Hematopathology / Umbilical Cord Blood Selection

Evaluation of Volume and Total Nucleated Cell Count as Cord Blood Selection Parameters

A Receiver Operating Characteristic Curve Modeling Approach

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Key Words: Umbilical cord blood banking; Cord blood selection; CD34+ cord blood cells; Cord blood total nucleated cell content; Cord blood volume; Receiver operating characteristic analysis; Sensitivity; Specificity

DOI: 10.1309/AJCPFB6EXO7BJVLR

Abstract

The objective of the study was to evaluate the current standard practice of using volume and total nucleated cell (TNC) count for the selection of cord blood (CB) units for cryopreservation and further transplantation. Data on 794 CB units whose CD34+ cell content was determined by flow cytometry were analyzed by using a receiver operating characteristic (ROC) curve model to validate the performance of volume and TNC count for the selection of CB units with grafting purposes. The TNC count was the best parameter to identify CB units having 2 × 10⁶ or more CD34+ cells, with an area under the ROC curve of 0.828 (95% confidence interval, 0.800-0.856; P < .01) and an efficiency of 75.4%. Combination of parameters (TNC/mononuclear cells [MNCs], efficiency 74.7%; TNC/volume, efficiency 68.9%; and volume/MNCs, efficiency 68.3%) did not lead to improvement in CB selection. All CB units having a TNC count of 8 × 10⁸ or more had the required CD34+ cell dose for patients weighing 10 kg or less.

Umbilical cord blood (UCB) is used as an alternative source of progenitors for hematopoietic stem cell (HSC) transplantation. Worldwide, more than 450,000 UCB units have been stored in CB banks (CBBs), and more than 10,000 UCB transplants have been performed in children and adults for a variety of disorders, including hematologic malignancies, bone marrow failure syndromes, selected hereditary immunodeficiency states, and metabolic disorders. The current status and future trends in this complex and rapidly evolving field have been recently reviewed.

HSCs are contained within a population of mononuclear CD34+ antigen-expressing cells, which typically represent fewer than 1% of the total leukocytes in CB. The CD34+ antigen is the accepted marker for determining the HSC content in bone marrow, peripheral blood, and UCB. Although no universally accepted guidelines exist, most CBBs use the combination of product weight (volume) and total nucleated cell (TNC) count as the main selection factors for cryopreservation, requiring a TNC content from 6 to 10 × 10⁸ for storage and a minimal volume between 40 and 60 mL. The CD34+ cell content has been shown to influence engraftment and survival after unrelated UCB transplantation, better predicting the hematopoietic potential of a CB unit than nucleated cell content.

For partially mismatched HLA transplants, the number of CD34+ cells infused is critical, with 1.7 × 10⁵ CD34+ cells per kilogram of the recipient’s body weight the threshold dose. Taking into account a 10% to 20% cell loss during freezing-thawing procedures, a dose of 2.0 × 10⁵ CD34+ cells per kilogram of recipient’s weight before cryopreservation seems to be more suitable for the selection of CB units for storage. To know the CD34+ cell number in a CB unit could...
be relevant in several aspects, including matching the weight of the intended recipient with the appropriate unit; also, it would allow more precise selection of CB units to perform double-unit transplants for adolescents and adults, as reported.\textsuperscript{14} However, owing to the lack of standardization of CD34+ cell counting methods, it is currently not possible to compare CD34+ cell counts among CBBs or transplant centers.

Receiver operating characteristic (ROC) curve analysis is a graphic representation of the reciprocal relationship between sensitivity and specificity and provides an objective measure of the overall diagnostic performance of a test; it has been frequently used to compare diagnostic tests.\textsuperscript{15,16} We were able to locate only 2 published reports on CB selection using ROC analysis; one focused on obstetric factors to establish selection criteria for CB cryopreservation,\textsuperscript{17} and the other dealt with fetal biometry in which prebirth assessment of biparietal diameter might allow the selection of donors who would yield CB units of sufficient volume and cell content.\textsuperscript{18}

We decided to use formal statistical analysis and ROC curve modeling to analyze the standard practice at most CBBs for using volume and/or the TNC count for the proper selection of suitable CB units for further transplantation.

**Materials and Methods**

The characteristics of 794 CB units received between May 2005 and May 2010 at the CBB of the Hematology Department, “Dr José E. Gonzalez” University Hospital, School of Medicine of the Universidad Autónoma de Nuevo León, Monterrey, México, were retrospectively analyzed for this study.

**UCB Collection**

Informed consent for UCB collection was obtained from healthy women with uncomplicated pregnancies receiving care at the Obstetrics Department, University Hospital “Dr. José Eleuterio González” and other regional hospitals. UCB was collected by gravity into a 150-mL sterile bag collection set (Grifols, Barcelona, Spain), containing 25 mL of citrate-phosphate-dextrose anticoagulant. The CB unit was then stored on wet ice for transport to the CBB for processing. As do others,\textsuperscript{10,11,19} at our CBB, we use volume and the TNC count as criteria for the selection of CB units for cryopreservation. UCB units were analyzed for nucleated cell counts and were cryopreserved if their TNC number was at least 8 × 10\textsuperscript{8} and their volume exceeded 80 mL, including the anticoagulant. The time from collection to processing was set at 48 hours or less.

