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Original Research

Analysis of predictive factors of complete blood count for a high-yield hematopoietic stem cell apheresis collection

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ABSTRACT

Introduction: In medical practice, quantification of peripheral blood CD34+ cells is a method of choice to calculate apheresis yield. Nevertheless, other predictive factors have been evaluated. The objective of this study was to investigate the association between CD34+ cells and number of apheresis collections from patients undergoing autologous bone marrow transplantation based on complete blood count parameters. **Methods:** Retrospective analysis of 113 patients of the Department of Pediatric Oncology of Hospital de Clínicas de Porto Alegre (HCPA) who had autologous bone marrow transplantation between 2004 and 2011 and underwent mobilization with granulocyte-colony stimulating factor in combination or not with chemotherapy. The following parameters were assessed: Total leukocyte count, platelets, hemoglobin, absolute neutrophil count, lymphocytes, monocytes, and immature granulocytes (IG). Statistical tests were used for asymmetric variables. **Results:** The correlation between CD34+ $\times$ 10^6/kg and leukocyte count ($r_s = 0.082$; $P = 0.394$), platelets ($r_s = 0.078$; $P = 0.418$), hemoglobin level ($r_s = -0.05$; $P = 0.564$), neutrophils ($r_s = 0.042$; $P = 0.665$), lymphocytes ($r_s = 0.048$; $P = 0.619$), and IG ($r_s = 0.165$; $P = 0.083$) revealed no significant result. In relation to monocytes, there was a weak but significant correlation ($r_s = 0.255$; $P = 0.007$). In addition, patients with leukocyte count higher than 30 $\times$ 10^9/L and monocyte count higher than 1.8 $\times$ 10^9/L had good collection yield. **Conclusion:** Although there was no significant association between CD34 $\times$ 10^6/kg and blood parameters, we found that leukocyte count higher than 30 $\times$ 10^9/L and monocyte count higher than 1.8 $\times$ 10^9/L may be predictive factors of efficient collection. However, these values cannot be considered absolute factors because patients with lower counts also had satisfactory collections.

KEY WORDS: Apheresis, autologous transplant, hematopoietic stem cell, CD34+ cells, mobilization

INTRODUCTION

Infusion of peripheral hematopoietic stem cells (HSCs) can restore hematopoiesis after myeloablative therapy. During apheresis, there is mobilization of HSCs with growth factors granulocyte-colony stimulating factor (G-CSF) alone or in combination with chemotherapy and inhibitors of cytokine receptors, which promote circulation of large amount of cells into peripheral blood [1-4]. Apheresis is very convenient and the method of choice for autologous bone marrow transplantation (BMT), used to treat various hematologic neoplasias [2-5]. Apheresis has many advantages when compared with autologous BMT whose source of HSCs is the bone marrow, such as reduced period of patient's aplasia, thus decreasing the number of transfusions of blood components and the length of hospital stay [5,6].

There is no consensus on the amount of progenitor cells to be infused to achieve adequate cell recovery. However, a minimum of 2-5 $\times$ 10^6 CD34+ cells per kilogram in adults have shown good results [7,8]. Recent studies have suggested that most patients who receive more than 5 $\times$ 10^6 CD34+ cells per kilogram show faster and long-lasting cell recovery. There has been much speculation on predictive factors for an effective yield. That is, parameters analyzed before the procedure that could indicate collection efficiency regarding the total number of CD34+ cells. Several different parameters have been suggested as possible predictive factors for apheresis yield, such as total leukocyte count [9], absolute number of lymphocytes [10], platelet count [11], and percentage of circulating immature granulocytes (IG) [12]. In medical practice, peripheral blood CD34+ cell count is accepted as the best indicator to start apheresis collection in patients

undergoing autologous BMT [13]. However, besides being highly specialized and having a high cost, the technique used to calculate CD34+ cell count is not universally available and takes longer to be conducted. Therefore, parameters that are more accessible have been evaluated as potential predictive factors [14]. Therefore, the objective of the present study was to identify a parameter of the complete blood count (CBC) of the candidates to autologous BMT that may be a potential predictive factor of apheresis yield, thus enabling the reduction of costs and patient exposure to growth factor.

