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The Cord Blood Apgar: a novel scoring system to optimize selection of banked cord blood grafts for transplantation

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Abstract

BACKGROUND—Engraftment failure and delays, likely due to diminished cord blood unit (CBU) potency, remain major barriers to the overall success of unrelated umbilical cord blood transplantation (UCBT). To address this problem, we developed and retrospectively validated a novel scoring system, the Cord Blood Apgar (CBA), which is predictive of engraftment after UCBT.

STUDY DESIGN AND METHODS—In a single-center retrospective study, utilizing a database of 435 consecutive single cord myeloablative UCBTs performed between January 1, 2000, to December 31, 2008, precryopreservation and postthaw graft variables (total nucleated cell, CD34+, colony-forming units, mononuclear cell content, and volume) were initially correlated with neutrophil engraftment. Subsequently, based on the magnitude of hazard ratios (HRs) in univariate analysis, a weighted scoring system to predict CBU potency was developed using a randomly selected training data set and internally validated on the remaining data set.

RESULTS—The CBA assigns transplanted CBUs three scores: a precryopreservation score (PCS), a postthaw score (PTS), and a composite score (CS), which incorporates the PCS and PTS values. CBA-PCS scores, which could be used for initial unit selection, were predictive of neutrophil (CBA-PCS 7.75 vs. <7.75, HR 3.5; $p < 0.0001$) engraftment. Likewise, CBA-PTS and CS scores were strongly predictive of Day 42 neutrophil engraftment (CBA-PTS 9.5 vs. <9.5, HR 3.16, $p < 0.0001$; CBA-CS 17.75 vs. <17.75, HR 4.01, $p < 0.0001$).

CONCLUSION—The CBA is strongly predictive of engraftment after UCBT and shows promise for optimizing screening of CBU donors for transplantation. In the future, a segment could be assayed for the PTS score providing data to apply the CS for final CBU selection.

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AUTHORSHIP

KMP conceptualized the study, collected clinical data, analyzed data, wrote the manuscript, and performed all coordination efforts; JK conceptualized the study, analyzed data, wrote the manuscript, and made a direct contribution in the generation of graft data; LZ, AM, SW, and SC were involved in statistical analysis, review of the data, and preparation of the manuscript; AEB conceptualized the study, analyzed data, and edited the manuscript; and KS and TG analyzed data and edited the manuscript.

CONFLICT OF INTEREST

The authors have no conflict of interest to disclose.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1. Cumulative incidence of neutrophil engraftment at day 42 and risk of neutrophil engraftment for training and testing datasets by CBA-PCS, PTS and CS score.

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Current selection of donor cord blood units (CBUs) for unrelated donor transplantation is primarily based on dosing of total nucleated cells (TNCs) measured on the CBU before cryopreservation and human leukocyte antigen (HLA) matching. Unfortunately, with this strategy, approximately 20% of patients experience primary engraftment failure or delays after single-unit umbilical cord blood transplantation (UCBT).¹⁻⁴ It is believed that a significant reason for this relates to the overall quality of the cord blood (CB) graft. Attempts at correlating other graft variables, for example, CD34+ cells or colony-forming units (CFUs), with engraftment have shown superior predictive value in limited studies,⁵⁻¹² but no single variable has a high enough correlation with engraftment or graft failure. Furthermore, individual CBU profiles may show discordant, rather than linear, correlations of one variable compared to another. This phenomenon may be further influenced by the type of CB processing used before cryopreservation. We recently reported that postthaw total CFUs most strongly correlated with neutrophil and platelet (PLT) engraftment after UCBT.¹³ We subsequently hypothesized that a composite analysis of multiple graft characteristics would provide a more sensitive method to assess potency of an individual CB graft. To this end, we now report the creation and retrospective validation of a novel scoring system, the Cord Blood Apgar (CBA) score, to optimize CB graft selection for transplantation.

