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Published in:
Journal of clinical apheresis

DOI:
10.1002/(SICI)1098-1101(1997)12:2<68::AID-JCA3>3.0.CO;2-7

Journal of Clinical Apheresis 12:68–73 (1997)

Adjustment of the Interface Detector (Location 71) to the Absolute Number of Mononuclear Cells in the Peripheral Blood: No Improvement of the Collection Efficiency of the Fenwal CS3000 Plus During Progenitor Cell Harvests

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Improvement of the collection efficiency (CE) of the Fenwal CS3000 plus in collecting circulating progenitor cells (CPC) might diminish the number of leukapheresis procedures (LP) required to obtain the CPC required to assure engraftment.

We analyzed whether adjustment of the optical setting (location 71,L71) to the number of MNC present in the peripheral blood could enhance the CE of the MNC. Thirty-five patients underwent 121 LP with an adjusted L71. We compared the results retrospectively with 26 LP performed with a fixed L71 (1:100) in 12 patients. The CPC were mobilized with chemotherapy followed by subcutaneous administration of granulocyte colony-stimulating factor (G-CSF) in both groups. Adjustment of the L71 did neither improve the CE of the MNC, the estimated CE of CD34+ cells nor diminished granulocyte contamination. For the total 121 LP with an adjusted L71 and for the total 26 LP with a fixed L71 the mean CE of MNC were, respectively, 44.6 ± 18.3% and 46.4 ± 14%. The mean granulocyte contamination, determined by manual white blood cell differentiation, was 1.7 ± 2.3% for the adjusted L71 group and 2.3 ± 3 for the fixed L71 group. There was no difference in the median number of LP required to obtain 3×10^6 CD34+ cells/kg between both groups. We found a weak significant correlation between WBC and pre-LP MNC count and the CE of MNC ($r = 0.36$, $P = 0.012$, resp.$r = 0.33$, $P = 0.023$), but no correlation between the CE of MNC and the estimated CE of CD34+ cells ($r = 0.24$, $P = 0.113$). In conclusion, adjustment of the L71 to the MNC did not improve the CE of MNC of the Fenwal CS3000. The lack of correlation between the CE and MNC and the estimated CE of CD34+ cells should be further explored. J. Clin. Apheresis 12:68–73, 1997.

Key words: leukapheresis; Fenwal CS3000 plus; collection efficiency; progenitor cell; adjustment interface detector

INTRODUCTION

Circulating progenitor cells (CPC) are successfully used as a source of hematopoietic stem cells for rapid and sustained hematological reconstitution after high dose chemotherapy [1–4]. Collections of CPC can be performed by automated cell separators [5,6]. The numbers and quality of CPC present in the harvests are influenced by patient characteristics like the extent of pretreatment and the mobilisation schedule [7–10].

Apart from these factors, the cell separator used for the collection of CPC has its own impact on the results of the harvests. Machine factors influencing the results of the CPC harvests include the type of cell separator used and the flow rate during the leukapheresis [5,6,11,12]. The parameter that is often used to express the efficacy of the cell separator is the collection efficiency of the mononuclear cells (MNC) [5,6,11,12]. Since the collection of CPC is labor-intensive, expensive, and time-consuming, it is desirable to optimize the collection efficiency, which might decrease the number and duration of the leukapheresis procedures, herewith saving costs and time of both patient and medical personnel.

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Received for publication 30 June 1996; Accepted 23 November 1996

We investigated whether the adjustment of the interface detector to the absolute number of mononuclear cells (MNC) in the peripheral blood of the patients might enhance the CPC collection efficiency of the Fenwal CS3000 plus, the continuous flow cell separator used in our institute for CPC harvests.

In addition, we compared the harvests obtained with and without the adjustment of the optical setting with relation to the extent of granulocyte contamination, the hematological reconstitution after circulating progenitor cell transplantation (CPCT), and several clinical parameters which might influence the collection efficiency, including the blood cell counts, plasma total protein, and erythrocyte sedimentation rate.

