Bone Marrow Transplantation (2002) 29, 893–897
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Progenitor cell mobilisation

A randomized trial of assessment of efficacy of leukapheresis volumes, 8 liters *vs* 12 liters

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Summary:

It is logical to expect that large-volume leukapheresis may be able to collect adequate numbers of PBSC with fewer procedures. To date, there is no agreement on the optimal volume of leukapheresis. Therefore, in this study we compared 8 l volume with 12 l and assessed whether a 50% increase in the blood volume processed would decrease the number of leukaphereses each patient needed to collect $\geqslant 2.5 \times 10^6$ CD34$^+$ cells/kg in normal mobilizers. PBSC mobilization was done with cyclophosphamide etoposide followed by rhG-CSF in all patients. Forty patients were randomized to undergo 8 l leukaphereses ($n = 20$ patients) or 12 l leukaphereses ($n = 20$). The median numbers of leukaphereses required in order to collect $\geqslant 2.5 \times 10^6$ CD34$^+$ cells/kg in patients processed with 8 l and 12 l were 1 (range 1–5) and 1 (1–4), respectively ($P = 0.50$). The median number of total nucleated cells (TNC) collected per patient was greater for the 12 l group (7.47×10^8/kg *vs* 3.90×10^8/kg, $P < 0.001$), as was the median number of total mononuclear cells (TMNC) (4.26×10^8/kg *vs* 2.16×10^8/kg, $P < 0.001$), whereas there was no difference between the two groups for the median number of CD34$^+$ cells collected per patient (8.94×10^6/kg *vs* 8.60×10^6/kg, $P = 0.85$). The TNCs and TMNCs collected per leukapheresis were again greater for the 12 l group (3.64×10^8/kg *vs* 1.91×10^8/kg, $P = 0.001$ and 2.17×10^8/kg *vs* 0.88×10^8/kg, $P < 0.001$), whereas there was no difference between the two groups for the median number of CD34$^+$ cells collected per leukapheresis (3.98×10^6/kg *vs* 3.26×10^6/kg, $P = 0.90$). This study showed that there is no difference between 8 l and 12 l volumes in regard to collected CD34$^+$ cells/kg and also the use of a 12 l leukapheresis volume did not decrease the number of leukaphereses performed compared with a 8 l leukapheresis volume. In fact, the use of the larger leukapheresis volume had the disadvantage of adding 60 min to the time the patient was on the machine.
Bone Marrow Transplantation (2002) **29,** 893–897. DOI: 10.1038/sj/bmt/1703571
Keywords: large-volume leukapheresis; 8 liters *vs* 12 liters

Autologous peripheral blood stem cell (PBSC) infusions are being used with increasing frequency following the administration of myeloablative chemoradiotherapy for the treatment of hematologic malignancies and solid tumors.[1] Two important reasons for their use are the faster hematopoietic recovery of mobilized PBSC compared with steady-state bone marrow support and the ability to collect PBSCs as an outpatient procedure.[1–3] The best way to mobilize and collect PBSCs is yet to be defined. It is logical to expect that large-volume leukapheresis (LVL) may be able to collect adequate numbers of PBSCs with fewer procedures. Therefore, LVL has been proposed as a way to collect adequate numbers of PBSC using fewer collection procedures.[4,5] Such an approach may be beneficial and cost-effective to the patient and hospital. Schwarer *et al*[6] compared standard volume with large volume in patients with a variety of malignant hematologic diseases undergoing PBSC mobilization and reported no difference between two groups regarding numbers of aphereses and collected CD34$^+$ cells.[6] Based on the limited data available on this issue in the literature, we decided to conduct a randomized comparison of leukapheresis volume 8 l with 12 l to evaluate whether or not the larger volume would decrease the number of leukaphereses by increasing the numbers of CD34$^+$ cells/kg collected.

