Journal of Clinical Apheresis 12:63–67 (1997)

Prospective, Concurrent Comparison of the Cobe Spectra and Haemonetics MCS-3P Cell Separators for Leukapheresis After High-Dose Filgrastim in Patients With Hematologic Malignancies

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A prospective study was undertaken to compare the mononuclear cell, CD34+ cell, and CFU-GM yields of the Haemonetics MCS-3P and the Cobe Spectra cell separators in ten patients (nine multiple myeloma and one non-Hodgkin lymphoma) on two consecutive days after mobilization with high-dose filgrastim (12–16 μg/k) for 4 days. All patients were harvested once on each machine, five starting on each machine. The target duration of the procedure on the Spectra was 160 minutes, and the target blood volume processed on the MCS-3P was 60–70 ml/kg body weight. Both machines were operating on the 1995 software versions supplied by the respective manufacturers. The time taken for the procedure was significantly longer with the Haemonetics machine. The volumes of blood processed and the product collected were significantly higher with the Spectra, as were the absolute mononuclear and CD34+ cell yields, and yields per unit time. Mononuclear and CD34+ cell yields per unit volume of blood processed were comparable for both machines. The differences in CFU-GM yields were not significant, largely because of wide interpatient variations. The extent of platelet depletion as a result of the procedure was greater with the Spectra because of the higher blood volume being processed. We conclude that the Cobe Spectra is a significantly faster machine than the Haemonetics MCS-3P; and consequently, its use is associated with higher mononuclear and CD34+ cell yields. J. Clin. Apheresis 12:63–67, 1997.

Key words: cell separators; Cobe Spectra; filgrastim; granulocyte colony-stimulating factor, Haemonetics MCS-3P; leukapheresis; peripheral blood stem cell transplantation

INTRODUCTION

Blood-derived stem cells have virtually replaced bone marrow as the source of autologous hematopoietic support after high-dose chemotherapy or chemoradiotherapy because of faster hematopoietic recovery [1]. Cell separators used for leukapheresis are either intermittent flow devices which require a single site of vascular access (Haemonetics V50 or Haemonetics MCS-3P) or continuous flow machines which require two sites of vascular access (Cobe Spectra or Fenwal CS-3000). If available sites of adequate peripheral venous access are limited, intermittent flow separators may have the advantage of obviating the insertion of an in-dwelling catheter specifically for leukapheresis. This may be especially attractive for normal donors in whom insertion of an in-dwelling catheter is not desirable. Continuous flow separators, on the other hand, are faster because the inflow and outflow lines are separate [2]. The requirement of two intravenous lines usually does not pose a problem in healthy donors and patients with good vascular access, but may be difficult in extensively pretreated patients [1].

There are limited data comparing collection efficiencies of various cell separators [2–4]. In 1993, we had compared the Cobe Spectra and Haemonetics MCS-3P cell separators for leukapheresis in patients with malignancies when the latter machine had just become available in the UK, and experience with its use was very limited [2]. Since then, our mobilization schedule has been modified with a different dose of growth factor [5], and the harvest software versions for both machines have been upgraded. We therefore undertook another prospective, concurrent comparison of both the machines in a group of patients with hematologic malignancies mobilized with a uniform high-dose filgrastim (granulocyte colony-stimulating factor, G-CSF, Amgen, Cambridge) regimen.

Contract grant sponsors: Cobe Laboratories and the Haemonetics Corporation.

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Received for publication 1 September 1996; Accepted 23 November 1996.

PATIENTS AND METHODS

Between October 1995 and February 1996, ten consecutive patients who underwent leukapheresis in our unit and fulfilled all the following criteria were enrolled into a prospective study comparing the Cobe Spectra™ (Cobe Laboratories, Lakewood, CO) and the Haemonetics MCS-3P™ (Haemonetics Corporation, Leeds, UK) machines concurrently: hematologic malignancy, scheduled peripheral blood stem cell autograft as part of planned therapy, normal leukocyte and platelet counts before starting mobilization, mobilization with a uniform high-dose G-CSF protocol without chemotherapy, no previous autograft, and no fever or active infection. All patients gave written consent for leukapheresis. Individual disease-specific high-dose chemotherapy and transplant protocols were approved by the local institutional review board.