**Laboratory Processing**

After removal of aliquots for routine testing, UCB in the collection bag was mixed with 6% hydroxyethyl starch (HES) in a 1:4 ratio, with 30 mL as the maximum. The buffy coat was then separated by centrifuging at 45g for 7 minutes at 10°C according to a method previously described.\textsuperscript{20} The UCB/HES mixture and the supernatant plasma were transferred into a second plasma transfer bag without severing the connecting tube.

A second centrifugation step was performed at 600g for 15 minutes at 10°C. The cell suspension was removed into a processing set (Pall Medical, Covina, CA) and adjusted to precisely 20 mL. The equipment used to perform the volume reduction was an automated closed system, Sepax-100 (Biosafe America, Houston, TX). The cryocyte bag was placed in the Coolmix-210 (Biosafe America), which kept it in constant agitation for adding the cryoprotective mixture, which consists of 2.5 mL of DMSO (dimethyl sulfoxide) 99.9% (Sigma-Aldrich, St Louis, MO) and 2.5 mL of Dextran 40 (Sigma-Aldrich). When the temperature reached 4°C, the cryocyte bag was placed in a protective aluminum cassette (Medical Technology Vertriebs, Altdorf, Germany) and then deposited horizontally on a level surface inside a controlled-rate freezer (Cryomed, Thermo Forma, Marietta, OH) until it reached –90°C. The units were then transferred into the liquid phase of a nitrogen freezer (Cryoplus, Thermo Forma) for long-term storage.

**Statistical Analysis**

Normality of the studied variables was assessed with the Kolmogorov-Smirnov test. Spearman correlation set at a significance level of .01 was performed to assess the correlation of volume and TNC and MNC counts with the CD34+ cell count.
cell content of the CB units. ROC curve analysis was applied to evaluate the performance of volume, TNC count, and absolute MNC counts as a method for identifying CB units with a CD34+ cell content of $2 \times 10^6$ or more, determining also the optimal operating point allowing the best performance for the CB parameter selected. We arbitrarily added different cutoff points for comparing the performance of the selected parameter at different values, determining their sensitivity, specificity, positive and negative predictive values, efficiency, and positive and negative likelihood ratios.

The analyzed units were divided in 2 groups depending on a CD34+ cell count, $2 \times 10^6$ or more or fewer than $2 \times 10^6$ as determined by flow cytometry. Descriptive statistics (percentages, mean, SD, median, and range) were used to analyze the UCB units group with a count of $2 \times 10^6$ or more CD34+ cells, presenting the number and total CD34+ cell content of CB units that would be suitable for transplantation based on the required dose of $2 \times 10^5$ CD34+ cells/kg, estimated for recipients weighing 10 to 40 kg. The statistical package used was SPSS, version 17.0 (SPSS, Chicago, IL). MedCalc statistical software (MedCalc, Mariakerke, Belgium) was used for ROC curve analysis.

**Results**

We studied 794 CB units received at our CBB during a 5-year period. By applying the current criteria, we found that our banking efficiency was 57.5%. The initial analysis revealed a direct correlation in volume, absolute TNC and MNC counts, and the total number of CD34+ cells. The TNC content had the most significant correlation with the number of CD34+ cells ($r = 0.681$; $P < .01$), compared with the MNC count ($r = 0.655$; $P < .01$) and volume ($r = 0.581$; $P < .01$).

ROC analysis showed the TNC count to be the parameter with a superior area under the ROC curve, 0.828 (95% confidence interval [CI], 0.800-0.856), with a significant difference compared with the MNC count (0.810; 95% CI, 0.780-0.840; $P < .05$) and volume (0.784; 95% CI, 0.753-0.816; $P < .001$)

**Table 1.** Performance Evaluation for Different ROC-Derived TNC Cutoff Values for Cord Blood Selection

<table>
<thead>
<tr>
<th>Performance Measure</th>
<th>TNC Cutoff Value ($\times 10^8$)</th>
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<tbody>
<tr>
<td></td>
<td>8.0</td>
</tr>
<tr>
<td>Sensitivity (%)</td>
<td>80.9</td>
</tr>
<tr>
<td>Specificity (%)</td>
<td>65.6</td>
</tr>
<tr>
<td>Negative predictive value (%)</td>
<td>70.7</td>
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<tr>
<td>Positive predictive value (%)</td>
<td>76.9</td>
</tr>
<tr>
<td>Efficiency (%)</td>
<td>74.5</td>
</tr>
<tr>
<td>Positive likelihood ratio</td>
<td>2.4</td>
</tr>
<tr>
<td>Negative likelihood ratio</td>
<td>0.3</td>
</tr>
</tbody>
</table>

ROC, receiver operating characteristic; TNC, total nucleated cell.

