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CIRCULATING PROGENITOR CELL COLLECTION: EXPERIENCE FROM 275 LEUKAPHERESES IN VARIOUS MALIGNANCIES AND IN HEALTHY DONORS

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ABSTRACT

Background. **Blood cell transplantation has become a new type of support in high-dose chemotherapy (HDC) for several oncologic and hematologic diseases. Over the last few years the demand for circulating progenitor cell (CPC) collection by blood cell separators has grown dramatically, and transfusion services must manage new CPC programs.**

Materials and Methods. **A protocol for optimizing the collection and clinical use of CPC is described. The results of 275 harvestings were studied: 128 patients were divided into 5 groups according to tumor type (A: breast cancer; B: Hodgkin's disease; C: non-Hodgkin lymphoma; D: multiple myeloma; E: various solid tumors). An additional group (F) consisted of 11 healthy donors. Factors affecting collection (mobilizing regimen or previous radiation therapy) and side effects were investigated.**

Results. **The mean values of mononuclear cells (MNC$\times 10^7$/kg) and $CD34^+$ cells ($\times 10^6$/kg) collected per leukapheresis in the 6 respective groups were: 31.4 and 4.6 in group A; 26.4 and 3.4 in group B; 21.8 and 5.8 in group C; 24.6 and 2.4 in group D; 26.8 and 2.9 in group E; 60 and 6 in group F. Previous chemotherapy and/or radiation therapy were the main factors influencing CPC harvesting. The different chemotherapy regimens employed demonstrated no significant differences in their mobilizing efficacy. Side effects related to leukapheresis were few (2.3% of the procedures) and manageable.**

Conclusions. **CPC collection is feasible in a wide range of clinical situations. Careful clinical evaluation of patients, accurate monitoring of progenitor cell release and collection timing are important for obtaining a sufficient number of CPC for hemopoietic recovery. Previous chemotherapy and radiotherapy are the main factors influencing CPC harvests. The mobilizing regimens employed showed no substantial differences in their efficacy.**

Key words: circulating progenitor cells, blood cell transplantation, high-dose chemotherapy, recombinant human granulocyte colony-stimulating factor

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Circulating progenitor cell (CPC) reinfusion after high-dose chemotherapy (HDC) has radically changed the therapeutic management of several oncologic and hematologic diseases.[1-3] The rising demand for leukapheresis procedures has produced remarkable growth in the activity of our Transfusion Center. Consequently we have been forced to develop a working strategy capable of facing the changing clinical scene. The wide range of patients with completely different types of tumors undergoing blood cell transplantation (BCT) has required the adoption of differentiated harvesting methods related to the particular disease in question. It is well known that chemotherapy (CT) associated with recombinant

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	n° of patients	Mean age	Male	Female	Disease
Group A	20	52±11	–	20	Breast cancer
Group B	25	29±12	19	6	Hodgkin's disease
Group C	41	39±13	30	11	Non-Hodgkin's lymphoma
Group D	12	45±8	10	2	Multiple myeloma
Group E	19	25±14	12	7	Miscellaneous solid tumors
Group F	11	28±11	7	4	Healthy donors

Table 1. Patient characteristics.

human growth factors (rhG-CSF or rhGM-CSF) dramatically augments the number of post-aplasia CPC able to sustain short and long-term hematopoiesis.[4-7] Harvesting and subsequent reinfusion of CPC permit the use of HDC regimens capable of exceeding the drug resistance of neoplastic cells.[3,8,9] Cyclophosphamide (at 4-7 g/m^2) was the first drug for which the ability to mobilize stem cells was documented; the demonstration that other drugs such as ara-C and epirubicin may also mobilize CD34$^+$ cells has led to disease-oriented CT that aims at an antitumor effect and a mobilizing effect at the same time.[10-12] Monitoring CD34$^+$ cells in the peripheral blood aids in identifying the time to harvest and provides information about the opportuneness of continuing leukapheresis.[13,14] The kinetics of CD34$^+$ mobilization is different and often individual in patients with solid tumors or with marrow diseases like multiple myeloma, and is affected by factors such as bone marrow involvement or previous CT or radiotherapy.[12,15]