MATERIALS AND METHODS

Patients

We conducted a retrospective analysis of 113 patients of the Department of Pediatric Oncology of Hospital de Clínicas de Porto Alegre who underwent autologous BMT from 2004 to 2011. Patients who had autologous BMT until 2004 underwent mobilization with chemotherapy regimen (cyclophosphamide, irinotecan, and etoposide) combined with G-CSF. After 2004, only G-CSF was used in all patients.

CBC

We used peripheral blood samples collected in K_2EDTA anticoagulant to perform CBC that were assessed by Pentra DX ABX® and Sysmex XE 2100® hematology analyzers (Sysmex Corporation, Japan). Results of CBC conducted up to 24 h before the apheresis procedure was included in the study. The hematology analyzers showed quality control, intra- and inter-assay with a good daily performance.

Mobilization Regimen

Mobilization was performed with G-CSF at a dose of 6 mg/kg twice a day (every 12 h) with a collection starting on the fourth day. For patients that had mobilization performed with G-CSF and chemotherapy, growth factor was administered on the fifth day after treatment, and collection started when total leukocyte count reached a minimum of 10×10^9/L.

Progenitor Cell Collection

Peripheral hematopoietic progenitor cell collection was performed using apheresis equipment (Cobe® or Baxter®) with specific kit processing 3-4 patient blood volumes. For patients weighing lower than 30 kg, a red blood cell (RBC) priming was used. After collection, the cells were cryopreserved with dimethyl sulfoxide at −80°oC. The apheresis equipment showed quality control, intra- and inter-assay with a good daily performance.

CD34+ Cell Quantification

CD34+ cells in bone marrow or mobilized peripheral blood were quantified using an FACSCalibur flow cytometer (Becton Dickinson Biosciences, San Jose, CA, USA) using double platform for absolute quantification. Cell viability was determined by trypan blue. CD45 and CD34 monoclonal antibodies (mAbs) conjugated with the fluorochromes fluorescein isothiocyanate (FITC) and phycoerythrin (PE) were used, respectively, according to the ISHAGE guidelines [15].

After adding the 10 μL mAbs in 1×10^6 cells, were homogenized and incubated for 15 min away from light, and RBCs were lysed and washed with phosphate buffered saline (PBS). Subsequently, cells were resuspended with PBS, and 200.000 events were acquired in the flow cytometer in order to ensure the acquisition of at least 100 CD34+ events. Results were obtained and analyzed using the *CellQuest* software (Becton Dickinson Biosciences, San Jose, CA, USA).

Statistical Analysis

Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) version 16.0 (SPSS Inc., Chicago, IL, USA). Descriptive analysis of the variables was expressed as median and interquartile range. The association between variables was assessed using the Spearman's correlation coefficient. The independent samples were compared using the Kruskal-Wallis test, and one-way analysis of variance for asymmetric samples was evaluated using the Mann–Whitney test.

Ethical Aspects

The present study was approved by the Research Ethics Committee of the Graduate and Research Group of Hospital de Clínicas de Porto Alegre in accordance with the International and National Guidelines and Standards, particularly the Resolution 196/6 and complementary resolutions of the Brazilian National Health Council.

The researchers signed a medical liability form and committed to protect patients' privacy.

RESULTS

We evaluated 113 patients. Their clinical diagnoses were: Neuroblastoma [43], Hodgkin's lymphoma (HL) [20], Ewing's tumor [13], medulloblastoma [10], Wilms' tumor [9], acute myeloid leukemia (AML) [6], pineoblastoma [3], germ cell tumor (GCT) [3], non-HL (NHL), retinoblastoma [2], central nervous system (CNS) GCT [1], and retinoblastoma with CNS involvement [1]. The patient's mean age was 8.6 years (4 months-31.9 years). 40 (35.4%) patients were female and 73 (64.6%) patients were male [Table 1].

