Prediction of peripheral blood progenitor cell collection by measurement of CD34+ cells in the preapheresis blood

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Summary

Preapheresis quantification of circulating CD34+ cells (CD34) may be used to predict peripheral blood progenitor cell (PBPC) yield in subsequent leukapheresis products. In this study, we retrospectively analyzed the results obtained from 113 PBPC leukapheresis procedures performed on two groups of patients (49 adults and 21 children) with malignant blood diseases. CD34 were analyzed by flow cytometry on blood samples taken immediately prior to leukapheresis and on the resulting harvest products. In both adults and children, preapheresis CD34/ml peripheral blood highly correlated with leukapheresis CD34/kg patient body weight (r=0.94, $P < 0.001$). Regardless of the patient's diagnosis or mobilization regimen, preapheresis blood samples containing $\geq 34.6 \times 10^3$ CD34/ml in adults and $\geq 17.6 \times 10^3$ CD34/ml in children provided more than 2×10^6 CD34/kg in 81% and 88.5% in the leukapheresis products respectively. Our results confirm that quantification of CD34 in preapheresis blood samples on the day of harvest is a reliable guide for predicting progenitor cell number in subsequent harvest products.

Résumé

La quantification des cellules CD34+ (CD34) du sang périphérique peut être utilisée pour prédire le rendement des cellules souches du sang périphérique (CSSP) dans les produits de cytaphérèse. Dans cette étude, nous avons analysé rétrospectivement les résultats obtenus à partir de 113 procédures de cytaphérèse réalisées chez deux groupes de patients (49 adultes et 21 enfants) atteints de diverses hémopathies malignes. Les cellules CD34 ont été quantifiées par cytométrie de flux sur des échantillons de sang prélevés immédiatement avant la cytaphérèse et sur les produits résultants de celle-ci. Dans des deux groupes de patients, les cellules CD34/ml dans le sang périphérique montrent une forte corrélation avec les produits de cytaphérèse exprimés en CD34/kg (r=0.94, $P < 0,001$). Indépendamment de la pathologie et du régime de mobilisation, les échantillons du sang périphérique contiennent plus de $34,6 \times 10^3$ CD34/ml chez les adultes et plus de $17,6 \times 10^3$ CD34/ml chez les enfants. Ces chiffres fournissent plus de 2×10^6 CD34/kg dans 81% et 88,5% des produits de cytaphérèse chez les adultes et les enfants respectivement. Nos résultats confirment que la quantification des cellules CD34 dans les échantillons du sang périphérique, le jour de la cytaphérèse, est un guide fiable pour prédire le nombre de CSSP dans les produits de cytaphérèse.

1 Introduction

Myeloablative therapy followed by peripheral blood progenitor cells (PBPC) infusion is now being widely used to treat patients with various malignant disorders[1-3]. This is because PBPC harvested by leukapheresis provides rapid hematopoietic recovery after conditioning regimens[4,5]. PBPC represent an interesting alternative to bone marrow (BM) as source of hematopoietic progenitor cells. One of the advantages of PBPC compared with autologous BM harvesting is avoidance of general anesthesia[6,7]. Additional benefits include a shorter hospital stay, decreased use of antibiotics and less transfusion needs[1,7-11]. From previous reports[3,11-14], the numbers of infused CD34 should range from 1 to 5 $x10^6$/kg patient body weight to achieve engraftment after myeloablative therapy. However, a higher threshold quantity of up to 8 $x10^6$ CD34/kg has also been proposed by some investigators[15,16]. The efficiency of PBPC mobilization varies greatly. Some patients have more than sufficient progenitors in a single leukapheresis while others mobilize poorly and require several apheresis procedures. Leukapheresis content of CD34 depends mostly on several factors, including timing of mobilization, the type of chemotherapy used for mobilization, the cytokine (s) added during the post chemotherapy phase to enhance the mobilization and their dosage, the duration of blood processed during the apheresis and the bone marrow rescue of the patient. The main difficulty was to establish the best time to start the CD34 collection after PBPC mobilization into the general circulation with standard chemotherapy and/or cytokines. Numerous parameters have been attempted to predict PBPC in leukapheresis products. So far, CD34 measurement in peripheral blood before leukapheresis procedures appears to be the most reliable and the most convenient guideline for PBPC harvesting[17-24]. Relatively little information is available on the comparative situation between adults and children regarding the predictive value of preapheresis CD34. To assess this question, we retrospectively analyzed the results recorded over a period of 3 years on 113 preapheresis blood samples and the corresponding leukapheresis products collected from both adults and children with malignant disorders.