CPC harvesting in healthy donors mobilized with growth factors alone represents a completely different question. In these subjects it is easier to predict and identify collection time and often a single leukapheresis may obtain the desired progenitor yield for a successful engraftment.[16] We report our experience with a large number of CPC collections in patients with various oncologic or hematologic diseases and in healthy donors, relatives of patients undergoing allogeneic bone marrow transplantation (BMT).

Patients and Methods

Between January 1994 and August 1995, 275 leukaphereses were performed for CPC harvesting in 128 subjects (117 cancer patients and 11 healthy donors) to meet the needs of various oncologic and hematologic Institutions.

In the present study we divided the patients (clinical characteristics are described in Table 1) in 5 groups according to disease:

– group A: 20 patients with breast cancer and more than 10 metastatic lymph nodes;
– group B: 25 patients with relapsed or refractory Hodgkin's disease (HD);
– group C: 41 patients with very poor prognosis or relapsed non-Hodgkin's lymphoma (NHL);
– group D: 12 patients with multiple myeloma (MM), stage III or IV;
– group E: miscellaneous group containing 19 patients with different solid tumors (ovarian and germinal tumors, rabdomyosarcoma, neuroblastoma).

An additional group (F) included 11 healthy CPC donors, all relatives of patients undergoing allogeneic transplantation from PB; in 6 cases HLA-identical CPC donors had already given BM to the same recipient, while the other 5 were first related donors partially matched with their recipients for 3 antigens.

Patients were considered eligible for CPC collection if they had no severe cardiovascular, liver or renal disease and no recent infectious febrile episodes. A careful evaluation of clinical condition, laboratory parameters and vascular accesses was performed by the transfusionist at two different times: the first about 2 weeks before the presumed time of leukapheresis, in order to verify the patient's eligibility for the procedure; the second the day before the collection time as a final examination of the patient and in order to select the most suitable harvesting technique to employ. Written informed consent was obtained from every subject according to insti-

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tutional guidelines. Patients were mobilized with different priming CT schedules, followed (24-48 hours later) by rhG-CSF (5 μg/kg) s.c. daily until the last apheretic procedure (Table 2). To mobilize CPC, healthy donors were treated only with rhG-CSF (10-12 μg/kg) s.c. daily for 3-5 consecutive days. Autologous blood cell transplantation was adopted as support following HDC in groups A-E; allogeneic CPC were employed as an alternative salvage procedure after classic BMT failure in group F.

Collection timing

Flow cytometry according to Siena *et al.*[17] was used for daily continuous monitoring of $CD34^+$ cells in the PB during the post CT hematopoietic recovery phase. Collection time was identified as the period when $CD34^+$ cells reached a value of 20/μL. The possibility of harvesting even though $CD34^+$ were below 20/μL (between 10 and 20/μL) was always considered in relation to the particular clinical history, state of disease and therapeutic strategy adopted for each patient.

CPC harvesting

Two different cell separators were employed for CPC collection: CS 3000 Plus Baxter and Spectra Cobe. With the former we used a granulocyte separation chamber combined with 1) an A-35 collection chamber if the platelet count was more than 100×10^9/L or if the whole blood volume of the patient was more than 3000 mL, or 2) a small volume collection chamber (SVCC) if the platelet count was under 100×10^9/L and/or the whole blood volume was less than 3000 mL.

With the Spectra Cobe separator we adopted the technique of harvesting mononuclear cells very close to the red cell layer, thus obtaining a final product with a 5-6% hematocrit. With CS 3000 Plus the hematocrit of the final product was between 7 and 9%. A minimum of two whole blood volumes was processed at each procedure.