Patients and methods

The purpose of this study was to evaluate the number of leukaphereses at each collection volume, 8 l or 12 l,

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Received 26 November 2001; accepted 20 March 2002

required to collect $>2.5 \times 10^6$ CD34$^+$ cells/kg body weight of the patient. Forty patients were randomly assigned to either a 8 l ($n = 20$ patients) or 12 l ($n = 20$ patients) leukapheresis volume. Collections of the assigned volume were to be continued until $>2.5 \times 10^6$ CD34$^+$ cells/kg were collected. If five collections had been performed and $<1 \times 10^6$ CD34$^+$ cells/kg had been collected, a BM harvest would be performed to supplement the stem cell number.

Patients

Between November 1999 and October 2001, 40 patients meeting protocol eligibility criteria for treatment with high-dose chemotherapy requiring PBSC transfusion received cyclophosphamide (CY), etoposide and rhG-CSF for mobilization of PBSCs.[1] Patients characteristics are listed in Table 1. Patients were eligible for PBSC mobilization and harvest if they had Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 2, were aged $\leqslant$65, and had evidence of adequate hepatic, renal and cardiac function.

Informed consent

Oral and written informed consent for PBSC collection was obtained from all patients or their guardians.

Mobilization regimen

PBSC mobilization was done with CY 4 g/m^2 $\times$ 1 on day 1 and etoposide 200 mg/m^2/day $\times$ 1 on days 1, 2 and 3 in all patients. All patients received MESNA for the prevention of hemorrhagic cystitis. The MESNA dose was 4 g/m^2 as equally divided administrations prior to infusion of CY and 4 and 8 h after. RhG-CSF (Amgen, Roche, Thousand Oaks, CA, USA) 16 μg/kg/day i.v. twice daily was started the day after completion of chemotherapy and continued until the last day of apheresis. Patients received antibiotic prophylaxis with ciprofloxacin 750 mg orally twice per day when the WBC count was less than 0.5×10^9/l. Apheresis was performed by using Fenwall CS3000+ (Baxter Health Care System, Deerfield, IL, USA) and started as soon as WBC counts reach $\geqslant 2 \times 10^9$/l following a nadir.

Leukapheresis procedure

All procedures were performed with a Baxter CS3000+ by nursing staff and procedures were run at 55 ml/min whole blood flow rate with acid citrate dextrose-A (ACD-A) formulation as the anticoagulant. The interface was set at 20 at commencement of the procedure and adjusted up or down by the operator to obtain the appropriate collection consistency as determined by the color of the component-rich plasma line. A draw line was usually obtained peripherally, and the return line was a Hickman catheter. All procedures gave a final leukapheresis product volume of 220 ml; this volume was independent of the volume of patient's peripheral blood processed during the procedure (8 l or 12 l).

Quantitation of CD34$^+$ cells

Circulating peripheral blood CD34$^+$ cell counts prior to apheresis and collected CD34$^+$ cell counts in the apheresis product after each collection were analyzed using flow cytometry. Erythrocytes were removed from samples of cells obtained before cryopreservation or after thawing and serial dilution by hypotonic lysis using ammonium chloride. The cells were then stained with a fluorescein isothiocyanate or phycoerythin-conjugated CD34 antibody (8G12; Becton Dickinson, San Jose, CA, USA) or an irrel-