2 Materials and methods

2.1 Patients and PBPC mobilization

From January 1995 to December 1997, 49 adults (22 male /27 female) and 21children (11 boys/10 girls) were treated with high dose chemotherapy and autologous PBPC transplantation. The patient diagnoses were non-Hodgkin's lymphoma (n=22), Hodgkin's disease (n=1), multiple myeloma (n=18), chronic lymphocytic leukemia (n=3), chronic myelogenous leukemia (n=3), chronic myelomonocytic leukemia (n=1), acute lymphocytic leukemia (n=11), acute myelogenous leukemia (n=3), neuroblastoma (n= 5) and other solid tumors (n= 3). The median age of the adult patients was 53 years (range 17 - 67) with a median weight of 69 kg (range 50 - 103). The median age of the children patients was 9 years (range 1 - 16) with a median weight of 23 kg (range 10 - 66).The decision to use human granulocyte colony stimulating factor (rhG-CSF; Neupogen®, Amgen, USA) alone or with chemotherapy for mobilization of PBPC was

based on clinical considerations. Patients received 5 µg/kg of rhG-CSF daily infused alone, subcutaneously, for 4 consecutive days. PBPC were collected on day 4 to 5 following rhG-CSF administration. In patients receiving chemotherapy and rhG-CSF, leukaphereses were performed between days 10 and 15. rhG-CSF was administered from day 5 post chemotherapy and until PBPC harvesting began. Chemotherapy regimens varied with the patients and the pathology type. Patient characteristics and PBPC mobilization schemes are summarized in Table 1.

Samples

Peripheral blood samples taken immediately prior to each apheresis procedure into EDTA anticoagulant are named preapheresis samples (PS). Samples taken from the leukapheresis products into ACD anticoagulant are named leukapheresis samples (LS). All samples were stored at room temperature and processed within 1 hour of collection for total white blood cell count (WBC) and CD34 analysis.

CD34 analysis

CD34 were analyzed by flow cytometry (FACS) as previously described[25]. Briefly, the cells were stained with PE-conjugated anti-CD34 moAbs (HPCA-2, Becton Dickinson, Germany). Mouse anti-IgG1 was used as irrelevant isotype control. After red cell lysis and two washes, the cells were counterstained with propidium iodide (Becton Dickinson) before being analyzed in an ELITE flow cytometer (Coulter). A minimum of 100,000 events was recorded for each sample acquisition.

Clonogenic assays

Granulocyte-macrophage colony forming units (CFU-GM) were assayed in GEM1a methyl cellulose semi-solid medium (GEM-CNRS, Villejuif, France). Samples were adjusted to 2×10^6 nucleated cells/ml with HBSS calcium and magnesium-free (Gibco, UK). Aliquots containing 10^5 cells were seeded in triplicate on a 35 mm culture dish (Nunc, Denmark). Filtered supernatant from the human bladder carcinoma cell line 5637 was used as source of growth factors. CFU-GM were scored after 14 days of incubation at 37°C in a humidified atmosphere with 5% CO_2.

Leukapheresis

Leukapheresis for PBPC collection started when the WBC was $\geq 2 \times 10^6$ /ml. All leukapheresis were performed using a COBE Spectra (Cobe Laboratories, Quedgeley, UK). In pediatric patients at least two blood-volumes are processed in each leukapheresis. In adult patients 10 to 20 litres of blood were processed for each leukapheresis as previously described[25]. The

leukaphereis procedure was repeated on the following days during maximum 6 consecutive days until the total number of CD34+ cell reached at least 2 x 10^6/kg.