PATIENTS AND METHODS

Patients and Mobilisation Schedules

A total of 47 patients were included in the study. Thirty-five patients underwent the leukapheresis procedures with an adjusted optical setting (see below). We retrospectively compared the results of the harvests of CPC with the results of CPC harvests obtained from 12 patients who underwent the leukapheresis procedures with a fixed optical setting and who were monitored in the same way as the patients who underwent the leukapheresis procedures with an adjusted optical setting. The CPC were mobilized with a schedule containing 500 mg/m^2 5-FU, 120 or 90 mg/m^2 epirubicine, and 500 mg/m^2 cyclophosphamide given by intravenous injection on day 1, followed by daily subcutaneous injections of 5 μg/kg granulocyte colony-stimulating factor (G-CSF, filgrastim) from day 2 till the end of the leukaphereses in patients with breast cancer. The patients with malignant lymphoma, chorion carcinoma, or germ cell tumors received a mobilisation schedule containing 4 g/m^2 ifosfamide on day 1 (with mesna prophylaxis) and 100 mg/m^2 etoposide on days 1, 2, and 3 given by intravenous infusion followed by daily subcutaneous injections of 10 μg/kg G-CSF (filgrastim) from day 4 till the end of the leukaphereses.

All patients scheduled to undergo high dose chemotherapy with CPC support were required to have adequate renal function (creatinine clearance >60 ml/min), adequate hepatic function (bilirubine < 25 μmol/L, ASAT < 60 U/L) and adequate bone marrow function (white blood cell count $\geqslant 3.5 \times 10^9$/L, platelets $\geqslant 100 \times 10^9$/L). All patients had WHO performance status 0 or 1.

The patients' characteristics are shown in Table I.

The study was approved by the medical ethical and scientific review committees of the Netherlands Cancer Institute. Informed consent was obtained from all patients.

TABLE I. Patients' Characteristics*

	Fixed L71	Adjusted L71
No. of patients	12	35
Male:female	4:8	16:19
Diagnosis		
Breast cancer	6	15
Germ cell tumor	4	6
Malignant lymphoma	2	13
Chorion carcinoma	0	1
Median age in years	41	41
Range	29–48	23–55
Pretreatment		
Median cycles of CT	4	4
Range	3–13	2–36
Prior irradiation	3	10
Median body weight in kg	68	72
Range	51–98	53–125
High dose regimen		
CTC	10	21
BEAM	2	13
TBI/Cyc	0	1

*CT = chemotherapy; CTC = carboplatin, thiotepa, cyclophosphamide; BEAM = BCNU, etoposide, Ara-C, melphalan; TBI/cyc = total body irradiation, cyclophosphamide.

CPC Collection

In our hands, there is a good correlation between the number of granulocyte/macrophage colony-forming units (CFU-GM) and the number of CD34+ cells [13,14]. We therefore used the CD34 assay to monitor and guide the CPC harvests, since this method is simpler and less time-consuming than the determination of the number of CFU-GM.

From the eighth day of G-CSF administration, the percentage of CD34+ cells was determined daily. As soon as the white blood cell count (WBC) exceeded 3 × 10^9/L and a consistent rise in the percentage of CD34+ cells (percentage of CD34+ cells in the peripheral blood >0.2% of the total WBC) was observed, the leukapheresis procedures were started and continued for 1–5 consecutive days depending on the number of CD34+ cells harvested, the number of CD34+ cells in the peripheral blood and the number of transplantation procedures. The target yield for the harvest was a minimum of 3 × 10^6 CD34+ cells/kg for each transplantation procedure, since it is known that with this quantity rapid and sustained bone marrow recovery can be achieved [1,9,13]. A second mobilisation procedure was performed and/or bone marrow was harvested if this target yield was not obtained.

Prior to the mobilisation procedure, a double lumen Hickman catheter (13.5 French) was inserted in the subclavian or femoral vein. The leukapheresis procedures were performed in an outpatient setting with the continuous flow blood separator Fenwal CS3000 plus (Baxter, Utrecht, the Netherlands). The total blood volume processed in each session was 10 L at a flow rate of 50–60 ml/min. The small volume collection container was used for all collections. All patients underwent the leukapheresis at a centrifuge speed of 1,600 rpm.