Those patients who had a clinical diagnosis of pineoblastoma, medulloblastoma, CNS-GCT, and retinoblastoma with CNS involvement were grouped into the category of CNS tumors for the analysis of the correlation between clinical diagnoses and number of CD34 $\times$ 10^6/kg cells. Patients with HL and NHL were grouped into the category of lymphoma. We found that the collections of CD34+ cells of patients diagnosed with AML and

Ewing's tumor were less efficient than those of other patients [Table 2]. There was a statistically significant correlation between diagnoses and number of CD34 × 10^6/kg ($P < 0.001$). Furthermore, considering the diseases, we found a significant difference between CNS tumors with AML ($P = 0.01$) and CNS tumors with Ewing's tumors ($P = 0.006$).

The results of patients' blood parameters are showed in Figure 1. No significant result was found after analyzing the correlation between CD34 × 10^6/kg and hemoglobin level, total leukocyte count, platelet count, absolute neutrophil count, absolute lymphocyte count, and IG count (myeloblasts to bands). In terms of absolute monocyte count, there was a weak correlation ($r_s = 0.255$; $P = 0.07$).

Subsequently, patients were divided into two groups according to the number of collections performed to assess the correlation with CD34 × 10^6/kg and the blood count parameters described above. The first group (86.7%) underwent up to two collections and the second group (13.3%) had more than two collections. There was a significant difference between platelet count ($P = 0.002$) and total leukocyte count ($P = 0.015$), as well as between absolute neutrophil count ($P = 0.035$), and absolute monocyte count ($P = 0.007$). The first group had the best counts considering all these blood count parameters [Table 3].

With the purpose of finding a cut-off point, we used leukocyte and monocyte counts as a comparison criterion. Thirty-five patients had leukocyte count above 30 × 10^9/L and monocyte count above 1.8 × 10^9/L. Among these, 22 (63%) underwent only one collection, and 13 (37%) underwent two collections, thus resulting in a median CD34 × 10^6/kg of 8.7 (4-48.7). The group of patients who had leukocyte counts lower than 30 × 10^9/L and monocyte counts lower than 1.8 × 10^9/L included 41 patients. Thirteen of them (31%) underwent only one collection, 16 (39%) underwent two collections, and 12 (29.2%) had three or more collections. The median CD34 × 10^6/kg for these patients was 7 (2.3-39).

Table 1: Patients' characteristics

	n
Gender (%)	
Female	40 (35.4)
Male	73 (64.6)
Age, years	8.6 (0.4-31.9)
Diagnoses	
Neuroblastoma	43
HL	20
Ewing's tumor	13
Medulloblastoma	10
Wilms' tumor	9
AML	6
Pinealoblastoma	3
GCT	3
NHL	2
Retinoblastoma	2
CNS GCT	1
CNS retinoblastoma	1
Total	113

HL: Hodgkin's lymphoma, NHL: Non-Hodgkin's lymphoma, CNS: Central nervous system, GCT: Germ cell tumor

Table 2: Diagnoses and collections of CD34+ × 10^6/kg

Diagnoses grouped into categories	*n*	CD34+ × 10^6/kg
Neuroblastoma	43	8.28 (3.10-33.0)
Lymphoma	22	8.85 (5.12-32.8)
CNS tumors	15	11.25 (6.43-39.7)
Ewing's tumour	13	5.6 (3.21-13.02)
Wilms' tumor	9	6.77 (6.0-8.74)
AML	6	4.86 (2.37-7.37)
GCT	3	6.62 (6.0-8.74)
Retinoblastoma	2	29.99 (11.28-48.7)

Data are expressed as median (minimum-maximum), CNS: Central nervous system, AML: Acute myeloid leukemia, GCT: Germ cell tumor

Table 3: Description of blood parameters and correlation with count of CD34×10^6/kg (Spearman)

