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$CD34^+$ cells

$CD34^+$ cell collection efficiency does not correlate with the pre-leukapheresis hematocrit

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Summary:

One hundred and seventy-seven large-volume leukapheresis procedures performed on 91 patients over a 15 month period were reviewed to see if the pre-apheresis hematocrit (Hct) affected the $CD34^+$ cell collection efficiency (CE) of the Fenwal CS 3000 Plus cell separator. The Hct was 0.174–0.461 (median 0.317), and the peripheral blood $CD34^+$ cell count 2–2487 per μl (median 21). The total $CD34^+$ cell quantity collected was 3.0–2677.2 $\times$ 10^6 (median 113.0). Based on the number of $CD34^+$ cells contained in the blood volume processed (23.3–37303.2 $\times$ 10^6; median 318.0), the CE was 1.7–87.5% (median 30.3). No correlation was found between the Hct and CE ($r^2 = 0.0034$; $P = 0.44$) or the total $CD34^+$ cell quantity collected ($r^2 = 0.0040$; $P = 0.40$). CEs for Hct <0.25 (median CE 36%), Hct 0.25–0.299 (median CE 30%) and Hct 0.30 (median CE 30%) were comparable. As expected, highly significant correlations were seen between the $CD34^+$ cell quantities collected and quantities processed ($r^2 = 0.59$; $P < 10^{-6}$) as well as the peripheral blood $CD34^+$ cell counts ($r^2 = 0.60$; $P < 10^{-6}$). We conclude that the minimum acceptable Hct or hemoglobin level for leukapheresis should be dictated by clinical circumstances because it does not affect stem cell collection. *Bone Marrow Transplantation* (2001) **28,** 597–601.

Keywords: apheresis; $CD34^+$ cells; cell separator; collection efficiency, FAHCT; hematocrit; leukapheresis

Peripheral blood has essentially replaced bone marrow as the source of hematopoietic stem cells for transplantation because of faster hematopoietic[1] and immune[2] recovery, and lower treatment-related mortality[3] and relapse rates.[2] The factors identified as important in determining $CD34^+$ cell yields have been studied extensively.[4–7] These include the peripheral blood $CD34^+$ cell count, the extent of prior therapy, diagnosis and mobilization technique, and have been reviewed in depth elsewhere.[8]

Technical issues surrounding the clinical aspects of leukapheresis have been studied less extensively.[9–22] Most of these studies have compared different cell separators or collection techniques. Data on collection efficiency (CE) of cell separators, especially with reference to the collection of $CD34^+$ cells, are limited.[18]

There are limited data on the impact of hematocrit (Hct) on CE.[18] Although Ford *et al*[18] found that higher leukocyte, Hct and albumin levels influenced CE inversely, there is a belief among some centers that higher Hct levels are associated with better $CD34^+$ cell collections. This coupled with varying perceptions of a 'safe' lower limit of Hct or hemoglobin during apheresis has resulted in a rather wide variation in the minimum acceptable Hb or Hct level to start leukapheresis amongst various centers. These levels range from Hb 8 g/dl or Hct 0.25 at the low end to Hb 10 g/dl or Hct 0.30 at the high (unpublished observations).

The standard operating procedure (SOP) at our institution has been to ensure a minimum Hct of 0.30 prior to leukapheresis from patients. However, a number of patients, usually mobilized with a combination of chemotherapy and growth factor, have been apheresed with lower Hct values. On the other hand, a number of patients have had their leukaphereses delayed on account of low Hct values despite being asymptomatic so that they could be transfused prior to apheresis. Such delays can potentially have a major adverse impact on patients and the transplant program in terms of scheduling, cost of additional growth factor, and the cost and consequences of transfusions.

Since no objective adverse clinical consequences were seen among patients apheresed with Hct values lower than 0.30 at our center, in accordance with requirements laid down by the Foundation for the Accreditation of Hematopoietic Cell Therapy (FAHCT), we sought to review and validate this SOP to see if the Hct limit needed to be modified. Our findings are presented here because they resulted in a modification of our SOP and have practical implications for other centers.