ACD-A (acid citrate dextrose, formula A) was employed at an anticoagulant/whole blood ratio of 1/11 with the Spectra Cobe cell separator and 1/10 with the CS 3000 Plus Baxter cell separator. Continuous monitoring of the patient's vital signs was carried out during the procedure.

Transfusion management in CPC collection

When patients presented severe thrombocytopenia (PLT $< 20\times10^9$/L), a transfusion with single-donor irradiated PLT concentrate was given one hour before the leukapheresis.

In patients with low body weight (< 30 kg), the cell separator was primed with previously irradiated red cells suspended in a 5% albumin solution to obtain the desired hematocrit in order to obtain an extracorporeal volume of less than 10% of the patient's blood volume.

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Group A - Breast cancer (20 patients)	a) FEC (5-fluorouracil+ epirubicin + cyclophosphamide) b) cyclophosphamide
Group B - HD (25 patients)	a) cyclophosphamide b) etoposide + ifosfamide c) vincristine + epirubicin
Group C - NHL (41 patients)	a) cyclophosphamide 7 g/m^2 b) etoposide c) Ara-C + mitoxantrone d) CHOP (cyclophosphamide + adriamycin + vincristine + prednisone)
Group D - MM (12 patients)	a) cyclophosphamide 4 g/m^2
Group E - Miscellaneous tumours (19 patients)	a) cisplatin + ifosfamide + etoposide b) vincristine + ifosfamide + etoposide c) adriamycin + ifosfamide d) ifosfamide + etoposide e) D-CECAT (desferrioxamine + thiotepa + cyclophosphamide + etoposide + paraplatin)

Table 2. Mobilization chemotherapy regimens used in the five groups of patients.

Notwithstanding red blood cell transfusion, sometimes patients got ready for collection with a low hematocrit (< 25%). In these cases as well, our strategy was to prime the separator with matched red cells (previously irradiated and suspended in a 5% albumin solution) to minimize the hemodynamic imbalance in the initial phase of the procedure.

CPC quality control and freezing

MNC count and $CD34^+$ flow cytometric analysis were performed for every product according to Siena *et al.*[17] Aliquots of mononuclear cells from leukapheresis products were assayed for CFU-GM colony growth by culture in semisolid medium (0.8% methylcellulose in alpha medium). MNC products were cryopreserved with a slow rate of cooling, using 10% dimethylsulfoxide (DMSO) as a cryoprotectant, then stored in liquid nitrogen until use.

Patient care and surveillance

Every patient was continuously monitored during leukapheresis for blood pressure and cardiac frequency. Each patient's peripheral venous accesses were carefully evaluated in advance; if they were adequate, we employed 16-17 G diameter needles or an intravenous cannula in antecubital veins. Whenever the accesses were unable to guarantee a good flow rate (40-60 mL/min) a central venous catheter (dual-lumen dialysis, 12 FR in size, 16 cm in length, Arrow-Howes) was inserted in the subclavian vein. After every procedure patients were checked for hemocytometric values, electrolyte, liver, renal status and coagulation parameters. We routinely administered 10% calcium gluconate in continuous infusion (3.3 mmol/L).

Adverse effects secondary to leukapheresis

Adverse effects were graded as mild, moderate or severe according to the following criteria adopted in our center:

1. mild effects were transient in nature, responded quickly to simple measures and had little or no clinical significance;
2. moderate effects where those that caused considerable discomfort to the patient and did not respond quickly to treatment;
3. severe effects occurred when the patient was clinically unstable and required vigorous resuscitation measures, and they generally required termination of the procedure.

We investigated the adverse effects related to collections as technical and processing complications, metabolic, hematologic, infectious and catheter related complications.

Statistical analysis

Descriptive statistical analysis of results, unpaired T-test for significance test on means, and linear regression were adopted for statistical analysis of results.

