Letter to the Editor

What is the adequate mononuclear cell content for selecting umbilical cord blood units for cryopreservation?

Over the past decade umbilical cord blood (UCB) has been used as an alternative source of hematopoietic stem cells (HSCs). Worldwide, more than 500,000 cord blood units (CBUs) have been cryopreserved and stored in banks. It has been shown that the CD34+ cell content influences engraftment and survival after UCB transplants. Although there are no fully agreed upon standardized criteria for selecting units for cryopreservation, most cord blood banks use a combination of the total nucleated cell (TNC) count and volume; a TNC count ≥8 × 10^8 has been considered the best predictor for CD34+ HSC content. No such parameter has been established for the mononuclear cell count (MNC).

Recently it has been proposed that in order to assure that stem cells will perform as intended, the quality and potency of UCB hematopoietic progenitor cells should be measured. This can only be done using the MNC fraction, not the TNC fraction. Given this recent development, we assessed the possibility of using the MNC to predict the CD34+ cell content in cord blood and its relationship to the TNC count.

We analyzed 857 CBUs received at the cord blood bank of the Hematology Department of the Hospital Universitario ‘Dr. Jose E. Gonzalez’, Universidad Autónoma de Nuevo León in Monterrey, México. TNC counts were determined with an automated hematology analyzer (Sysmex XT 2000, Sysmex, Mundelein, IL, USA) using a multangle polarized scatter separation technique which provides the primary TNC count. Total TNC counts were calculated by multiplying the primary TNC count (per microliter) by the total volume of the CBU. Total MNC counts were calculated by adding the absolute lymphocyte and monocyte counts (per microliter) reported in the complete blood count and then multiplying this value by the total volume of the bag. CD34+ cell counts were determined by employing fluorescein isothiocyanate labeled anti-CD45+ monoclonal antibodies (FITC; Becton Dickinson, San Jose, CA, USA) and monoclonal anti-human CD34+ Class III/FITC, Clone BIRMA K-3 (DAKO, Denmark) in a FACScalibur flow cytometer (Becton Dickinson). The mean collected volume of the CBUs was 96.98 ± 28.88 mL and the mean TNC, MNC and CD34+ counts were 9.95 ± 4.97 × 10^8, 5.10 ± 2.65 × 10^8 and 2.95 ± 2.33 × 10^6, respectively. We divided the CBUs into two groups depending on the CD34+ cell count (less than or greater than 2 × 10^6). Receiver operating characteristic (ROC) curve analysis comparing TNC and MNC to select CBUs with a CD34+ cell count of ≥2 × 10^6 were designed. The optimal operating points for TNC and MNC that best correlated with a CD34+ count ≥2 × 10^6 were determined (Table 1; Figure 1). A significant difference between TNC and MNC was not found (p-value = 0.059). In addition, a TNC >7.94 × 10^8 was selected by ROC analysis as the point that best reflects the MNC guaranteeing a CD34+ count ≥2 × 10^6 (Table 1).

Previously, we applied a ROC model to analyze the correlation between volume, TNC and MNC with the CD34+ count, and established the TNC content as the most significant parameter. In the present report, we evaluated the degree to

![Figure 1](image-url)
which a TNC count $\geq 8 \times 10^8$ reflects a MNC content to meet the required CD34$^+$ count and found that the mean MNC count was $5.10 \times 10^8$ (63.75% of the mean TNC count). Moreover, ROC analysis selected a MNC of $4.05 \times 10^8$ as the cutoff point to achieve an optimal CD34$^+$ count ($\geq 2 \times 10^8$).

Thus, our findings confirm that the current TNC standard for UCB cryopreservation gives reliable information on the MNC content and suggests a cutoff point for the lowest fraction for transplantation purposes.

## Conflicts of interest

The authors declare no conflicts of interest.

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## REFERENCES


José Carlos Jaime-Pérez *, Gisela García-Arellano, Alejandra Celina Esparza-Sandoval

Universidad Autónoma de Nuevo León, Monterrey, Mexico

*Corresponding author at: Servicio de Hematología, Edificio “Dr. Rodrigo Barragan”, 2° piso, Hospital Universitario “Dr. Jose E. Gonzalez”, Av. Madero y Gonzalitos S/N, Colonia Mitras Centro, Monterrey, Nuevo Leon C.P. 64460, Mexico.
E-mail address: carjaime@hotmail.com (J.C. Jaime-Pérez).

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