Table 1 Patient characteristics

Variable	*8 l (n = 20)*	*12 l (n = 20)*	*P value*
Sex			
Male	13 (65%)	12 (60%)	0.74
Female	7 (35%)	8 (40%)	
Age (years) (median, range)	44.5 (16–62)	43 (17–61)	0.97
Weight (kg) (median, range)	69 (51–91)	67.5 (49–94)	1
Previous treatment			
Chemotherapy alone	17 (85%)	15 (75%)	
Chemotherapy and radiotherapy	3 (15%)	5 (25%)	0.69
PBSC mobilization regimen			
Chemotherapy+G-CSF (16 μg/kg/day)	20 (100%)	20 (100%)	—
WBC counts on the first day of apheresis ($\times 10^9$/l) (median, range)	6.67 (2.49–13.60)	5.7 (2.10–14.18)	$>$0.05
Platelet counts on the first day of apheresis ($\times 10^9$/l) (median, range)	43.85 (10.76–117 000)	48.4 (18.21–131 000)	$>$0.05
Number of heavily pre-treated patients	7/20 (35%)	5/20 (25%)	0.65
Diseases			
Lymphoma	9 (45%)	8 (40%)	0.74
Breast cancer	5 (25%)	6 (30%)	
Multiple myeloma	5 (25%)	6 (30%)	
Other	1 (5%)		

evant isotype control, washed and counterstained with propidium iodide (PBI; Becton Dickinson) or 7-aminoactinomycin D (7AAD; Sigma Chemical, St Louis, MO, USA). After an additional wash and within 2 h of staining, the proportions of viable (PI or 7AAD excluding) cells, mononuclear cells and $CD34^+$ cells in the fresh and thawed specimens were determined by flow cytometric analysis (FACScan, Becton Dickinson). $CD34^+$ cells showed fluorescence of >99.8% of isotype control-stained cells. The quantities of these cell populations in each product before and after freezing were determined.[7]

Statistics

The Mann–Whitney *U* test and a two sample, two-tailed *t*-test were used to test for differences in collection numbers and cell yields between the two treatment arms. Student's *t*-test was also used to compare body weight and age of the patients in both groups. Gender, disease type and previous chemotherapies were compared with chi-square analyses.

Results

Patient details

Twenty patients were randomized to a 8 l leukapheresis volume and 20 patients to a 12 l leukapheresis volume. The groups were evenly matched for clinical parameters (Table 1). Median numbers of WBC and platelet counts on the first day of apheresis in patients processed with 8 l and 12 l were 6.67×10^9/l (range 2.49–13.60), 43.85×10^9/l (10.76–117.000) and 5.7×10^9/l (range 2.10–14.18), 48.4×10^9/l (18.21–131.000) ($P > 0.05$), respectively (Table 1).

Number of leukapheresis per patient

Total numbers of leukaphereses required to collect $\geq 2.5 \times 10^6$ $CD34^+$ cells/kg in patients processed with 8 l and 12 l were 32 and 30, respectively (Table 2). Eleven of 20 (55%) patients in the 8 l group and 13 of 20 in the 12 l group (65%) reached the minimal requirement of $CD34^+$ cells ($\geq 2.5 \times 10^6$/kg) in one collection ($P = 0.52$) (Table 2). There was no difference between the two groups regarding the number of leukaphereses performed per patient. The median numbers of leukaphereses performed per patient required to collect $\geq 2.5 \times 10^6$ $CD34^+$ cells/kg in 8 l and 12 l groups were 1 (range 1–5) and 1 (1–4), respectively ($P = 0.50$) (Table 3).

Table 2 Numbers of leukaphereses required to collect $\geq 2.5 \times 10^6$ $CD34^+$ cells/kg

Leukaphereses (n)	*8 l (n = 20)*	*12 l (n = 20)*
1	11 (55%)	13 (65%)
2	6 (30%)	5 (25%)
3	3 (15%)	1 (5%)
4	0	1 (5%)

P value = 0.52.

Cells collected per patient

The total numbers of TNCs collected per kilogram of patient body weight ($P < 0.001$) and TMNCs collected per kilogram of patient body weight ($P < 0.001$) were greater in the 12 l collection group, whereas there was no statistical difference in $CD34^+$ cell numbers collected per patient ($P = 0.85$) (Table 3).

Cells collected per leukapheresis

The per leukapheresis number of TNCs collected per kilogram of patient body weight ($P = 0.001$) and MNCs collected per kilogram of patient body weight ($P < 0.001$) were greater in the 12 l collection group, whereas there was no statistical difference in $CD34^+$ cell numbers per kilogram of patient body weight collected ($P = 0.90$) (Table 4).