Study Design

Each patient was harvested twice, once on each machine. The first five patients started on the Spectra, and the next five on the MCS-3P. For each patient, the harvest machine was changed on the second day. For each apheresis product, the following values were measured or calculated: daily pre- and post-harvest automated total and differential blood counts, daily total and differential automated cell counts on the apheresis product (bag count), the number of CD34+/CD33+ and CD34+/CD33−cells, and the number of granulocyte-macrophage colony-forming units (CFU-GM). The lymphocyte and monocyte fraction of the total nucleated cells was considered the mononuclear cell population. Red cell content of the harvested product was not studied. Engraftment endpoints were not studied because both collections were pooled for transplantation.

Mobilization

A variable dose of filgrastim (12–16 μg/kg daily), rounded off to the nearest vial size such that the actual delivered dose was not less than 12 μg/kg or more than 16 μg/kg [5], was administered subcutaneously once a day on four consecutive days (days 1–4) to mobilize stem cells in the basal state after complete hematopoietic recovery from any preceding chemotherapy.

Leukapheresis

Indwelling vascular catheters were not inserted in any patient specifically for the purpose of harvesting stem cells. Existing Hickman catheters were used to return processed blood during harvests on the Spectra, but were not utilized for drawing blood on either machine because of problems with intermittent luminal collapse due to the Bernoulli effect. Large-bore peripheral venous cannulas were inserted in all patients as required to draw blood.

Leukapheresis was performed using the manufacturer's recommended technique for each machine in a consistent fashion. On the Spectra, the duration of the procedure was fixed at 160 ± 10 minutes which usually permitted 200–250% of the patient's calculated blood volume to be processed during each procedure. For the MCS-3P, in accordance with the manufacturer's recommendations, 60–70 ml of blood was processed per kilogram patient body weight. The Spectra harvest software was Version 4, and the MCS-3P software was Revision E. All procedures were performed by one of two senior, qualified apheresis nurses (S.C. or H.P.).

Pulse and blood pressure were closely monitored during the procedure, and patients were asked to report any adverse experiences or unusual sensations. At the end of the second procedure, patients were asked in general terms about which machine they preferred.

Flow Cytometry

All blood samples were counted and used for flow cytometric analysis within 2 hours of collection. The antibodies used were mouse anti-human CD45-FITC and CD14-RPE (Sigma, UK), and CD34-FITC and CD33-PE (Becton Dickinson, UK). The progenitor subsets assayed were CD34+/CD33− and CD34+/CD33+.

Aliquots of 50 μl were dispensed into tubes of 10 μl ready-conjugated antibody agitated on a mixer and incubated at 4°C for 20 minutes. One milliliter of lysis solution (Ortho Diagnostics, UK) was added to each tube and incubation continued at room temperature for 10 minutes. The tubes were transferred to an ice bath and analyzed immediately.

Samples were analyzed using an Ortho Cytoronabsolute (Ortho Diagnostics, UK) flow cytometer with gating for lymphocytes. The absolute number of lymphocytes within the gate was determined from the number of cells expressing CD45. Calibration for the lymphocyte gate ensured that >99% of the gated cells were lymphocytes. The machine was calibrated before use with normal donor blood that had been assessed for the differential count. Data with experimental samples were collected in list mode file and analyzed using the software package supplied by the manufacturer. All subsequent calculations were based on the total white count assayed by a Coulter counter to provide absolute counts of individual progenitor subsets. The CD34+ cell subset results were derived by adding the two subsets that were assayed.