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Patients and methods

Sample selection

One hundred and seventy-seven consecutive leukapheresis procedures performed on 91 patients (1–6 procedures per patient; median 1) over a 15-month period (December 1999 to February 2001) were studied. Patient characteristics are shown in Table 1. Most patients with malignant diseases had non-Hodgkin's lymphoma, Hodgkin's disease, or multiple myeloma.

Availability of the following data was essential for inclusion in the study: peripheral blood $CD34^+$ cell count prior to apheresis on the day of apheresis (not routinely done prior to all apheresis procedures) and Hct prior to apheresis on the day of apheresis. Normal donors were excluded. Patients who were transfused prior to apheresis (because of low Hct) were included if Hct was repeated prior to apheresis, but were not included if these data were not available. Since 98% of leukapheresis procedures performed over this time period were performed using Fenwal CS 3000 Plus (Baxter, Deerfield, IL, USA) machines and only 2% on Cobe Spectra (Gambro BCT, Lakewood, CO, USA) machines, procedures performed on the Cobe machines were not included in the analysis.

Thus, not all patients apheresed during the specified time period were studied. For patients who were studied, not all procedures performed were studied.

Apheresis technique

All patients had rigid or semi-rigid 2- or 3-lumen in-dwelling catheters inserted for apheresis. The blood flow rate used for apheresis was 85 ml/min, and usually 15 l blood was processed. No adjustment was made to the collection technique based upon Hct. All patients provided informed consent for the procedure, and all experimental or research protocols were approved by the institutional review board.

Table 1 Patient characteristics

Number of patients	91
Number of procedures	177
Procedures per patient	1–6 (median 1)[a]
Diagnosis	
Malignant disorders	83 (163 procedures)
Auto-immune disorders	8 (14 procedures)
Mobilization	
Chemotherapy-growth factor	47 (94 procedures)
Growth factor alone	44 (83 procedures)
Pre-apheresis Hct	0.174–0.461 (median 0.317)
<0.25	8 (5%)
0.25–0.299	48 (27%)
0.30	121 (68%)
Absolute peripheral blood CD34 count (per μl)	2–2487 (median 21)
Blood volume processed	8–15 l
<15 l (8–14; median 11)	7 (4%)
15 l	170 (96%)

[a]These figures apply only to procedures for which all data were available (see 'Sample selection' under 'Patients and methods'). The actual number of aphereses performed on individual patients was higher.

Table 2 $CD34^+$ cell yields and collection efficiency

Total number of $CD34^+$ cells processed (10^6)	23.3–37303.2 (median 318.0)
Total number of $CD34^+$ cells collected (10^6)	3.03–2677.2 (median 113.0)
Collection efficiency (%)	1.7–89.3 (median 30.3)

Flow cytometry

$CD34^+$ cells were enumerated using standard techniques[13] on the peripheral blood prior to leukapheresis and on the apheresis product prior to processing and cryopreservation.

Data analysis

The $CD34^+$ cell CE was the proportion (expressed as %) of the total $CD34^+$ cells processed (ie passing through the cell separator) that was harvested, and was calculated as follows: {Total number of $CD34^+$ cells collected $\times 10^{-4}$}/{Absolute peripheral blood $CD34^+$ cell count per μl $\times$ Blood volume processed (l)}.

Regression analysis was used to assess the correlation between Hct and CE, Hct and the total number of $CD34^+$ cells collected, the absolute peripheral blood $CD34^+$ cell count and the total number of $CD34^+$ cells collected, and the total number of $CD34^+$ cells processed (ie the $CD34^+$ cell content of the total blood volume flowing through the machine) and the total number of $CD34^+$ cells collected. These are depicted in Figures 1–4. The figures are plotted on a logarithmic scale for all CD34 values and on an arithmetic scale for Hct and CE. The Wilcoxon rank-sum test was used to compare CEs for different Hct ranges.