Discussion

The convenience and cost of PBSC collection are influenced by the number of leukaphereses required to collect a target number of hematopoietic progenitors to support one or more cycles of dose-intensive cytotoxic chemotherapy. One difficulty in assessing the efficiency of a leukapheresis procedure to collect PBSC is the wide variability among patients in the concentration of PBSCs as a consequence of underlying disease and the extent of prior cytotoxic therapy. Some studies have shown that $CD34^+$ cells are mobilized into the peripheral blood during LVL.[8,9] On the basis of those observations, some authors have suggested that processing larger volumes of peripheral blood during each leukapheresis procedure may collect a larger number of $CD34^+$ cells than processing of one to two patient blood volumes.[5,6,8,9] LVL take a proportionally longer time for the patient and are more likely to be associated with adverse events, such as citrate reactions, intravenous access difficulties, patient discomfort, anemia and thrombocytopenia. Kobbe *et al*[5] investigated the effect of LVL on the collected stem cell yields of allogeneic donors and showed that LVL is safe and effective in maximizing the total number of harvested progenitor cells in healthy individuals. In that study, authors strongly recommended large volume processing in the poor mobilizers, who are characterized by low levels of circulating $CD34^+$ cells but LVL seemed to be of limited clinical value in individuals with very high (>80–100/μl) peripheral $CD34^+$ cell counts.[5]

Eight liter, 10 l and 12 l leukaphereses were the most frequently processed blood volumes at our institution. Initially, we compared the 8 l volume with 10 l to assess whether or not the larger blood volume processed would increase the number of $CD34^+$ cells in each collection in normal mobilizers.[10] In that study, there was no difference between the two groups regarding number of aphereses and collected $CD34^+$ cells.[10] Because of the low volume difference between 8 l and 10 l, and to see whether our previous

Table 3 Median cell yields per patient

	Leukapheresis volume		*P value*
	8 l (n = 20) median (range)	*12 l (n = 20) median (range)*	
TNCs $\times 10^8$/kg	3.90 (1.56–10.03)	7.47 (2.82–12.50)	<0.001
TMNCs $\times 10^8$/kg	2.16 (0.54–4.82)	4.26 (1.53–6.52)	<0.001
$CD34^+$ cells $\times 10^6$/kg	8.60 (3.77–41.49)	8.94 (3.60–28.80)	0.85
No. aphereses[a]	1 (1–5)	1 (1–4)	0.50

[a]In order to collect $\geqslant 2.5 \times 10^6$ $CD34^+$ cells/kg.

Table 4 Median cell yields per leukapheresis

	Leukapheresis volume		*P value*
	8 l (n = 20) median (range)	*12 l (n = 20) median (range)*	
TNCs $\times 10^8$/kg/day	1.91 (0.52–4.20)	3.64 (0.94–7.35)	0.001
TMNCs $\times 10^8$/kg/day	0.88 (0.42–2.41)	2.17 (0.51–5.80)	<0.001
$CD34^+$ cells $\times 10^6$/kg/day	3.26 (0.94–14.29)	3.98 (1.33–16)	0.90

observation is confirmed or not, we decided to compare the 8 l processed with the larger 12 l processed volume in a randomized trial to assess whether or not a 50% increase in the blood volume processed would increase the number of $CD34^+$ cells in each collection and thus decrease the average number of leukapheresis needed to reach the goal of $\geqslant 2.5 \times 10^6$ $CD34^+$ cells/kg patient body weight. However, the 12 l collection volume also did not decrease the number of leukapheresis needed to reach the prescribed $CD34^+$ cell number (Table 2). The median number of leukapheresis needed in order to collect $\geqslant 2.5 \times 10^6$ $CD34^+$ cells/kg was 1 for the 8 l and 12 l groups ($P = 0.50$), and there were no differences in the proportion of patients who required one, two or three leukapheresis to reach the minimal required number of $CD34^+$ cells. Fifty-five and 65% of patients in one collection and 30 and 25% of patients in two collections reached the target number of $\geqslant 2.5 \times 10^6$ $CD34^+$ cells/kg in 8 l and 12 l groups, respectively ($P = 0.52$) (Table 2).

