphereses | 40 |
| Median | 2 |
| Range | 1-4 |
| Blood volume processed (mL/kg) | |
| Median | 303.5 |
| Range | 146.5 -560 |
| TBV processed (fold-basis) | |
| Median | 4.2 |
| Range | 3.0 - 8.9 |
| Inlet flow (mL/min) | |
| Median | 50 |
| Range | 10 - 95 |
| Time | |
| Median | 246 |
| Range | 170 - 376 |

*TBV; total blood volume.*

$CD34^+$ cells ≥30/μL would be necessary. A threshold number of $5\times10^6$ $CD34^+$ cells /kg necessary for rapid engraftment after myeloablative therapy corresponded to a pre-leukapheresis concentration of 54.2 $CD34^+$ cells/μL. This $CD34^+$ cell target was achieved in 80% of patients if a ≥ 50 $CD34^+$ cells/μL concentration existed (median, 8.52; range, 2.85-18.5) processing a median blood volume of 438.7 mL/kg (range, 207-560) with a median time of 232.5 min (range, 182-376).

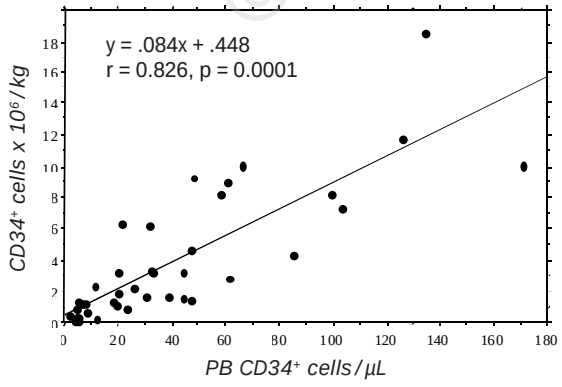


*Figure 1. Correlation between absolute peripheral blood (PB) $CD34^+$ cell count on harvest day and $CD34^+$ cells collected.*

## Discussion

PBPCs harvested after G-CSF mobilization provide rapid and long-term hematopoietic recovery after myeloablative therapy.[10] As the leukapheresis procedure is expensive, time-consuming and involves several technical and psychological problems, especially in pediatric patients, it is important to reduce the number and duration of procedures. Large-volume leukapheresis is a feasible method for collecting a large number of PBPC for autologous transplantation in children.[2] This method reduces the number of leukaphereses needed to achieve a target PBPC count for transplantation. As $CD34^+$ cell measurement by flow cytometry is a reliable and rapid way of quantifying PBPC, our purpose in this study was to determine whether PBPC yield can be predicted from pre-apheresis $CD34^+$ cell concentration. The study included 23 pediatric patients, with different malignancies, who underwent mobilization by G-CSF alone.

Confirming results from Kanold *et al.*,[11] we have found a wide inter-individual variation in $CD34^+$ cell concentration on the harvest day. This $CD34^+$ cell concentration did not correlate with patient age, body weight or underlying disease. However, the duration of previous chemotherapy had a negative influence on this parameter. Based on linear regression analysis, the quantity of circulating pre-apheresis $CD34^+$ cells, expressed as an absolute number, is a reliable parameter for predicting $CD34^+$ cell yield. The results indicate that collection of at least $1\times10^6$/kg $CD34^+$ cells is achieved in all patients if this value is ≥ 30 $CD34^+$ cells/μL and a $CD34^+$ cell target of ≥ $5\times10^6$/kg is achieved in at least 80% of patients if this value is ≥ 50 $CD34^+$ cells/μL. We also found a correlation between MNC count and $CD34^+$ cell yield. This fact is not surprising since $CD34^+$ cells are contained within the MNC fraction. However, MNC pre-leukapheresis concentration is a less predictor of PBPC harvest than $CD34^+$ cell concentration, reflected by their respective correlation coefficients. These findings are in accordance with those from several reports in adults.[5-7] However, there is little information on pediatric patients regarding this issue. Leibundgut *et al.*[12] reported that the number of circulating $CD34^+$ cells/μL prior to apheresis correlated strongly with and was predictive of the number of collected CFU-GM cells/kg collected from 20 children after chemotherapy + hemopoietic growth factor mobilization. Recently, Kanold *et al.*[11] found that the day-of-collection peripheral blood $CD34^+$ cell concentration was related to leukapheresis product $CD34^+$ cell and CFU-GM content, and measurement of circulating $CD34^+$ cells allowed the prediction of harvest magnitude in 42 pediatric patients after G-CSF mobilization.

In summary, our results taken together with those previously published strongly suggest that in pediatric patients the PBPC yield can be predicted from circulating $CD34^+$ cell concentration on the day of

collection. A CD34+ cell target of ≥ $5\times10^6$/kg is obtained in the majority of patients if a concentration of ≥ 50 CD34+ cells/μL is present, with a large-volume leukapheresis procedure lasting less than four hours.

*Contributions and Acknowledgments*

*MAD was the main investigator and designed the study. He wrote the article, managed statistical data and along with MGV reviewed the literature. MGV cared for the patients before mobilization and during apheresis. FGS and RL performed the CD34 cell analyses. JLV and LM reviewed the article and were the main co-ordinators in, respectively, the laboratory and clinical areas.*

*Disclosures*

*Conflict of interest: none.*

*Redundant publications: no substantial overlapping with previous papers.*

*Manuscript processing*

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