CFU-GM Measurements

The number of CFU-GMs harvested was assayed by a modification of the method of Pike and Robinson [6]. Instead of leukocyte feeder layers to provide colony-stimulating activity, conditioned medium from the 5637 bladder carcinoma cell line was used which contains GM-CSF and G-CSF amongst other growth factors. The cells were plated out at the concentration of 5×10^4 cells per dish into 35 mm Petri dishes in 1 ml of the alpha

TABLE I. Patient Characteristics*

No. of patients	10
Male/female	5/5
Age (yr)	45–58 (median 51)
Weight (kg)	57–96 (median 75.5)
Diagnosis	
Multiple myeloma	9
Non-Hodgkin lymphoma	1
No. of previous chemotherapy regimens	1–3 (median 2)
No. of previous chemotherapy cycles	5–14 (median 9)
Pre-harvest leukocyte counts (10^9/L)	
First day	9.4–61.9 (median 24.5)
	$P = .98$ (NS)
Second day	8.7–61.8 (median 24.4)
Spectra harvest days	12.2–61.9 (median 25.3)
	$P = .88$ (NS)
MCS-3P harvest days	8.7–61.8 (median 23.6)
Pre-harvest platelet counts (10^9/L)	
First day	83–537 (median 169.5)
	$P = .09$ (NS)
Second day	55–240 (median 125)
Spectra harvest days	835–537 (median 144)
	$P = .47$ (NS)
MCS-3P harvest days	55–240 (median 162)

*The pre-harvest platelet and leukocyte counts have been compared for the first versus second and the Spectra versus MCS-3P days.

modification of Eagle's minimum essential medium containing 0.3% agar and 100 μl of 5637 conditioned medium. The cultures were then incubated in a humidified incubator with 5% CO_2, and scored after 14 days under a low power microscope counting all colonies with 50 cells or more. The average of four replicates was calculated and the result expressed as the number of CFU-GM/10^5 nucleated cells plated.

Statistical Analysis

All statistical comparisons were performed using Student's paired t-test, and all *P* values shown are two-tailed.

RESULTS

Patient characteristics are shown in Table I. All patients had received considerable prior chemotherapy (median of two different chemotherapy regimens). None of the patients received platelet or packed red cell transfusions during the 2 days of the apheresis procedure. As Table I shows, the pre-harvest leukocyte counts were comparable for both days. The platelet counts, as expected, were lower on the second day of the procedure although not significantly so.

The time taken to process the specified blood volume on the MCS-3P was significantly longer than the fixed time on the Spectra, despite which the latter machine processed significantly greater amounts of blood (Table II). Both the quantity of the final product collected and the cell count on the harvest were greater with the Spectra. Unlike in our previous study [2], the percentages of

TABLE II. Comparison of Harvest Parameters and Volumes

Parameter	Cobe Spectra	Haemonetics MCS	*P* (t test)
Time taken per harvest session (minutes)			
Mean ± SD	162 ± 14.8	202 ± 16.9	.0001
Range	127–181	181–235	
Volume of blood processed per harvest session (ml)			
Mean ± SD	11,364 ± 2,184	4,690 ± 466	.000004
Range	6,685–14,323	3,870–5,565	
Volume of blood processed per kg body weight (ml/kg)			
Mean ± SD	152 ± 25	64 ± 9	.000002
Range	117–193	52–79	
Volume of blood processed per minute (ml/min)			
Mean ± SD	70.1 ± 12.2	23.2 ± 1.3	.000008
Range	52.6–82.9	20.4–24.9	

mononuclear cells in the product were comparable for the two machines (Table III).

As Table IV shows, the absolute mononuclear and CD34+ cell yields and yields per unit time were significantly higher with the Spectra. CFU-GM yields were not significantly different owing to the large inter-patient variations. Table IV also shows that cell yields per unit blood volume processed were comparable for both machines.

Table V shows that although pre- and post-harvest platelet counts were comparable with the two machines, platelets tended to decrease more after harvests on the Spectra than on the MCS-3P. However, the maximum decrease in the platelets was by 65% of the baseline value, and the lowest post-procedure platelet count seen was 38×10^9/L. Bleeding complications were not seen in any patient.