Parameters	*n*	Median (P25-P75)	*P*	r_s	R^2
Hemoglobin (g/dL)	113	10.7 (9.9-11.4)	0.564	−0.05	−0.025
Leukocytes (×10^9/L)	113	27.8 (17.7-42.1)	0.394	0.082	0.007
Platelets (×10^9/L)	112	167.0 (100.2-226.7)	0.418	0.078	0.006
Neutrophils (×10^9/L)	112	21.3 (13.2-33.9)	0.665	0.042	0.002
Monocytes (×10^9/L)	111	1.9 (0.9-2.8)	0.007	0.255	0.065
Lymphocytes (×10^9/L)	112	1.8 (1.0-2.8)	0.619	0.048	0.002
IG (×10^9/L)	113	0.0 (0.0-0.8)	0.083	0.165	0.027

IG: Immature granulocytes, Data are expressed as median (interquartile range: P25 and P75). $P<0.05$ was considered significant

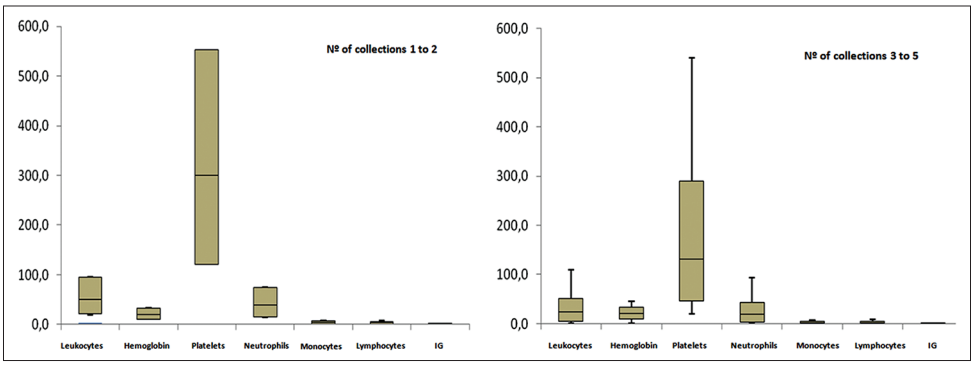

Figure 1: Correlation between blood parameters and count of CD34 × 10^6/kg according to the number of collections, IG: Immature granulocytes, Data are expressed as median (Md): (interquartile range: P25 and P75). $P<0.05$ was considered significant. P = leucocytes: 0.015; hemoglobin: 0.206; platelets: 0.002; neutrophils: 0.0035; monocytes: 0.007; lymphocytes: 0.104 and IG: 0.132

DISCUSSION

The factors that have an influence on HSC mobilization have been extensively studied to improve the efficacy and safety of apheresis. Although there are several predictive factors, the most accepted in clinical practice is the peripheral CD34 + cell count [13]. Thus, compared to CD34 + cell count with various blood count parameters.

Our findings did not reveal a significant association between CD34+ $\times 10^6$/kg and platelet count, hemoglobin level, total leukocyte count, absolute neutrophil count, absolute lymphocyte count, and absolute IG count. Other studies also found no significant correlation between CD34+ and leukocyte [6,18,19], neutrophil, and lymphocyte counts [12]. However, some authors demonstrated a significant correlation between CD34+ and platelet count [11,20], hemoglobin level [16], total leukocyte count [7,9,17], and IG count [7,12,16]. Hansson *et al.* (1995) showed that there is a relationship between the peak level of CD34+ cells and an increase in the absolute number of monocytes [10]. This is in agreement with our findings showing a significant although weak correlation between CD34+ cells and monocytes.