The interface detector is an optical sensor which mea-

sures the amount of light transmitted through a segment of the component rich plasma tubing. By this method, the microcomputer monitors the component rich plasma tubing for the presence of cells and regulates the frequency of the spill-overs (see the manufacturer's manual and description of the Fenwal CS3000, Baxter B.V., October, 1990).

Modified procedure 1 (as advised by the manufacturer) with the interface offset setting of 100 (location 71, L71) was used in 12 patients. The 1-120 program (L71) was used in 35 patients. The 1-120 program includes changes to reduce the blocked line alarms at spill over and incorporates setting according to the hematocrit and the absolute number of MNC of the patients. This program can be used at draw rates of 20–80 ml/min by changing the draw rate with the front panel keys. The adjustment of L71 to the number of MNC present in the peripheral blood was performed according to the following schedule:

MNC	$<0.5 \times 10^9$/l	L71 = 100
MNC	$0.5 - <3 \times 10^9$/l	L71 = 120
MNC	$3 - <5 \times 10^9$/l	L71 = 140
MNC	$\geqslant 5 \times 10^9$/l	L71 = 160

Changes in L68 as a function of patient hematocrit for CPC collection were made in order to reduce the contamination of the MNC with other formed elements as previously reported [14]. Anticoagulant citrate dextrose (ACD) solution formula A was used as anticoagulant delivered at 11:1 ratio. No prophylactic calcium drip was used. Collection efficiency (CE) was determined using the following formula:

$$\text{CE in \%} = \frac{\text{Yield of MNC}}{\text{No. of MNC processed}} \times 100\%$$

The number of MNC processed is the mean of precollection and postcollection blood MNC counts multiplied by the volume of blood (without the anticoagulant) processed.

In addition, we determined the estimated collection efficiency of the Fenwal CS3000 plus with the relation to the CD34+ cells harvested. We had to use a simplified formula, since we did not perform CD34+ cell assays in the peripheral blood after the leukapheresis procedures. The estimated CE of CD34+ cells in %:

$$\frac{\text{Total number of CD34+ cells harvested}}{\text{Number of CD34+ cells present in the peripheral blood at the start of the leukapheresis multiplied by the volume of blood (without the anticoagulant) processed.}} \times 100\%$$

TABLE II. Wilcoxon Test for Differences Between the First Leukapheresis Procedures in the Adjusted and Fixed L71 Group*

	Adjusted L71 (N = 35)	Fixed L71 (N = 12)	*P* value
CE of MNC (%)	46.9 ± 22.9	47.9 ± 8.5	0.6961
CE of CD34+ cells (%)	34.6 ± 14.1	47.8 ± 13.4	0.0137
Granulocyte contamination (%)	1.7 ± 2.3	2.7 ± 3.0	0.3572

*The results are given as mean values and standard deviations.

Blood Cell Counts

Complete blood cell counts, including a five part white cell differential count, were performed before all procedures to assess the baseline values and to determine the adjustment of L68 and L71 to the blood cell counts of the patients. After each procedure the determination of the blood counts was repeated. The blood cell counts were performed by an automated blood cell counter (Coulter^R STKS, Coulter Corporation, Miami, FL). Manual white cell differential counts were performed on each leukapheresis product. Lymphocytes, monocytes, blast cells, myelocytes, and metamyelocytes were considered to be MNC. We included the granulocyte precursors in the MNC because, according to our experience, it is rather difficult to distinguish by manual differentiation granulocyte precursors from true mononuclear cells in CPC harvests obtained by a small collection container due to the fact that a large amount of cells are packed together in a small field.

Progenitor Cell Assays and Cryopreservation Procedure

The determination of the CD34+ cell content of the peripheral blood and leukapheresis products and the cryopreservation procedure were performed by standard flow cytometric methods as previously described by our group [13,14].

Statistical Analysis

Results were given as median and range or as mean and standard deviation. Nonparametric tests were performed, so distributional assumptions were not involved.

The Wilcoxon two-sample test was used to test whether a significant difference could be found in the CE of the MNC, the estimated CE of CD34+ cells and granulocyte contamination between the two treatment groups (Table II). A *P*-value of <0.05 was considered to be significant.

