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Collection Efficiency on the Fenwal CS3000 When Using Filgrastim (Recombinant Methionyl Human Granulocyte Colony-Stimulating Factor) as a Peripheral Blood Stem Cell Mobilization Agent

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The collection efficiency (CE) of the Fenwal CS3000 in collecting peripheral blood stem cells during post-chemotherapy recovery phase ranges from 58% to 73%. Recently filgrastim (recombinant methionyl human granulocyte colony-stimulating factor [G-CSF]) has also been shown to be effective as a mobilization agent although mobilization occurs during elevated and not low normal leukocyte counts. We compared the mononuclear cell (MNC) CE and the myeloid progenitor cell (CFU-GM) CE among 11 patients with G-CSF mobilization (33 procedures) and 19 patients during recovery following myelosuppression chemotherapy (93 procedures). Pre-apheresis leukocyte, neutrophil, MNC, and PB CFU-GM counts were significantly higher in the G-CSF group, while the granulocyte percentage in the apheresis products was similar in both groups. Both MNC CE (81.8 ± 4.5% vs. 64 ± 2.4%) and CFU-GM CE (79.5 ± 10.5% vs. 55.8 ± 3.5%) were higher in the G-CSF group. Only the pre-apheresis MNC count showed an independently significant correlation for both CE ($P < .001$). The higher CE in the G-CSF group can only be partly explained by a rise in MNC count during apheresis. These data suggest that the blood cell separator works better with leukocytosis, and especially with a higher MNC count. The improvement in CE is another benefit of G-CSF mobilization over chemotherapy mobilization.

Key words: peripheral blood stem cells, leukapheresis, G-CSF, collection efficiency

INTRODUCTION

Peripheral blood stem cell transplant (PBSCT) is a therapeutic approach that is being performed with increasing frequency [1,2] because it accelerates the haematological recovery after high-dose chemo-radiotherapy with reduction in cytopenic-associated complications and the duration of hospital stay [3].

Under normal conditions stem cells are found in peripheral blood in very low numbers [4,5]; therefore it is necessary to stimulate their release from the bone marrow to the peripheral blood by high-dose chemotherapy and/or haematopoietic growth factors [6–9]. Mobilised stem cells are then collected by leukapheresis. The FENWAL CS3000 is one of the most commonly used continuous flow blood cell separators for this purpose. The mononuclear cell (MNC) collection efficiency (CE) on the FENWALL CS3000 ranges from 58% to 73% [10–12] and shows a good correlation with the myeloid progenitor cell (CFU-GM) CE as reported by Haylock et al. [13].

Recently filgrastim (recombinant methionyl human granulocyte colony-stimulating-factor [G-CSF]) has been shown to be an effective mobilization agent. G-CSF mobilization is different from chemotherapy mobilization because no myelosuppression or cytopenia is involved. In contrast, the leukocyte count is often elevated to 30 × 10^9/L or more with a major increase in granulocytic cells. Furthermore, it is not clear whether these leukocytes would behave like their non-mobilized counterparts during leukopheresis. We have therefore compared retrospectively the MNC CE and the CFU-GM CE on the FENWAL CS3000 between a group with G-CSF induced leukocytosis and a group with leukopenia during recovery following myelosuppression chemotherapy [13].

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MATERIALS AND METHODS

G-CSF Group

Eligible patients for G-CSF mobilization were those with poor prognosis non-Hodgkin's lymphoma, Hodgkin's disease, acute lymphoblastic leukaemia, and germ cell tumour as previously reported [14]. Briefly, Filgrastim (recombinant methionyl human granulocyte colony-stimulating factor, G-CSF, AMGEN, Thousand Oaks, CA) was given as a continuous subcutaneous infusion (12 μg/kg daily) for 6 days. Leukapheresis were performed on days 5, 6, and 7 or on days 4, 6, and 8 depending on the cohort of a G-CSF multicentre trial.

Chemotherapy Group

Criteria for selection of patients included the following:

1. Patients with acute myeloblastic leukaemia, who had aphereses performed during the recovery phase following induction or consolidation chemotherapy [10];
2. Patients with lymphoma, advanced ovarian carcinoma, multiple myeloma, and breast carcinoma, who had aphereses performed during the recovery phase following single high-dose cyclophosphamide (3–7 g/m^2) as described previously [15].

Leukapheresis was commenced when the leukocyte and platelet counts rose above 1×10^9/L and 50×10^9/L, respectively, during the recovery phase following chemotherapy.