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Results

The mean number of leukapheresis required per patient was 2.2 in groups A and C, 2.1 in group B, 2.3 in groups D and E, 1.4 in healthy donors. The mean blood volume processed each procedure was 9 L (range 3L-12.5L), with a whole blood flow rate of between 30 and 65 mL/min. The mean number of MNC ($\times 10^7$/kg) collected at each leukapheresis was quite similar in groups B, C, D and E (respectively, 26.45, 21.8, 24.6, 26.8). Group A (breast cancer) achieved a mean of 31.4×10^7/kg and group F a mean of 60×10^7.

Mean collection efficiencies were: 69% (range 58-77%) with the Spectra Cobe cell separator, 65% (range 50-75) with the CS 3000 Plus cell separator (program 6 Special, Small Volume Collection Chamber combined with Granulo chamber), 62% (range 55-74) with the CS 3000 Plus cell separator (program 7 Special, A-35 as Collection Chamber combined with Granulo chamber).

We obtained a mean number of $CD34^+$ cells/kg from each leukapheresis in groups A, B, C, D, E of 4.6×10^6, 3.4×10^6, 5.8×10^6, 2.4×10^6, respectively. In Group F (healthy donors) the mean was 6.0×10^6/kg. CFU-GM expressed as colonies $\times10^4$/kg were 16.5 in group A, 9.35 in group B, 37.6 in group C, 10. 3 in group D, 29.8 and 38.3 in groups E and F, respectively.

Table 3 shows the number of leukapheresis per patient, the $CD34^+$ peak value days, the

amount of MNC cells, CD34+ cells and CFU-GM colonies for each collection expressed as mean values, standard deviations, minimum and maximum values.

From statistical analysis within groups A, B and D, we found that CD34+ cell collection was more difficult in intensively chemo-radiotreated patients. In group A, patients with more than 6 chemotherapy cycles provided a mean of 2.6×10^6 CD34+ cells/kg versus 5.23×10^6/kg in patients with fewer than 6 cycles (p=0.02). Significant statistical differences in group B occurred between patients who had been previously irradiated and those who had not (CD34+ cells 1.93 vs 6.57, p=0.003). Even in patients with MM the number of CT cycles (fewer or more than 6) and IFN administration influenced the yield of CD34+ cells: 5.9 vs 0.8×10^6/kg (p< 0.001). Within groups A, B, C, evaluation of CD34+ collections according to different types of mobilizing regimens showed no significant differences. Within group A two subgroups were characterized on the basis of the mobilizing agent employed. Subgroup A1, mobilized with cyclophosphamide 7 g/m^2, furnished a mean of 4.3×10^6 cells/kg versus subgroup A2 (mean 4.2×10^6/kg CD34+ cells) mobilized with FEC; no significant difference was found. However, within group A, patients previously heavily chemotreated (more than 6 cycles) provided a mean of 2.6×10^6 CD34+ cells versus a mean of 5.2×10^6 cells from the lightly treated ones (fewer than 6 cycles) (p=0.02). Within group B (Hodgkin's disease), 19 patients previously submitted to radiotherapy yielded a mean of 1.93×10^6/kg CD34+ cells, while non irradiated patients obtained a mean of 6.57^610^6 CD34+ cells/kg at each leukapheresis, (p=0.003). Within group D, ten patients with stage IV myeloma previously intensively chemotreated (> 10 cycles and/or associated IFN therapy) harvested a mean of 0.817×10^6 CD34+ cells. The other two MM patients (previously lightly treated with fewer than 4 cycles) were able to harvest many more cells per procedure (mean 7.46×10^6/kg) (p<0.001).

Adverse effects related to the apheresis procedures occurred in 3 patients (2.3%). One patient presented a moderate grade hypovolemia that required medical attention. Two others showed symptomatic hypocalcemia with perioral paresthesia and chills (mild grade complication) that promptly resolved upon additional infusion of calcium gluconate. One collection was not successful because a clotting problem in the collection bag caused the loss of the product.

A central venous catheter was implanted in the subclavian vein of 30 patients (23%). Complications related to catheter occlusion occcurred in 1 patient and required catheter repositioning, thus delaying collection.