Several different factors may have an influence on HSC collection; however, consensus cannot be reached because of the heterogeneity of the populations studied and the characteristics of the diseases. A study examining previous chemotherapy and administration of alkylating agents showed the harmful effect of these treatments on the product of the collection [21,22]. An analysis of the number of collections considering platelet count, total leukocyte count, absolute neutrophil and monocyte count showed that the patients who had the best counts underwent fewer apheresis procedures. In addition, patients with leukocyte count higher than 30×10^9/L and monocyte count higher than 1.8×10^9/L had efficient collections. In medical practice, it is important to find a cut-off point that is able to determine collection efficiency. In the present study, we found that these two parameters (leukocyte and monocyte) might be considered as predictive factors, although counts lower than the values mentioned above cannot necessarily be defined as unsatisfactory collections.

Regarding diagnosis, we found a significant difference between the diseases and CD34+ $\times 10^6$/kg, which is in agreement with other studies that also found such difference [1,6,21]. In our study, CNS tumors and retinoblastoma had a higher yield of CD34+ $\times 10^6$/kg followed of neuroblastoma and lymphoma. Patients with AML and Ewing's tumor had a collection of CD34+ cells lower than the other groups. We also found a significant difference between patients who had CNS tumors with AML and those who had CNS tumors and Ewing's tumor. Some of the diseases showed a small number of the sample, which can affect the results. Perseghin *et al.* (2009) investigated the impact of diagnosis in a series of patients and found that patients diagnosed with AML had ineffective mobilization [1]. Even though, we did not assess the correlation with type of treatment, some studies have suggested that the reason for inefficient collection is related to the refractory potential of some diseases because some chemotherapy protocols affect mobilization [23].

The impact of the use of G-SCF in patients and the costs with apheresis procedure and quantification of CD34+ cells, which is a specialized and high-cost technique [14], warrant the investigation of new parameters that may predict an efficient collection. Further studies should be conducted, including a larger number of patients in each group stratified by age, gender, and diagnosis, with the aim of identify new parameters which may be incorporated in future in the routine laboratory.

REFERENCES

1. Perseghin P, Terruzzi E, Dassi M, Baldini V, Parma M, Coluccia P, *et al*. Management of poor peripheral blood stem cell mobilization: Incidence, predictive factors, alternative strategies and outcome. A retrospective analyses on 2177 patients from three major Italian institutions. Transfus1. Apher Sci 2009;41:33-7.
2. Fruehauf S, Seggewiss R. It's moving day: Factors affecting peripheral blood stem cell mobilization and strategies for improvement [corrected]. Br J Haematol 2003;122:360-75.
3. Ikeda K, Kozuka T, Harada M. Factors for PBPC collection efficiency and collection predictors. Transfus Apher Sci 2004;31:245-59.
4. Greinix HT, Worel N. New agents for mobilizing peripheral blood stem cells. Transfus Apher Sci 2009;41:67-71.
5. Liu JH, Chen CC, Bai LY, Chao SC, Chang MS, Lin JS. Predictors for successful mobilization of peripheral blood progenitor cells with ESHAP + G-CSF in patients with pretreated non-Hodgkin's lymphoma. J Chin Med Assoc 2008;71:279-85.
6. Pastore D, Specchia G, Mestice A, Liso A, Pannunzio A, Carluccio P, *et al.* Good and poor CD34+ cells mobilization in acute leukemia: Analysis of factors affecting the yield of progenitor cells. Bone Marrow Transplant 2004;33:1083-7.
7. Sautois B, Fraipont V, Baudoux E, Fassotte MF, Hermanne JP, Jérusalem G, *et al.* Peripheral blood progenitor cell collections in cancer patients: Analysis of factors affecting the yields. Haematologica 1999;84:342-9..
8. Barnett D, Janossy G, Lubenko A, Matutes E, Newland A, Reilly JT. Guideline for the flow cytometric enumeration of CD34+ haematopoietic stem cells. Prepared by the CD34+ haematopoietic stem cell working party. General Haematology Task Force of the British Committee for Standards in Haematology. Clin Lab Haematol 1999;21:301-8.
9. Marques JF, Vigorito AC, Aranha FJ, Lorand-Metze I, Miranda EC, Lima Filho EC, *et al.* Early total white blood cell recovery is a predictor of low number of apheresis and good CD34(+) cell yield. Transfus Sci 2000;23:91-100.
10. Hansson M, Svensson A, Engervall P, Björkholm M, Gruber A, Söderström T. Increase of monocytes predicts mobilization of peripheral stem and progenitor cells after chemotherapy followed by G-CSF administration. Eur J Haematol 1995;54:321-8.
11. Zubair AC, Grant R, Wu W, Tun H, Rivera C, Moreno-Aspitia A, *et al.* Platelet count is a sensitive predictor of autologous peripheral blood progenitor cell collection yield in previously treated plasma cell disease patients. Transfusion 2008;48:1106-14.
12. Kozuka T, Ikeda K, Teshima T, Kojima K, Matsuo K, Bessho A, *et al.* Predictive value of circulating immature cell counts in peripheral blood for timing of peripheral blood progenitor cell collection after G-CSF plus chemotherapy-induced mobilization. Transfusion 2002;42:1514-22.
13. Sinha S, Gastineau D, Micallef I, Hogan W, Ansell S, Buadi F, *et al.* Predicting PBSC harvest failure using circulating CD34 levels: Developing target-based cutoff points for early intervention. Bone Marrow Transplant 2011;46:943-9.
14. Dunlop LC, Cohen J, Harvey M, Gallo J, Motum P, Rosenfeld D. The immature reticulocyte fraction: A negative predictor of the harvesting of CD34 cells for autologous peripheral blood stem cell transplantation. Clin Lab Haematol 2006;28:245-7.