In this study, overall and per leukapheresis, the patients undergoing the 12 l procedure had more TNC and MNC collected, although this did not translate into more $CD34^+$ cells collected. The numbers of $CD34^+$ cells collected overall and per leukapheresis were not significantly different between the two groups. This may be related to release kinetics of $CD34^+$ cells into peripheral blood which may be partially or completely independent of processed volume. It is also plausible to think that release kinetics of TNC and MNCs may be different from that of $CD34^+$ cells. It has to be noted that this study was conducted in normal mobilizers. Therefore, the results do not imply that in the poor mobilizer setting, large volume procedure is useless.

In summary, in the current study, the larger leukapheresis volume of 12 l provided no advantages over the 8 l leukapheresis volume in normal mobilizers. There was no decrease in the absolute number of leukapheresis procedures performed for patients having the larger, 12 l, blood volume processed when compared with patients having the 8 l blood volume processed. An adequate number of $CD34^+$ cells was collected in a median of one procedure with both leukapheresis volumes. In fact, the use of the larger leukapheresis volume had the disadvantage of adding 60 min to the time the patient was on the machine.

References

1 Demirer T, Bensinger WI, Buckner CD. Peripheral blood stem cell mobilization for high-dose chemotherapy. *J Hematother* 1999; **8**: 103–113.
2 Demirer T, Buckner CD, Appelbaum FR *et al*. Rapid engraftment after autologous transplantation utilizing marrow and recombinant granulocyte colony-stimulating factor-mobilized peripheral blood stem cells in patients with acute myelogenous leukemia. *Bone Marrow Transplant* 1995; **15**: 915–922.
3 Gianni A, Siena S, Bregni M *et al*. Granulocyte–macrophage colony-stimulating factor to harvest circulating haematopoietic stem cells for autotransplantation. *Lancet* 1989; **2**: 580–583.
4 Passos-Coelho JL, Machado AM, Luco P *et al*. Large-volume leukapheresis may be more efficient than standard-volume leukapheresis for collection of peripheral blood progenitor cells. *J Hematother* 1997; **6**: 465–474.
5 Kobbe G, Soehngen D, Heyll A *et al*. Large volume leukapheresis maximizes the progenitor cell yield for allogeneic peripheral blood progenitor donation. *J Hematother* 1997; **6**: 125–131.
6 Schwarer AP, Messino NM, Gibson M *et al*. A randomized trial of leukapheresis volumes, 7 L versus 10 L: an assessment of efficacy and patient tolerance. *J Hematother Stem Cell Res* 2000; **9:** 269–274.
7 Demirer T, Buckner CD, Bensinger WI *et al*. Peripheral blood stem cell (PBSC) collections after paclitaxel, cyclophosphamide and recombinant human granulocyte colony-stimulating factor (rhG-CSF) in patients with breast and ovarian cancer. *J Clin Oncol* 1995; **13**: 1714–1719.

8 Cull G, Cannell P. Recruitment of $CD34^+$ cells during large-volume leukapheresis. *Transfusion* 1997; **37**: 672–673.
9 Lefrere F, Makki J, Marolleau JP *et al*. CD34+ cells during leukapheresis procedures: relationship of volume processed and quantity of peripheral blood progenitor cells collected. *Transfusion* 2000; **40**: 493–495.
10 Demirer T, Ayl M, Günel N *et al*. A randomized trial of assessment of efficacy of leukapheresis volumes, 8 L versus 10 L. XIII Congress of the European Society for Haemapheresis. Rýva del Garda, Italy, abstract, page 67, 2001.