Results

Table 2 shows the total number of $CD34^+$ cells processed, collected and CE. There was no significant correlation between the pre-apheresis Hct and CE ($r^2 = 0.0034$; $P = 0.44$; Figure 1) or the total $CD34^+$ cell quantity collected ($r^2 = 0.0040$; $P = 0.40$; Figure 2). The latter is not an

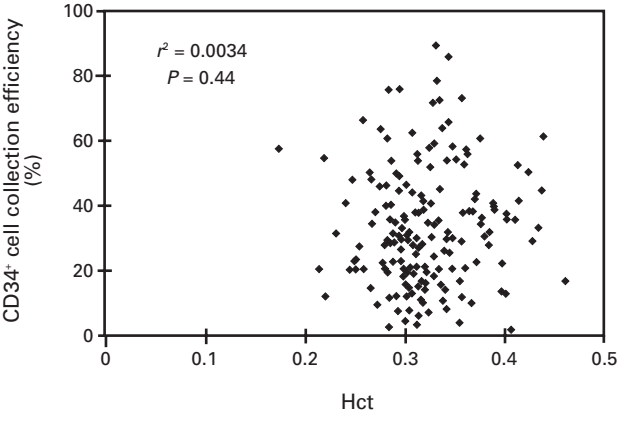

Figure 1 Lack of significant correlation between the pre-apheresis hematocrit and collection efficiency.

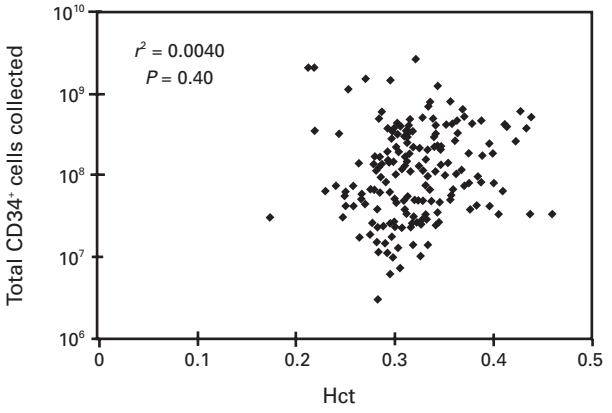

Figure 2 Lack of significant correlation between the pre-apheresis hematocrit and the total number of CD34+ cells collected.

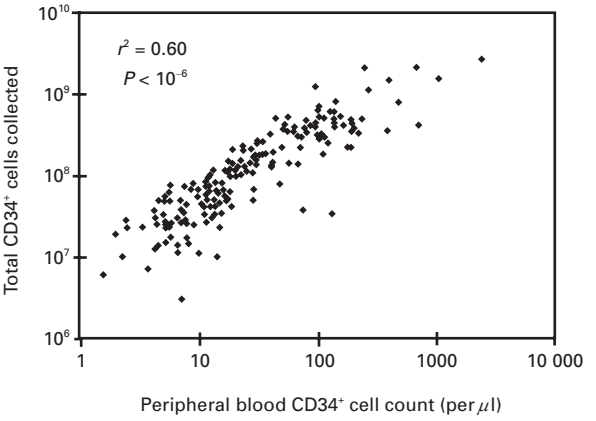

Figure 3 Highly significant correlation between the absolute peripheral blood CD34+ cell count and the total CD34+ cell quantity collected.

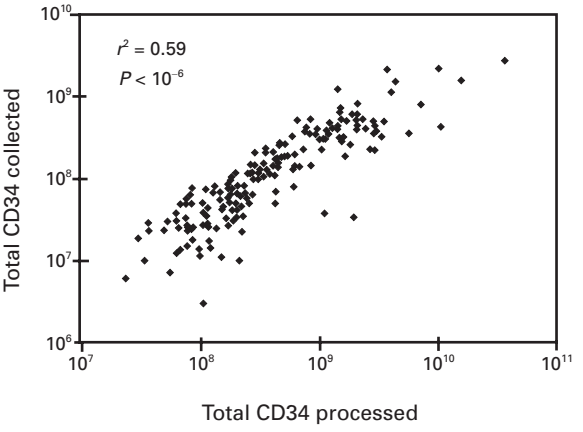

Figure 4 Highly significant correlation between the total number of CD34+ cells processed and the total quantity collected.

optimal comparison because it does not adjust for the absolute peripheral blood CD34+ cell count or the blood volume processed.

As Table 3 shows, CE was comparable with Hct ranges of <0.25, 0.25–0.299, and 0.30. Interestingly, the patient with the lowest Hct (0.174) appeared to have started leukapheresis through an oversight. The Hct was noticed when 8 l blood had already been processed without any adverse clinical consequences – and the apheresis was discontinued. The CE for this procedure was 57.5%.