Patients found the MCS-3P attractive because of its single vascular access and the Spectra because of the shorter procedure duration. No adverse reactions were encountered in any of the patients.

DISCUSSION

This study shows that both Haemonetics MCS-3P and Cobe Spectra cell separators are suitable for harvesting stem cells in patients with hematologic malignancies. The main issue addressed in this study was that of cell yields, and transplant-related end-points such as engraftment were not studied because collections from both machines were pooled for transplantation.

There are important methodologic differences between our previous study [2] and this one. The procedure was not time-bound for both machines in this study. We chose to follow the recommendation of the Haemonetics Corporation to process 60–70 ml blood per kg body weight, allowing a higher volume to be processed per harvest on the MCS-3P compared with our previous study [2]. As a result of this, the time taken on the MCS-3P was significantly longer than on the Spectra, but the volume processed was still lower than that on the Spec-

TABLE III. Volume, Total and Differential Count, and CD34 Cell Content of the Harvest

Parameter	Cobe Spectra	Haemonetics MCS	*P* (t-test)
Volume of product collected per harvest session (ml)			
Mean ± SD	151.7 ± 20.7	101.6 ± 19.0	.0003
Range	103–169	71–135	
Total nucleated cell count of harvested product (10^9/L)			
Mean ± SD	201.9 ± 50.6	150.8 ± 42.0	.001
Range	106.4–268.9	89.2–228	
Purity of harvested product (% mononuclear cells)			
Mean ± SD	73.8 ± 17.8	72.8 ± 15.8	.89 (NS)
Range	43–98	41–89	
CD34+ cell content of harvested product (%)			
Mean ± SD	0.30 ± 0.22	0.22 ± 0.23	.001
Range	0.1–0.85	0.02–0.82	
CD34+/CD33− cell content of harvested product (%)			
Mean ± SD	0.21 ± 0.18	0.14 ± 0.19	.07 (NS)
Range	0.06–0.64	0–0.65	

TABLE IV. Comparison of Absolute Cell Yields, Cell Yields Per Unit Time, and Cell Yields Per Unit Blood Volume Processed

Parameter	Cobe Spectra	Haemonetics MCS	*P* (t-test)
Total mononuclear cell yield (10^8/kg)			
Mean ± SD	3.0 ± 1.0	1.5 ± 0.6	.001
Range	1.5–4.5	0.7–2.8	
Total CFU-GM yield (10^4/kg)			
Mean ± SD	11.5 ± 20.5	1.9 ± 1.7	.17 (NS)
Range	0–67.6	0.03–5.4	
Total CD34+ cell yield (10^6/kg)			
Mean ± SD	1.35 ± 1.26	0.50 ± 0.62	.003
Range	0.23–4.50	0.03–2.14	
Mononuclear cell harvest rate (10^8/min)			
Mean ± SD	1.4 ± 0.5	0.5 ± 0.2	.0002
Range	0.7–2.1	0.3–0.8	
CFU-GM harvest rate (10^4/min)			
Mean ± SD	5.3 ± 9.6	0.7 ± 0.6	.16 (NS)
Range	0–31.7	0.01–1.7	
CD34+ cell harvest rate (10^6/min)			
Mean ± SD	0.62 ± 0.58	0.18 ± 0.25	.003
Range	0.10–2.11	0.0099–0.85	
Mononuclear cell yield per unit volume of blood processed (10^8/L)			
Mean ± SD	20.1 ± 6.9	23.2 ± 7.4	.17 (NS)
Range	8.5–31.2	12.4–35.1	
CFU-GM yield per unit volume of blood processed (10^4/L)			
Mean ± SD	76.5 ± 142.6	29.6 ± 25.6	.30 (NS)
Range	0–472.1	0.5–73.6	
CD34+ cell yield per unit volume of blood processed (10^6/L)			
Mean ± SD	9.1 ± 8.9	8.4 ± 12.1	.62 (NS)
Range	1.8–31.5	0.4–41.5	

tra. The software used on the Cobe Spectra for the first study was Version 3.6. This version was later replaced by Version 4, which has been used for the present study. The software used on the MCS-3P for the first study was Revision D2, which was upgraded to Revision E for the present study.

