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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.[1] It has been shown that the $CD34^+$ cell content influences engraftment and survival after UBC transplants.[2] 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 $\geq 8 \times 10^8$ has been considered the best predictor for $CD34^+$ HSC content.[3] 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.[4,5] 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 multiangle 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 $9.95 \pm 4.97 \times 10^8$, $5.10 \pm 2.65 \times 10^8$ and $2.95 \pm 2.33 \times 10^6$, 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 $\geq 2 \times 10^6$ were designed. The optimal operating points for TNC and MNC that best correlated with a $CD34^+$ count $\geq 2 \times 10^6$ were determined (Table 1; Figure 1). A significant difference between TNC and MNC was not found (p-value = 0.059). In addition, a TNC $>7.94 \times 10^8$ was selected by ROC analysis as the point that best reflects the MNC guaranteeing a $CD34^+$ count $\geq 2 \times 10^6$ (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.[3] In the present report, we evaluated the degree to

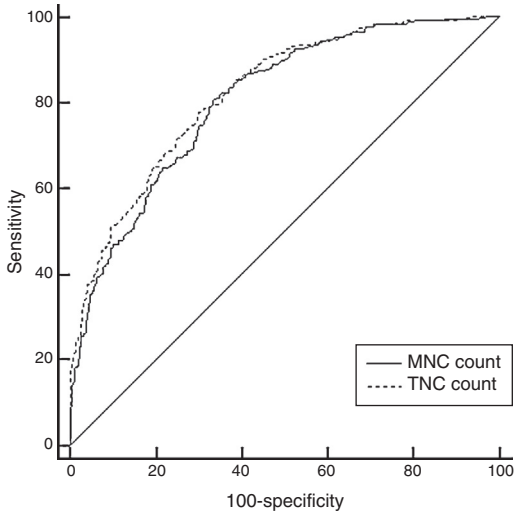

Figure 1 – Receiver operating characteristic (ROC) curve analysis comparing the total nucleated cell (TNC) count [area under the curve (AUC) = 0.822] vs. the absolute mononuclear cell (MNC) count (AUC = 0.806) as criteria for selecting cord blood units suitable for cryopreservation, that is, a $CD34^+$ cell content $\geq 2.0 \times 10^6$. No significant difference was found (p-value = 0.059).

Table 1 – A comparison between total nucleated cells and mononuclear cells for the selection of cord blood units with $CD34^+$ counts $\geq 2 \times 10^6$.

	Performance evaluation						Comparison of ROC curves		
	Sensitivity	Specificity	−PV	+PV	+LR	−LR	AUC	Difference between areas	*p*-Value
TNC cutoff ($\times 10^8$)							0.822		
>8.33	77.86	69.88	67.70	79.60	2.59	0.32			
>7.94*	80.97	64.33	69.20	77.40	2.27	0.30			
MNC cutoff ($\times 10^8$)							0.806		
>4.05	80.78	66.37	69.60	78.30	2.40	0.29		0.0159	0.059

TNC: total nucleated cell; MNC: mononuclear cell; ROC: receiver operating characteristic; −PV: negative predictive value; +PV: positive predictive value; +LR: positive likelihood ratio; −LR: negative likelihood ratio; AUC: area under the curve.

* Optimal operating point chosen by ROC analysis for TNC.

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 5.10×10^8 (63.75% of the mean TNC count). Moreover, ROC analysis selected a MNC of 4.05×10^8 as the cutoff point to achieve an optimal $CD34^+$ count ($\geq 2 \times 10^6$).

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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