As expected, the correlation between the absolute peripheral blood CD34+ cell count and the total CD34+ cell quantity collected was strong and highly significant ($r^2 = 0.60$; $P < 10^{-6}$; Figure 3). Similarly, the correlation between the total number of CD34+ cells processed and the total quantity collected was also strong ($r^2 = 0.59$; $P < 10^{-6}$; Figure 4).

No adverse events apart from symptomatic hypocalcemia requiring parenteral calcium supplementation were encountered.

Discussion

Our data show that the pre-apheresis Hct has no impact on CE or the total CD34+ cell numbers collected. As expected, there was strong correlation between the absolute peripheral blood CD34+ cell count and the total CD34+ cell quantity collected, and the total number of CD34+ cells passing through the machine and the total quantity collected.

The other reason for defining a threshold Hb or Hct level is patient safety. Leukapheresis results in depletion of red cells, and, consequently, lowered Hct and Hb. In normal donors undergoing one cycle of leukapheresis, the median decline in Hb has been found to be 1.2 g/dl.[22] It is reasonable to avoid starting leukapheresis with too low an Hb or Hct level in anticipation of this drop.

Since our threshold for transfusing patients under normal clinical circumstances is 8 g/dl (this limit is higher in patients with sepsis, bleeding or cardiorespiratory distress), based upon the findings of this analysis, we have lowered the minimum requirement for leukapheresis to a hemoglobin level of 9 g/dl which would correspond to an Hct value of 0.26–0.27.

Unlike Ford *et al*,[18] we did not detect an inverse relationship between Hct and CE. The other biologic factors influencing CE in their experience were albumin and the total

Table 3 Comparison of collection efficiencies for different hematocrit ranges

Group	*Hematocrit*	*Collection efficiency (%)*	
		Range	*Median*
A (n = 8)	<0.25	12.0–57.5	36.0
B (n = 48)	0.25–0.299	2.9–75.8	29.5
C (n = 121)	0.30	1.7–89.3	30.3
Comparison	*P*		
A vs B	0.69		
B *vs* C	0.92		
A *vs* C	0.72		

leukocyte count. However, they found that these three factors accounted for only a small part in the variability in CE. These biologic factors cannot be controlled or modified the way Hb or Hct can be through transfusions, and were not studied in the current analysis which was aimed at studying a factor (Hb/Hct) which could be changed in clinical practice (ie through transfusions).

In fact, there are few technical or procedural factors which can be modified to change the CE. In the case of the Fenwal CS 3000 Plus machine, changing the blood flow rate affects the CE.[12] Lin *et al*[12] showed that increasing the flow rate beyond the recommended 50 ml/min decreased CE for mononuclear cells and the total number of mononuclear cells collected. It is noteworthy that our procedures were performed at 85 ml/min – a rate far in excess of what is recommended by the manufacturer – which allowed 15 l blood to be processed in 3 h. This probably accounts for the wide range and relatively low median CE seen in this study. We could probably improve the median CE of 30% by slowing the flow rate – which would prolong the time taken to perform a 15 l apheresis to almost 6 h. An alternative is to use a machine like the Cobe Spectra which, when using the mononuclear cell collection protocol (version 4.7), can keep the CE constant at flow rates of 20–150 ml/min.[11]

Would the conclusions of the study hold true with optimum flow rates or different machines? While they probably would, this is a matter for future work.

Our experience also underscores an important quality management issue that is being brought into sharp focus through valuable inspections carried out by review and accrediting organizations such as FAHCT. That is all SOPs must be reviewed on a periodic basis for their validity, and should be modified as indicated. Measured by our previous standard, 32% of the procedures performed were 'deviations' despite not being associated with poor collections or adverse clinical consequences. Measured by our new standard, only 8% (Hct <0.26) to 10% (Hct <0.27) of the procedures are 'deviations'.

We conclude that the pre-apheresis Hct does not have any impact on $CD34^+$ cell collections or CE of the cell separator. In addition to an obvious factor such as the absolute peripheral blood $CD34^+$ cell count, the decision on commencement of leukapheresis should be based upon the clinical condition of the patient rather than the Hb or Hct level.

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