Statistical analysis

Data were analyzed for statistical correlation using the NCSS 6.0.21 software. Results are expressed as median ± range. A P value ≤ 0.05 was considered statistically significant.

3 Results

Independently of the CD34 yield in PS, all of the patients mentioned in this study underwent apheresis procedure when their total WBC count was ≥2 x 10^6 /ml. A total of 113 leukapheresis procedures were performed, with a median of one collection per adult (range 1-6) and two (range 1-4) per child. From our results, CD34 analysis of PS by FACS gave a very good indication of the PBPC collection quality. Indeed, a highly significant correlation was found between the absolute numbers of circulating CD34/ml in the PS and the CD34/kg in the LP as indicated by the correlation coefficient of r = 0.96 (P < 0.001) for adults (Figure 1A) and r = 0.94 (P < 0.001) for children (Figure 1B). Regression analysis indicated that the minimum threshold of 2 x10^6 CD34/kg may be attained with a single leukapheresis if the CD34/ml in the PS on the day of collection were ≥34.6 x10^3 in adults and ≥17.6 x10^3 in children. Regarding to these values, 81% of the harvest products from adults contained ≥ 2 x 10^6 CD34 /kg. The corresponding value reached 88.5% in children. A similar high correlation (Figure 2A and B) was found between CD34/kg and CFU-GM/kg in the two groups of patients (r = 0.80; P<0.001 for adults, and r = 0.95 for children; P < 0.001).

4 Discussion

After myeloablative therapy, threshold values of infused PBPC providing successful hemopoietic recovery should range from 1 to 5 x10^6 CD34/kg patient body weight[3,13,25]. It is crucial to attain such values with the minimum of apheresis procedures in order to minimize patient discomfort and overall handling cost. In agreement with previous studies[19-21,26], we confirm that flow cytometry analysis of CD34 in preapheresis circulating blood is a reliable and rapid method for predicting the number of collected CD34/kg. Recently, Leibundgut *et al* [23] reported that circulating CD34 + blood cells predict CFU-GM/kg of leukapheresis products in children. Prediction of harvested CD34/kg has been mentioned by some investigators[27,28]. Marson *et al*[27] suggest that the leukapheresis should be initiated when the count of CD34+ elements is at least 45/µl in PS; while Urban *et al*[28] indicate that the optimal time for collecting PBPC is when a PS contains at least 30 circulating CD34+/µl. Theses differences can reflect difficulties in standardizing the quantitative assay used for CD34+ cell measurement. It is important that each center establishes their own PS CD34 level for PBPC harvests and uses

published data as a guide. From our children results, ≥ 17.6 $x10^3$ CD34/ml of blood in preapheresis samples yielded at least 2 x 10^6 CD34/kg in the harvest products. This level is approximately twice lower than that of adults and may be due to the fact that hematopoeisis in children is more active. Otherwise, according to the results of our study, the level of 17.6 $x10^3$ CD34/ml determined in pediatric patients is two times lower than that reported by other authors[27,28]. This can be explained in part by differing mobilization techniques and by the differences in methodology for quantification of CD34+ cells. However, it is important to note that in a proportion of patients a sufficient progenitor cell yield cannot be obtained even by repeated leukapheresis procedures. In agreement with previous reports[24,29,30], our study also shows a close correlation between CD34 and CFU-GM results. However, the predictive role of CFU-GM assays is of limited value because the results can be obtained only after 14 days of collection. Nevertheless, the fact that CFU-GM/kg correlated well with CD34/kg strengthens the complementary role between these two tests. Because of its reliability, rapidity and simplicity in performance, flow cytometry CD34 analysis is particularly indicated for preapheresis monitoring to determine the best time to start PBPC harvesting. But this test only gives quantitative information's on the CD34 cell population yield in the leukapheresis products. Proliferative capacity of the collected progenitor cells is obtained with the more time-consuming clonogenic assay, which may directly reflect their ability of engraftment in vivo. Hence, CD34 testing by FACS should be performed in conjunction with CFU-GM assays in order to obtain both quantitative and qualitative data on the harvested PBPC.

