

## References

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### Enumeration of CD34<sup>+</sup> cells in fresh and thawed/washed placental/umbilical cord blood units and thawed unit segments

Sir,

The current perspective of expanding the clinical use of human placental/umbilical cord blood (PB) for transplantation requires standardization of methods for its collection, processing, characterization and cryopreservation. In this regard, the flow cytometric enumeration of hematopoietic progenitor cells by detection of the CD34 antigen is a critical step. A number of assays to count CD34<sup>+</sup> cells have been proposed. Most express the result as a percentage of total WBC or nucleated cells, which are usually counted with a hematology cell counter. This may be a cause of inaccuracy of the absolute CD34<sup>+</sup> cell count, especially in thawed/washed samples.

Recently, new procedures which allow the determination of the absolute CD34<sup>+</sup> cell count have been developed to overcome this problem. To our knowledge, limited information is available on the validity of these methods with fresh and frozen PB.

In this study we determined CD34<sup>+</sup> cell counts in 10 routine PB units before and after thawing/washing,<sup>1,2</sup> with three methods that do not require a separate

WBC count. We used, in parallel, the commercial IMAGN 2000 STELLer assay,<sup>3</sup> (Biometric Imaging, Mountain View, CA, USA), the ProCOUNT system, (Becton Dickinson Immunocytometry Systems, San Jose, CA, USA) and our local flow-cytometric method.<sup>4,5</sup> Our local method is a lyse and wash procedure in which, just before acquisition, a known number of Flow-Count™ fluorospheres (Coulter Corporation, Miami, FL, USA) are added to each sample in order to obtain the number of CD34<sup>+</sup> cells/μL directly.

Moreover, we evaluated whether segments from the cryopreservation bag tube, which can be used for quality control before transplantation, allow an accurate estimation of the unit's CD34<sup>+</sup> cell content and cell viability. The segments were stored in a pouch of the freezing bag and thawed at 37°C. Their content was slowly diluted with 10 mL of RPMI/25 percent fetal calf serum and centrifuged at 300 g x 10 min. The cell button was resuspended to 500 μL of RPMI/25 percent fetal calf serum and evaluated at the same time as the bag sample.

The CD34<sup>+</sup> cell counts obtained with the three methods were compared with Friedman's test.<sup>6</sup> *p* values below 0.05 were considered statistically significant. Furthermore, we performed Wilcoxon's test<sup>6</sup> to determine whether, within each method, CD34<sup>+</sup> cell counts and cell viability obtained in thawed/washed segments were different from those obtained in thawed/washed units.

Differences of CD34<sup>+</sup> cell counts did not reach statistical significance when the methods were used on fresh PB samples. Conversely, differences between methods reached statistical significance when specimens from thawed/washed units and from segments were used (Table 1). Mean and SD cell viabilities of thawed/washed unit and segment specimens were 97±6 percent and 69±10 percent respectively. Cell viability and CD34<sup>+</sup> cell counts computed from thawed/washed segment samples were significantly lower (*p*<0.05) than those found with each of the three methods in the corresponding thawed/washed units. These data suggest that the three methods pro-

**Table 1. Median (and range) number of CD34<sup>+</sup> cells/μL determined with 3 methods in 10 PB units before freezing (fresh units) and after freezing/thawing/washing (thawed units) and in 10 thawed/washed segments from the same units (thawed segments).**

Method	STELLer	ProCOUNT	Local method	Friedman test
Fresh units	24 (8-81)	19 (4-83)	20 (4-81)	r = 0.95, p>0.05
Thawed units	20 (9-74)	18 (6-89)	15 (6-59)	r = 12.4, p<0.05
Thawed segments	11 (4-23)	4 (2-21)	5 (2-18)	r = 10.4, p<0.05

duce comparable results with fresh samples, but do not so with thawed/ washed ones. Furthermore, we would caution against extrapolation of unit counts from segment specimens.

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### Key words

Cord blood units, CD34<sup>+</sup> cell count

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### GM-IVA, a short induction course for *de novo* acute myeloid leukemia, suitable for the elderly

Sir,

The number of older patients with AML is progressively increasing due to longer life expectancy and increased exposure to mutagenic agents.<sup>1</sup> The therapeutic approach in these patients is uncertain<sup>2-4</sup> and prognosis is generally poor both due to the patients' conditions and to unfavorable biologic features.<sup>5</sup>

We report on our experience with a short regimen that includes GM-CSF, Ara-C, VP-16 and idarubicin, followed by the FLANG protocol, a fludarabine containing salvage regimen, for patients with residual disease. Between May 1994 and January 1996, 21 consecutive patients with non M3 *de novo* acute myeloid leukemia entered the study. Poor performance status

was not considered an exclusion criterion.

Clinical and hematologic features are summarized in Table 1. Mean age was 64 years (range 29-85) and fourteen patients were over 60 years of age.

Diagnosis and classification of AML were performed according to the FAB criteria. Cytogenetic analysis was performed for all subjects at diagnosis on 24 h cultured BM cells according to standard procedures. At least 10 Q-banded metaphases were examined for each sample. Five (26%) abnormal karyotypes were found. Patients in complete remission also had cytogenetic follow up.

The GM-IVA scheme included idarubicin (12 mg/m<sup>2</sup>/day, by rapid infusion), etoposide (100 mg/m<sup>2</sup>/day by 30 minute infusion) and cytosine arabinoside (250 mg/m<sup>2</sup>/die in a continuous infusion preceded by a 100 mg i.v. bolus on day 1). All drugs were administered for three days. GM-CSF (300 mg) was given 12 hours before the start of therapy.<sup>6</sup> Patients with a blast count exceeding 100×10<sup>9</sup>/L were not primed with GM-CSF. Three patients died during the induction therapy. Patients with blastic regrowth after one course of GM-IVA were considered resistant and salvaged with FLANG.<sup>7</sup>

Patients under 60 years old achieving complete remission received 2 courses of FLANG and autologous or allogeneic BMT whenever possible.

Patients over 60 in CR received a second course of GM-IVA whenever possible. The results are reported in Table 2.

The therapeutic strategy itself was effective since 65% of patients achieved CR. The complete remission rate obtained in the younger (< 60 years) subset of patients (85%) is comparable to what has been reported in recent trials.<sup>10</sup> In elderly patients the final CR rate (62%) is noteworthy, considering that 6 patients were above 70 years of age and 3 were above

**Table 1. AML treated with GM-IVA: clinical features.**

Treated patients	21
M/F	12/9
Median age (range)	64 (29-85)
WHO performance status:	
0	5
1	10
2	4
3	2
FAB subtype	
M0	1
M1	3
M2	7
M4	6
M5	4
Karyotype	
interm/unfav. prognosis :	5
normal/favor. prognosis:	16

Abbreviations: favorable prognosis: t(8:21), inv16; interm/unfavorable prognosis: +8, complex, -5, del 5, -7, del 7.