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	leukaphereses/ patient	day of CD34+ peak value	MNC x 10^7/kg	CD34+ x 10^6/kg	CFU-GM x 10^4/kg
Group A Breast cancer	2.2 ± 0.9 (1-4)	12 ± 1 (11-13)	31.4 ± 19 (5.6-68)	4.6 ± 4.1 (0-19)	16.5 ± 21.5 (0-71.2)
Group B HD	2.1 ±1.1 (1-5)	13 ± 1.6 (11-16)	26.45 ± 22.1 (1.6-83)	3.4 ± 4 (0-4.3)	9.35 ± 11.9 (0.01-37)
Group C NHL	2.2 ± 0.8 (1-4)	12 ± 1.5 (10- 14)	21.8 ± 15.8 (4.3-70)	5.8 ± 10.1 (0.02-67.8)	37.6 ± 150 (0-971)
Group D MM	2.3 ±1.2 (1-5)	13 ± 1.8 (11-17)	24.6 ± 19 (4-71)	2.4 ± 2.7 (0.2-8.7)	10.3 ± 20 (0.1-64)
Group E Miscellaneous tumors	2.3 ± 1 (1-4)	11 ± 1.6 (10-13)	26.8 ± 18.9 (7.4-94)	2.9 ± 1.9 (1-7.7)	29.8 ± 43.4 (5-182)
Group F Healthy donors	1.4 ± 0.6 (1-2)	5 ± 1 (4-6)	60 ± 46 (7.1-152)	6 ± 7.1 (1.4-25.8)	38.3 ± 50 (0.4-122)

Table 3. Results of 275 PBSC collections expressed as mean value, standard deviation, minimum and maximum values obtained per leukapheresis.

Discussion

The growing demand for PBSC to support HDC in oncologic patients has recently involved our Transfusion Center in the management of these diseases. During the last two years the dramatically increased number of leukaphereses has forced us to develop guidelines for managing extremely different kinds of patients in the best way and for performing successful, non-time-consuming procedures.

A wide range of neoplastic diseases is treated with HDC and different drugs are employed for CPC mobilization.[8,18-20] Antineoplastic agents other than cyclophosphamide can successfully mobilize CPC and are also utilized as disease-specific CT.[12] The use of rhGM-CSF has been reported to be effective in increasing the absolute number of circulating mononuclear cells and of CFU-GM progenitors after 7g/m^2 cyclophosphamide mobilized patients.[21,22] In our experience only rhG-CSF was combined with chemotherapy, because of its good tolerability and ability as mobilizing agent. Harvesting time and collection yield are influenced by many variables such as BM involvement, patient age, previous CT and/or radiotherapy, time between last CT and priming, type of CT (i.e. toxicity on stem cell pool).[5,23] Our results in patients with breast cancer showed that high-dose cyclophosphamide is a proven mobilizing agent able to induce severe aplasia and consequently provide a good boost in the amount of hemopoietic progenitors. Furthermore, FEC, a polychemotherapy regimen widely used in the treatment of advanced breast cancer, also proved to have good mobilizing capacity, allowing collection of an adequate number of progenitors to support a dose-intensive program.[24] A statistically significant difference was found within group A between heavily pretreated (more than 6 cycles) and lightly CT-treated patients (fewer than 6 cycles). Within group B a great difference in CPC collection was observed between previously irradiated and non irradiated patients. The permanent marrow injury due to irradiation determines a reduction in hematopoietic marrow reserve, thereby necessitating more procedures per patient.