Stem Cell Apheresis

Leukapheresis was performed on the FENWAL CS3000 (FENWAL, Baxter Healthcare, Round Lake, IL) in the Clinical Aphereses Unit, Royal Adelaide Hospital, as previously reported [10]. Procedure 3 with the following settings was used: blood flow rate at 50–70 ml/min, interface detector at 20, and the target blood volume processed 7 litres.

Collection Efficiency

Blood counts with differential counting (Coulter S Plus and manual method respectively) and CFU-GM assay were determined in the peripheral blood immediately pre-apheresis and in the leukapheresis product. PB CFU-GM assay was performed as previously reported [16].

The CE for MNC and CFU-GM was calculated according to the following formula [17]:

$$CE\% = \frac{\text{Concentration in bag (/L)} \times \text{Volume in bag (/L)}}{\text{Concentration in blood pre-apheresis (/L)} \times \text{Blood volume processes (/L)}} \times 100$$

TABLE I. Pre-Apheresis Blood Counts in Chemotherapy and G-CSF Groups*

	Chemotherapy group, n = 93 (mean ± 1 SD)	G-CSF group, n = 33 (mean ± 1 SD)	Significance (unpaired t-test)
Leukocytes ($\times 10^9$g/L)	2.5 ± 0.1	49.2 ± 3.7	$P < .001$
Neutrophils ($\times 10^9$/L)	2.1 ± 0.1	44.4 ± 3.5	$P < .001$
Mononuclear cells ($\times 10^9$/L)	0.4 ± 0.2	3.8 ± 0.4	$P < .001$
Platelets ($\times 10^9$/L)	156 ± 9	149 ± 12	(NS)
CFU-GM ($\times 10^3$/L)	1,839 ± 208	5,202 ± 372	$P < .001$

*NS = non-significant difference.

Statistical Analysis

The Student t-test (paired and unpaired) was used to test for significant differences between two groups, except for CFU-GM CE. CFU-GM CE did not show a normal distribution so the Mann-Whitney test was used for analysis. Multiple regression and Spearman's correlation co-efficient were calculated to compare the measured variables. Results are given as mean ± 1 SEM unless otherwise specified.

RESULTS

Eleven patients (4 female, 7 male; mean age of 42 ± 3 years) with lymphoproliferative disorders (7 non-Hodgkin's lymphoma and 4 Hodgkin's disease) received G-CSF for PBSC mobilization. Three leukaphereses were completed for each patient, in seven on days 5, 6, and 7 and in four on days 4, 6, and 8. The blood volume processed was 7 litres in 24 procedures and 8 litres in nine procedures.

Data from 93 recovery phase stem cell aphereses in 19 patients (11 female, 8 male; mean age of 49.4 ± 3) were analyzed in the chemotherapy group. Diagnoses included acute myeloid leukaemia (3), multiple myeloma (1), non-Hodgkin's lymphoma (9), ovarian carcinoma (4), and breast carcinoma (2). The blood volume processed was 7 litres in 81 procedures, 4.3 litres in one procedure, 8.3 litres in one procedure, and 10 litres in ten procedures. These data had been previously reported by Haylock et al. [13].

The pre-apheresis blood cell counts are shown in Table I. The leukocyte, neutrophil, MNC, and CFU-GM levels were significantly higher in the G-CSF group than in the chemotherapy group.

MNC CE was 81.8 ± 4.5% in the G-CSF group (n = 32) and 64.0 ± 2.4% (n = 93) in the chemotherapy group ($P = .002$; Fig. 1). One G-CSF procedure was

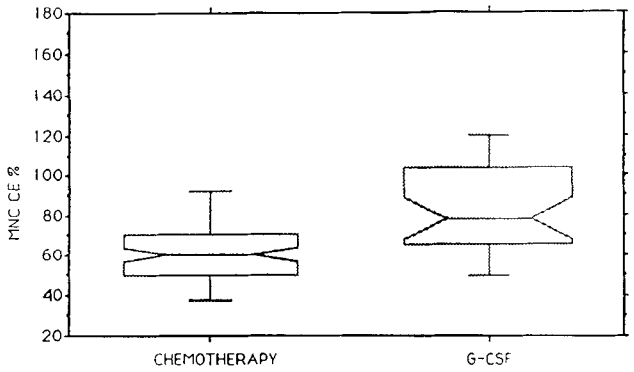

Fig. 1. Mononuclear cell collection efficiency (MNC CE) in chemotherapy and G-CSF groups. Notched box plot graph. The lines in the middle of the boxes represent the median for MNC CE. The top and bottom of the boxes represent the 75th and 25th percentiles, respectively. The top and bottom "whiskers" represent the 95th and 5th percentiles, respectively. There is a significant difference between the MNC CE in the two groups (P = .002).