The Spearman rank correlation coefficient was calculated to study the association between the CE of MNC with: the estimated CE of CD34+ cells, hematocrit, erythrocytes, WBC for the start of the leukapheresis procedure, thrombocytes, absolute number of MNC present in the peripheral blood before the start of the leukapheresis procedure, total protein, and albumine and to study

TABLE III. Association Between CE of the MNC and Clinical Parameters During the First Leukapheresis Procedures (12 Procedures With a Fixed L71 and 35 Procedures With an Adjusted L71)

	CE of MNC Spearman rank correlation coefficient	*P* value
CE of CD34+ cells	0.24	0.1132
Hematocrit	0.19	0.1931
Erythrocyte counts	0.29	0.0604
WBC before the LP[a]	0.36	0.0122
Thrombocyte counts	−0.05	0.7160
Absolute MNC before LP[a]	0.33	0.0236
Total protein	−0.20	0.2141
Albumine	−0.19	0.2213
Erythrocyte sedimentation rate	0.19	0.2131

[a]Leukapheresis procedure.

the association between WBC and granulocyte contamination of the leukapheresis products as calculated by the manual white blood cell differential. A *P*-value of <0.05 was considered to be significant.

RESULTS

Effect of Adjustment of the Interface Detector on the CE of MNC and Granulocyte Contamination of the Harvests

In the 12 patients who underwent the leukaphereses with a fixed L71, a total of 26 (median 2, range 1–3) procedures were performed; the 35 patients with adjustment of the L71 underwent 121 (median 2, range 1–9) procedures.

The leukapheresis procedures were well tolerated by the patients. No procedure had to be interrupted due to complications or citrate toxicity. The alarms for blocked lines were always caused by catheter problems and were not due to problems with the cell separator.

There was no significant difference in the number of leukaphereses necessary to obtain the target yield of 3×10^6 CD34+ cells/kg body weight for each transplantation procedure (several patients underwent two or three transplantation procedures) between the patients who underwent their procedures with a fixed L71 (median 1, range 1–3) and those who underwent the procedure with an adjustment to the absolute number of MNC present in the peripheral blood (median 1, range 1– 7).

The difference in CE of MNC, the estimated CE of CD34+ cells, and granulocyte contamination of the first leukapheresis procedures of both groups are given in Table II. There was neither a statistical difference between the CE of MNC of the Fenwal CS3000 plus between the leukaphereses performed with a fixed and adjusted L71 nor a statistical difference in the contamination of granulocytes between the two groups.

In Table II and III we compared the CE of the first leukapheresis of each patient to rule out that certain (unknown) patient characteristics of the patients who had to undergo more than one leukapheresis procedure might influence the results.

The analysis of the total number of leukapheresis procedures showed the same results: there was no significant difference with relation to granulocyte contamination or the CE of MNC between the leukaphereses performed with a fixed and adjusted L71.

The mean CE of the MNC of the 26 leukapheresis procedures performed with a fixed L71 was 46.4% (standard deviation 14%), the median CE was 48%, range 10–77%. The mean CE of the MNC was 44.6% (standard deviation 18.3%) for the 121 leukapheresis procedures performed with an adjusted L71, the median CE was 48%, range 6–100%. There was a marked difference in the CE of MNC between different patients independent of the performed leukapheresis procedures (either fixed or adjusted L71). In addition, in some patients there was a marked difference in the CE of the MNC of consecutive performed leukapheresis procedures that was not related to differences in the WBC or MNC present in the peripheral blood or difficulties during the leukapheresis procedures.

Six out of the total 26 leukapheresis products (23%) harvested with a fixed L71, contained >5% granulocytes. The median granulocyte contamination was 1%, range 0–32%. Eleven of the 121 leukapheresis products (9.2%) harvested with an adjusted L71 contained >5% granulocytes. The median granulocyte contamination was 1%, range 0–50%.

Correlation Between CE of MNC and Estimated CE of CD34+ Cells and the Effect of the Adjustment of the Interface Detector on the Estimated CE of CD34+ Cells

The estimated CE of CD34+ cells in the group of patients who underwent the leukapheresis procedures with a fixed L71 was significantly higher compared to the group of patients who underwent the procedures with an adjusted L71 (Table II). Surprisingly, there was no significant correlation between the CE of the MNC and the estimated CE of the CD34+ cells (Table III).