We have increased the dose of filgrastim used for mobilization of stem cells from 250 μg/m^2 [2] (corresponding to approximately 8 μg/kg) to 12–16 μg/kg, and the number of aphereses has been decreased from 4 to 2.

TABLE V. Comparison of Platelet Depletion

Parameter	Cobe Spectra	Haemonetics MCS	*P* (t test)
Pre-harvest platelet counts (10^9/L)			
Mean ± SD	178.4 ± 131.2	154 ± 52.7	.24 (NS)
Range	83–537	55–240	
Post-harvest platelet counts (10^9/L)			
Mean ± SD	96.2 ± 49.9	105.2 ± 40.1	.34 (NS)
Range	38–218	44–192	
Percent drop in platelet counts due to the procedure			
Mean ± SD	42.2 ± 12.6	30.9 ± 10.7	.04
Range	26.4–63.5	19.9–46.2	

The previous schedule resulted in a higher number of nucleated cells and a lower number of progenitor cells being harvested [5,7], whereas the current schedule results in a lower number of nucleated cells being harvested without decreasing the number of progenitor cells. This is important to minimize collection of contaminating malignant cells which could result in increased relapse rates [8].

Finally, because no gates were used for the acquisition and analysis of the flow cytometry data in the previous study [2], the exact CD34+ progenitor yields with the two machines could not be compared accurately. This study allows the progenitor yields to be compared.

The present study confirms that the Cobe Spectra is a significantly faster machine which processes a greater amount of blood per unit time. This results in significantly higher mononuclear cell and CD34+ cell yields; in absolute terms as well as per unit time. Although the CFU-GM yields also tend to be higher with the Spectra, the difference is not statistically significant. The technical differences between continuous flow and intermittent flow procedures [2] can explain the differences in cell yields. The basic ability of the two machines to collect progenitor cells is comparable because the progenitor cell yields per unit blood volume processed are similar.

Unlike our previous study [2], the mononuclear cell purity of the MCS-3P collections was comparable to those with the Spectra in this study. In the previous study, the Spectra yielded a significantly purer product (81 ± 15% vs. 42 ± 15, $P < .001$). This difference may be the result of the software revision with the MCS-3P machine or the higher dose of the growth factor used here for mobilization of stem cells.

The minimum number of CD34+ cells required to obtain consistent (although not necessarily rapid) engraftment is probably 1×10^6/kg. In the present study, only one of ten MCS-3P runs resulted in a CD34+ yield of $\geqslant 1 \times 10^6$/kg compared with four of ten with the Spectra ($P = 0.3$, Fisher's exact test). Three of ten MCS-3P runs resulted in a CD34+ yield of $\geqslant 0.5 \times 10^6$/kg compared with nine of ten with the Spectra ($P = 0.02$, Fisher's exact test). This suggests that by using high-dose filgrastim for mobilization in this type of patients, an adequate number of CD34+ cells can be obtained with two

aphereses on the Spectra in 90% of patients. The generally poor CD34+ cell yields are not surprising because most patients were extensively pre-treated. We routinely obtain much higher CD34+ cell numbers with a single apheresis on the Spectra from healthy donors for allogeneic transplantation after stimulation with lower doses of G-CSF [9].

We conclude that the Cobe Spectra is a significantly faster machine than the Haemonetics MCS-3P, and consistently collects higher numbers of mononuclear and CD34+ cells by virtue of its speed. As a result of this, a smaller number of harvests are required on the Spectra to reach a minimum collection target in extensively pre-treated patients.

ACKNOWLEDGMENTS

This work was supported by grants and technical assistance from Cobe Laboratories and the Haemonetics Corporation.

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