In conclusion, our results show that CD34 count in preapheresis blood samples by flow cytometry on the day of harvest was highly predictive for CD34 yield in the leucapheresis products in both adults and children.

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Number of patients	70
adults /children	49/21
Sex	
adults (male/ female)	22/27
children (boy/ girl)	11/10
Median age (range), years	
adults	51(17-67)
children	9(1-16)
Diagnosis	
NHL	22
HD	1
MM	18
CLL	3
CML	3
CMML	1
ALL	11
AML	3
NBL	5
Other solid tumors	3
Mobilization schemes	
rhG-CSF alone	24
rhG-CSF plus chemotherapy	46

***Abbreviations*:** NHL= non-Hodgkin's lymphoma ; HD= Hodgkin's disease ; MM= multiple myeloma ; CLL= chronic lymphocytic leukemia ; CML= chronic myelocytic leukemia ; CMML= chronic myelomonocytic leukemia ; ALL= acute lymphocytic leukemia ;AML= acute myelogenous leukemia ; NBL= neuroblastoma ; rhG-CSF= recombinant human granulocyte colony stimulating factor.

Table 1: Patient characteristics and mobilization schemes

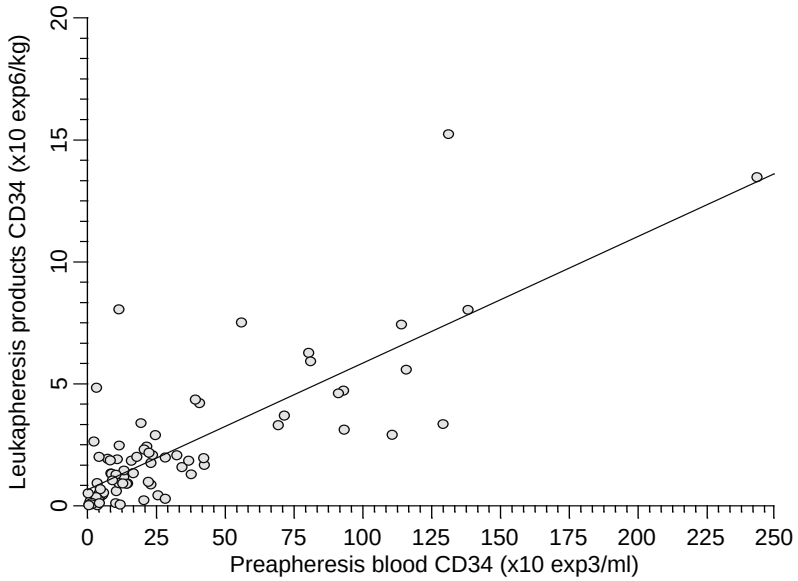

Figure 1(A): Correlation between preapheresis blood CD34 and leukapheresis products CD34 in adults patients (r=0.96 ; *P*<0.001).