* Optimal operating point chosen by ROC analysis.
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The characteristics of the CB units meeting a CD34+ cell content of $2 \times 10^6$ or more ($n = 466 \left[58.7\%ight]$) were further analyzed. The median volume obtained was 106.3 mL (range, 48.0-213.2 mL) with a median TNC content of $11.0 \times 10^8$ (range, $2.5 \times 10^8$-$36.6 \times 10^8$) and a median CD34+ cell count of $3.4 \times 10^6$ (range, $2.0 \times 10^6$-$19.4 \times 10^6$) after volume reduction and before cryopreservation. Based on the requirement of $2 \times 10^5$ CD34+ cells per kilogram of recipient weight before freezing the CB, this approach would make it possible to use 100%, 37.3%, 15.2%, and 7.5% of CB units for patients weighing 10, 20, 30, or 40 kg, respectively (data not shown).

It is interesting that when applying the ROC-selected TNC cutoff as selection criterion to the 466 CB units having a CD34+ cell content of $2 \times 10^6$ or more, the CD34+ cell median content increased by 11%, from $3.4 \times 10^6$ to $3.8 \times 10^6$, although at the expense of a 21% ($n = 99$) decrease in the number of CB units available for transplantation. This approach would lead to an increase in the percentage of units stored that could be safely grafted, particularly for patients weighing 15 to 25 kg.

Discussion

Although different studies have been performed regarding selection, collection, and processing of CB, improvement in methods is needed to maximize resource utilization without compromising quality for clinical use, to increase efficacy, and to reduce the cost of CB grafting.

The TNC count had the largest area under the ROC curve, confirming that it is the primary driver of the CD34+ cell content in CB. Combining 2 parameters to select CB did not lead to an improvement in the relationship between sensitivity and specificity; therefore, the TNC count as a single criterion seems to be sufficient for selecting CB units for cryopreservation. Most of the CB units not meeting the CD34+ cell content of $2 \times 10^6$ or more had a TNC count below the ROC-selected value. These units would not have been processed for CD34+ cell content had this cutoff point been applied. It is worth mentioning that of all CB units with a TNC count more than the ROC-selected value, only 20% had a CD34+ cell count of less than $2 \times 10^6$. These units, however, could potentially be used for double partially HLA-matched UCB transplantation in adults who otherwise are not eligible for allogeneic grafting owing to the lack of a suitable single-UCB unit, as previously reported.

Table 2

<table>
<thead>
<tr>
<th>TNC Cutoff ($\times 10^8$)</th>
<th>Mean ± SD</th>
<th>Median</th>
<th>Patient Weight (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>8.0</td>
<td>4.4 ± 2.5</td>
<td>3.7</td>
<td>377</td>
</tr>
<tr>
<td>8.32‡</td>
<td>4.5 ± 2.6</td>
<td>3.8</td>
<td>367</td>
</tr>
<tr>
<td>9.0</td>
<td>4.6 ± 2.6</td>
<td>3.9</td>
<td>325</td>
</tr>
<tr>
<td>10.0</td>
<td>4.9 ± 2.7</td>
<td>4.2</td>
<td>278</td>
</tr>
<tr>
<td>11.0</td>
<td>5.2 ± 2.9</td>
<td>4.6</td>
<td>231</td>
</tr>
<tr>
<td>12.0</td>
<td>5.3 ± 3.0</td>
<td>4.6</td>
<td>188</td>
</tr>
<tr>
<td>13.0</td>
<td>5.7 ± 3.0</td>
<td>4.9</td>
<td>155</td>
</tr>
</tbody>
</table>

MNC, mononuclear cells; TNC, total nucleated cell.

‡ Optimal operating point chosen by receiver operating characteristic analysis.

For 20-40 kg, data are given as number (percentage) in relation to the value in the 10-kg column.

All units met the required dose for patients weighing 10 kg; 89 units containing $\geq 2 \times 10^6$ CD34+ did not meet the $8 \times 10^8$ TNC content.

Figure 2 Receiver operating characteristic (ROC) curve analysis of absolute total nucleated cell count with an area under the ROC curve of 0.828 (95% confidence interval, 0.800-0.856; $P < .01$). The optimal ROC-derived cutoff (square) shown has a sensitivity of 78.7% and a specificity of 70.7%.

Table 2

Descriptive Statistics for Cord Blood Units With a CD34+ Cell Count of $2 \times 10^6$ or More According to TNC Cutoff Selection Criterion and the Requirement of $2 \times 10^6$ CD34+ Cells per Kilogram of Patient Weight, Estimated for 10 to 40 kg ($n = 466$)

![Figure 2](https://example.com/figure2.png)
Because CD34+ cell determination demands significant time and resources, we used our data for applying ROC curve analysis to validate current CB parameters as surrogates for CD34+ cell counting and ability to provide reliable, effective, and cost-effective criteria for the appropriate identification of CB units suitable for transplantation, avoiding the cost of processing units with a high probability of not having the required CD34+ cell content for cryopreservation and grafting purposes. We confirmed that the TNC count is the parameter that better correlates with the CD34+ cell content in UCB, in agreement with previous reports.26,31,32

ROC curve analysis validated the current standard of TNC content of 8 × 10^8 or more as an appropriate cutoff parameter for the selection of CB units containing an adequate number of CD34+ MNCs for cryopreservation for grafting purposes.

References