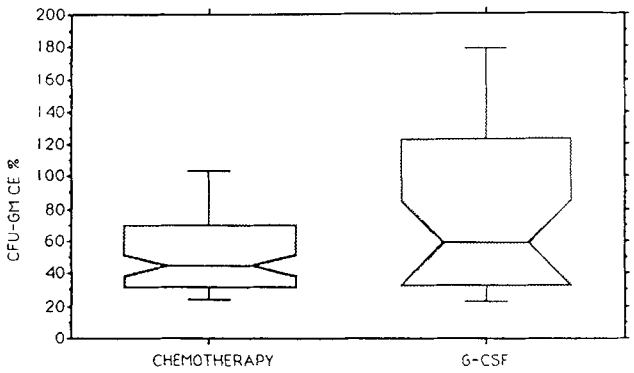

Fig. 2. CFU-GM CE in chemotherapy and G-CSF groups. Notched box plot graph. The lines in the middle of the boxes represent the median for CFU-GM CE. The top and bottom of the boxes represent the 75th and 25th percentiles, respectively. The top and bottom "whiskers" represent the 95th and 5th percentiles, respectively.

excluded as the MNC CE was more than 3 standard deviations (SD) from the mean. CFU-GM CE was 79.5 ± 10.5% in the G-CSF group (n = 30) and 55.8 ± 3.5% (n = 91) in the chemotherapy group (P = not significant; Fig. 2). Three procedures in the G-CSF group and two procedures in the chemotherapy group were excluded because they were 3 SD from the mean. There was a significant correlation between MNC CE and CFU-GM CE ($P < .01$). Only the pre-apheresis MNC count showed an independently significant correlation for both MNC CE and CFU-GM CE ($P < .01$). The following parameters did not show any significant correlation with the MNC CE or CFU CE: age, sex, disease type, previous chemotherapy, bone marrow involvement, blood volume processed, or pre-apheresis platelet and red cell counts.

The pre- and post-apheresis MNC counts were compared in 18 procedures in the chemotherapy group and 22 procedures in the G-CSF group. The mean of the post-apheresis MNC level was 91% and 108% of the pre-apheresis MNC level in the chemotherapy and G-CSF groups, respectively. The difference between the two groups was significant (P = .004).

The percentage of MNC in the leukapheresis product was 84 ± 2.7% in the G-CSF group, not significantly different from the chemotherapy group 89 ± 2.5% (P = .121).

DISCUSSION

The collection target of MNC and/or CFU-GM to achieve a rapid, complete, and sustained haematological recovery after PBSCT is higher than that required for ABMT [18].

We report here that the MNC CE is significantly higher in patients undergoing G-CSF mobilization than in those undergoing chemotherapy mobilization. The CFU-GM CE is also higher in G-CSF mobilization although the difference is not statistically significant probably because of the non-normal distribution of the data. The higher leukocyte and neutrophil counts in the G-CSF group did not adversely affect the machine performance, and the percentages of granulocytic cells in the apheresis product are similar in the two groups.

It is possible that in patients with leukopenia or normal white cell counts, the interface detector oscillates across a narrow MNC layer exceeding the edges, thereby reducing "the purity" of the product. In contrast, when the leukocyte and especially the MNC count is high, the interface detector may be able to work optimally. This is supported by our finding that the MNC count showed an independent correlation co-efficient with both MNC CE and CFU-GM CE.

We have previously reported that MNC levels dropped throughout leukapheresis during recovery from chemotherapy and that this drop contributed to an apparently low overall CE because of the way CE is calculated based on the pre-apheresis count [13]. The mean post-apheresis count was 91% of the pre-apheresis count. An opposite trend was observed among G-CSF mobilization with the post-apheresis count higher than the pre-count (108%). This can be explained based on the progressive rise in cell counts seen in such patients so that leukapheresis did not deplete the circulating counts. We applied a correction factor of 108/91, i.e., 1.19 to the CE in the chemotherapy mobilization group. This brought the MNC CE to 76% and the CFU-GM to 66%. They are still lower than the 81.8% and 79.5% seen in the G-CSF group. Therefore the higher CE in the G-CSF group can only be partly explained by the rise in circulating counts during apheresis.

G-CSF mobilization has several advantages over the chemotherapy mobilization [14]: There are neither associated pancytopenia complications nor non-haematological chemotherapy-associated toxicities (e.g., cyclophosphamide cardiotoxicity), and usually the patients do not require hospitalization. The improvement in the CE is another benefit. It is yet to be determined if such improvement in the CE is observed with other haemotopoietic growth factors.

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