Particular consideration must be given to group D (12 MM patients who obtained a low mean number of CD34$^+$ cells per procedure).[25] Ten of these patients suffered from stage IV myeloma, had been previously intensively chemotreated (> 10 cycles and/or associated IFN therapy), and furnished a mean of 0.817×10^6/kg CD34$^+$ cells per harvest. The other two patients, who were lightly pretreated (fewer than 4 cycles), were able to achieve a very high yield per procedure, similar to collections in healthy donors. Our results suggest that it is advisable to start collecting CPC in the early stage of the disease, when the patient's marrow is less contaminated by neoplastic cells.[26]

At the beginning of our experience three leukaphereses failed: one because of clotting in the collection bag and two others due to an inaccurate preliminary count of CD34$^+$ cells in the peripheral blood. In our strategy we consider it crucial to monitor peripheral CD34$^+$ cells daily by means of flow cytometry, in order to identify the exact moment in which leukapheresis must start. Our borderline value for CD34$^+$ is at least 20 cells/μL. Sometimes patients with a history of intensive CT or with an exhausted BM are unable to reach the value of 20 cells/μL, so the opportuneness of harvesting is discussed with the attending physician every time.

Our Institution considers as safe for engraftment a dose of CD34$^+$ cells per kg $> 2.5\times 10^6$ which represents the minimum target for every collection. Our aim is to obtain a minimum of 4×10^6/kg CD34$^+$ cells, which represents our collection target and was reached in all patients.[27,28]

Many authors discuss the CFU-GM/kg threshold dose required for engraftment, especially as far as the platelet lineage is concerned, with reported values ranging between 5 and 50×10^4 CFU-GM/kg.[17,29] As reported by other authors and confirmed by our experience, there is a wide range in the CFU-GM count (Table 3).[27,30] In the absence of a standardized method, every Institution must define its own CFU-GM/kg dose required for safe engraftment.

Group F (healthy donors) merits a separate discussion. RhG-CSF administration to a healthy individual seems to be safe and capable of mobilizing a good number of progenitors.[31-35] The data reported in Table 3 are expressed in

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relation to the weight of the recipient. We obtained abundant MNC harvestings, generally with a single leukapheresis performed after 5 days of rhG-CSF administration. The mean $CD34^+$/kg value of our collections was altered because in one case we had an enormous difference between donor and recipient body weights (70 vs 137 kg!); in another case we stopped the procedure after one blood volume had been processed because the young donor (4 years old) presented severe nausea, vomiting and chills. Our preliminary experience with 10 allotransplants suggests the feasibility of this strategy.

The two-step evaluation of the patient permits us to know the exact clinical condition in order to avoid problems that could delay or prevent harvesting. A careful estimate of peripheral vascular accesses is essential for assuring that leukapheresis can be performed. In our experience, placing a dual-lumen dialysis catheter (12 FR) in the subclavian vein when peripheral accesses cannot guarantee a good flow rate has permitted all programmed leukaphereses to be carried out. Subclavian access, when positioning is carried out by experienced personnel, offers less risk of infection and is tolerated better by patients than the femoral site. In contrast with Goldberg *et al.*, we observed no catheter infectious complications and encountered only one occlusive episode.[36,37] Hypovolemia due to extracorporeal circulation during leukapheresis has been reduced considerably with the advent of third generation cell separators. Starting hypotension represents a rare event: less than 1% of all harvestings; however, patients with anemia (hemoglobin < 10 g/dL) or with a low body weight may experience hypovolemia-related symptoms. The strategy of priming the apheresis kit with irradiated red cells suspended in a 5% albumin solution avoids this problem entirely. Administration of a large amount of anticoagulant (600-1000 mL of ACD-A1) may induce hypocalcemia-related symptoms; perioral paresthesia was the most common manifestation among our patients, and it was easily controlled by infusing calcium gluconate, which also avoided more severe symptoms like muscle twitching in the extremities, chills, pressure in the chest, nausea and vomiting.

In conclusion, the growing need for CPC as HDC support has radically changed the therapeutic choices in oncological patients.[38] Transfusion centers will have to deal with an increasing demand for leukapheresis and will actively participate in the strategy of submitting patients for auto- and allotransplantation with CPC. Furthermore, the development of new techniques for selecting, purging and providing CPC as vehicles for gene therapy makes stem cells a truly new blood component.[39-41]

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