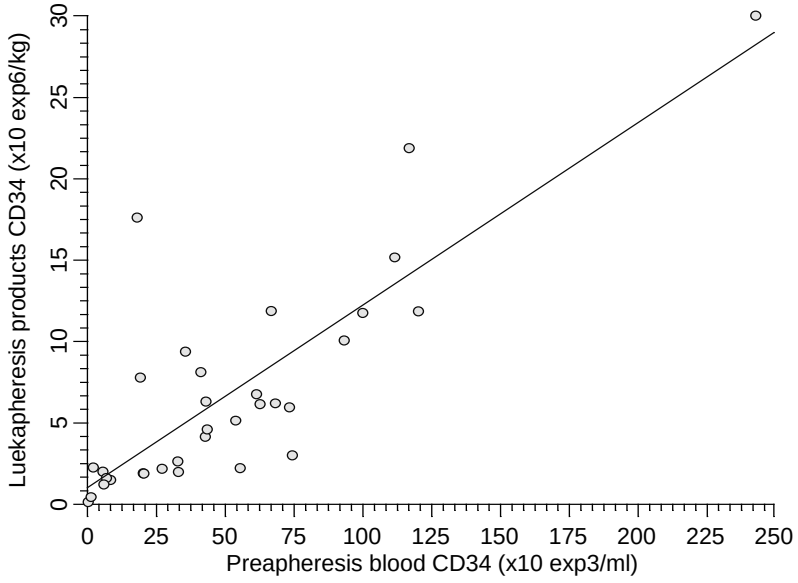

Figure 1(B): Correlation between preapheresis blood CD34 and leukapheresis products CD34 in children patients (r=0.94 ; *P*<0.001).

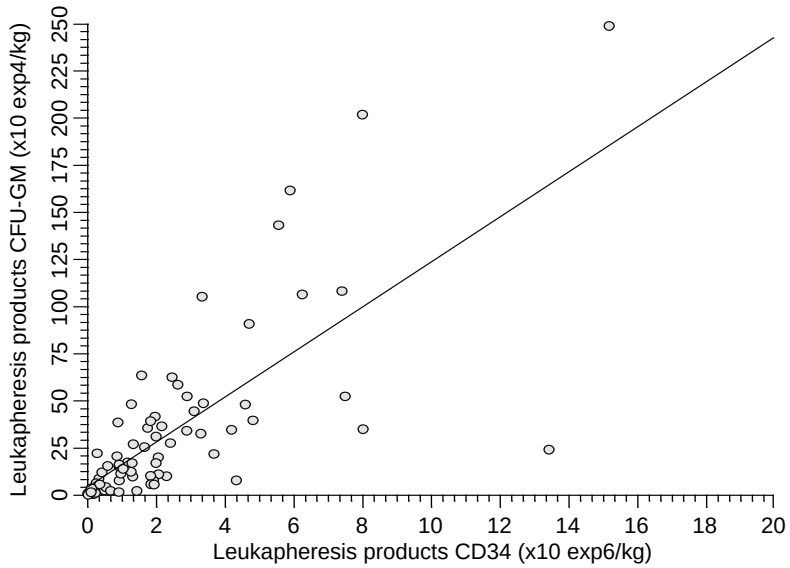

Figure 2 (A): Correlation between leukapheresis products CD34 and CFU-GM in adult patients (r=0.80 ; P<0.001).

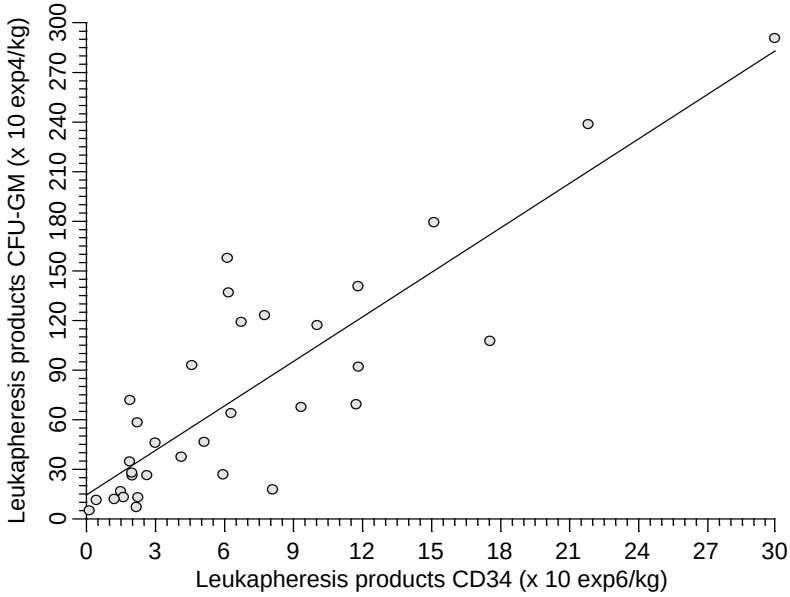

Figure 2 (B): Correlation between leukapheresis products CD34 and CFU-GM in children pateints (r=0.95 ; P<0.001)