15. Sutherland DR, Anderson L, Keeney M, Nayar R, Chin-Yee I. The ISHAGE guidelines for CD34+ cell determination by flow cytometry. International Society of Hematotherapy and Graft Engineering. J Hematother 1996;5:213-26.
16. Sakashita AM, Kondo AT, Ribeiro AA, Cipolleta, AN, Colesanti MV, Hamerschlak N, *et al*. Factors affecting autologous peripheral blood hematopoietic stem cell collections by large-volume leukapheresis: A single center experience. Einstein 2011;9:196-200.
17. Delamain MT, Metze K, Marques JF Jr, Reis AR, De Souza CA, Lorand-Metze I. Optimization of CD34+ collection for autologous transplantation using the evolution of peripheral blood cell counts after mobilization with chemotherapy and G-CSF. Transfus Apher Sci 2006;34:33-40.
18. Ford CD, Chan KJ, Reilly WF, Petersen FB. An evaluation of predictive factors for CD34+ cell harvest yields from patients mobilized with chemotherapy and growth factors. Transfusion 2003;43:622-5.
19. Gutensohn K, Magens MM, Kuehnl P, Zeller W. Increasing the economic efficacy of peripheral blood progenitor cell collections by monitoring peripheral blood CD34+ concentrations. Transfusion 2010;50:656-62.
20. Morton J, Morton A, Bird R, Hutchins C, Durrant S. Predictors for optimal mobilization and subsequent engraftment of peripheral blood progenitor cells following intermediate dose cyclophosphamide and G-CSF. Leuk Res 1997;21:21-7.
21. Ketterer N, Salles G, Moullet I, Dumontet C, ElJaafari-Corbin A, Tremisi P, *et al.* Factors associated with successful mobilization of peripheral blood progenitor cells in 200 patients with lymphoid malignancies. Br J Haematol 1998;103:235-42.
22. Morris CL, Siegel E, Barlogie B, Cottler-Fox M, Lin P, Fassas A, *et al*. Mobilization of CD34+ cells in elderly patients (>/= 70 years) with multiple myeloma: Influence of age, prior therapy, platelet count and mobilization regimen. Br J Haematol 2003;120:413-23.
23. Wuchter P, Ran D, Bruckner T, Schmitt T, Witzens-Harig M, Neben K, *et al.* Poor mobilization of hematopoietic stem cells-definitions, incidence, risk factors, and impact on outcome of autologous transplantation. Biol Blood Marrow Transplant 2010;16:490-9.

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