Association Between CE of MNC and Clinical Parameters

We looked for patient characteristics which might have impact on the CE of the MNC. The results are summarized in Table III. The absolute MNC and the WBC for the start of the leukapheresis procedure were (borderline for the MNC) significantly associated with the CE of the MNC. Patients with a high WBC and absolute MNC had a high CE of the MNC.

There was no correlation between the WBC and the granulocyte contamination of the leukapheresis product

(Spearman rank correlation coefficient = 0.12, P = 0.3987).

Hematological Reconstitution After the CPCT

The results of the hematological reconstitution after the CPCT have been described in references 13 and 14 and will not be repeated in this article. There was no difference in the recovery rate between the group of patients who underwent the leukaphereses with a fixed or adjusted L71. As we have published elsewhere, the rate of recovery was dependent on the number of CD34+ cells reinfused [13,14] and is not influenced by the way the CPC were harvested.

DISCUSSION

This paper shows that adjustment of the optical setting to the absolute number of MNC did not lead to an enhanced CE of the MNC of the Fenwal CS3000 plus. This finding is consistent wtih the results of other authors [16]. The estimated CE of CD34+ cells was even worse in the group of patients who underwent the leukapheresis procedures with an adjusted optical setting.

In both groups there was a marked difference in the CE of MNC between different patients. In addition, in some patients there was a large difference in the CE of MNC of consecutive performed leukapheresis procedures that could not be explained by difficulties during the leukapheresis procedures or changes in the WBC or MNC present in the peripheral blood of that particular patient.

Apart from the failure to enhance the CE of the MNC, adjustment of the optical setting to the MNC of the patients did not result in a significant less contamination with granulocytes. The hematological reconstitution after the CPCT was similar in both groups of patients. Based on these results, it cannot be recommended to perform the leukapheresis procedures with the Fenwal CS3000 plus with an adjusted optical setting.

It is generally assumed that the specific gravity of MNC is similar to CPC. Up until recently there was no simple staining or counting method available for CPC. Therefore, the CE of MNC is often used to monitor if sufficient CPC have been collected by the leukapheresis machines.

In our patient patient population, however, we did not find a significant correlation between the CE of the MNC and the estimated CE of CD34+ cells. This could be due to the fact that the determination of the CE of MNC relies upon manual differentiation and/or determination of the blood cell counts by an automated blood cell counter, which are fairly rough methods. According to our experience, it is rather difficult to determine the degree of granulocyte contamination of the CPC harvests obtained with the small collection container by manual differentiation because a large amount of cells are packed together in a small field. In these harvests, it is sometimes difficult to distinguish true mononuclear cells from precursors of the granulocytes (myelocytes, metamyelocytes) and this can influence the estimation of the CE of the MNC and the estimation of the degree of granulocyte-contamination of the CPC harvests.

Since the number of CD34+ cells is a better parameter for circulating progenitor cells than MNC [1], it would be better to use the CE of CD34+ cells instead of the CE of MNC to determine the efficacy of cell separators. It would also be better to use flow cytometric methods to determine the degree of granulocyte contamination of the CPC harvests, since this is more reliable than determination by manual differentiation.

We were also interested in clinical parameters that might influence the CE of MNC of the Fenwal CS3000 plus. Of the clinical parameters studied, only the preleukapheresis count of the MNC and the WBC showed a weak but significant positive correlation with the CE of the MNC. This is in accordance with the results of To et al. [17]. It can be hypothesized that in patients with leucopenia or normal white blood cell counts, the interface detector oscillates across a thin layer of MNC exceeding the edges, herewith reducing the purity of the leukapheresis product [17]. When the MNC is high, the interface detector might therefore work better than when this count is low [17].

In summary, a standard optical setting at location 71 of 1:100 is appropriate for the harvests of CPC with the Fenwal CS3000 plus, since adjustment of the L71 to the MNC of the patients neither improved the CE of the MNC or the estimated CE of CD34+ cells, nor diminished the granulocyte contamination. In our opinion, the results of the CPC harvests should also be monitored by standard flow cytometric methods.

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