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 ≥8×10^6 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 multivariate 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 ×10^8, 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 ×10^6 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 – 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 ≥2.0×10^6. No significant difference was found (p-value = 0.059).
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 ($\geq 2 \times 10^6$).

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 frac-
tion for transplantation purposes.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgement

We thank Sergio Lozano-Rodriguez M.D. for his review of the manuscript.

REFERENCES

1.azzocchetti D, Berti AM, Sartini R, Lucarini A, Ragusa G, Caroli M, et al. Total nucleated cells as a sole predictor of
distinct targets of hematopoietic potential (CD34+ cells) in cord
blood units: the results of a large series analysis in autologous
2. Solves F, Carbonell-UBeros F, Mirabet V, Roig R. CD34+ cell
content for selecting umbilical cord blood units for
3. Jaime-Perez JC, Monreal-Robles R, Rodriguez-Romo LN,
Mancias-Guerra C, Herrera-Garza JL, Gomez-Almaguer D.
Evaluation of volume and total nucleated cell count as cord
blood selection parameters: a receiver operating characteristic
721–6.
4. Patterson J, Moore CH, Palser E, Hearn J, Dumitru D, Harper
HA, et al. Detecting primitive hematopoietic stem cells in total
nucleated and mononuclear cell fractions from umbilical cord
5. Rich IN. Improving quality and potency testing for umbilical
cord blood: a new perspective. Stem Cells Transl Med.
2015;4(9):967–73.

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

Universidad Autónoma de Nuevo León, Monterrey, Mexico

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

Received 18 December 2015
Accepted 9 January 2016
Available online 9 February 2016

https://dx.doi.org/10.1016/j.bjhh.2016.01.003
1516-8484/© 2016 Associação Brasileira de Hematologia,
Hemoterapia e Terapia Celular. Published by Elsevier Editora
Ltda. All rights reserved.