The CBA scoring system was developed and validated using a database of single cord, myeloablative unrelated UCBT performed at our center from 2000 to 2008. The score was defined as the weighted summation of selected precryopreservation or postthaw graft characteristics based on the hazard ratio (HR) magnitude in the univariate analysis of neutrophil engraftment. The CBA score assigns three scores to each transplanted CBU; a precryopreservation score (PCS) using precryopreservation characteristics, a postthaw score (PTS) using values measured on the actual CBU at the time of thawing, and a composite score (CS) based on combined precryopreservation and postthaw graft characteristics. The scoring system was developed from a randomly selected training data set from the database (68%) and validated by an independent testing data set derived from the remaining patients in the database (32%). Cumulative incidence curves of engraftment were compared between dichotomized CBA groups using Gray's test. Multivariate models were developed by considering clinical characteristics with the CBA together. Taken together, the CBA should optimize selection of a donor CBU for transplantation.

MATERIALS AND METHODS

Study design and eligibility

We performed a retrospective analysis of 435 consecutive UCBT procedures performed at Duke University using a single nonmanipulated CBU after full myeloablative conditioning as a first transplant between January 1, 2000, and December 31, 2008. Study subjects meeting these criteria were selected from a pool of consecutive patients transplanted for a malignancy, metabolic disorder, hemoglobinopathy, immunodeficiency, or marrow failure syndrome. Additionally, identified technical graft characteristics including precryopreservation and postthaw TNC count, mononuclear cell (MNC) count, and CD34+ and total CFU content needed to be available. All patients were enrolled in a Duke University Medical Center Institutional Review Board–approved protocol or treatment plan. Written informed consent was obtained from all patients or legal guardians. Of the patients included in this study, 132 patients were previously reported as part of Cord Blood Transplantation Study (COBLT) study^{2,3,14} and 10 patients were enrolled on the current Blood and Marrow Transplant Clinical Trials Network 0501 study. A subset of the study population has been previously reported in other reports from our center.¹⁵⁻²⁰

Donor selection

Initial donor screening used intermediate-resolution typing for HLA Class I (A and B) and high resolution typing for HLA-DRB1. High-resolution HLA typing was obtained for confirmatory typing and final unit selection. The CBU with the highest number of nucleated cells/recipient body weight and closest HLA match was selected. Priority was given to units with a TNC count of more than 3×10^7 cells/kg and a minimum of a four of six HLA match. Donor sex, race/ethnicity, and ABO typing were provided for all CBUs. Matching for race/ethnicity and ABO types were also prioritized when feasible.

CBU collection, processing, thawing, and infusion

CBUs were collected and processed according to local bank procedures. Upon selection for transplantation, they were shipped to the Duke Stem Cell Laboratory in a dry shipper that maintained temperatures below -150°C . Upon receipt at the Duke Stem Cell Laboratory, CBUs were removed from the dry shipper and quickly transferred to a liquid nitrogen freezer where they were stored under liquid nitrogen until the day of transplant. One to two hours before transplant, all CBUs were thawed using a modified version of the dextran-albumin wash per Rubinstein and colleagues.²¹ The washed cells were resuspended in dextran 40–5% albumin in a final volume adjusted not to exceed 5 mL/kg patient's body weight. Thirty minutes before infusion, patients were medicated with acetaminophen, diphenhydramine, and methylprednisolone. The CBU was infused through the patient's central venous line over 15 minutes. Aliquots (0.5 mL) of the product were obtained after washing (postthaw) and analyzed for sterility, TNCs, MNCs, viability, CFUs, and CD3+ and CD34+ content.

CBU characteristics

Precryopreservation data were provided by the CB bank supplying the unit as part of routine reporting procedures. The available data included precryopreservation TNCs, MNCs, CD34+ cell dose, CFU content, volume collected, and cell viability (trypan blue dye exclusion). Postthaw testing was performed at Duke Stem Cell Laboratory using samples obtained after thawing and washing with dextran-albumin, and included TNC, MNC, CD34+, CFU, CD3+ and cell viability. CFU subsets were enumerated, but not included in this analysis. ABO typing and microbial cultures (bacterial and fungal) were provided for all CBUs before cryopreservation and after thawing.

Graft characterization

TNCs and MNCs—For CBUs collected and processed by the Carolinas Cord Blood Bank (CCBB) at Duke, and all thawed units, TNC counts were obtained on an automated cell counter (Sysmex K-1000 from January 2000–April 2008, Sysmex XE5000 for precryopreservation analysis from April 2008–present, Sysmex XS-1000i for postthaw analysis from April 2008–present, Sysmex America, Inc., Mundelein, IL). Manual differentials were performed for calculation of the total MNCs [(%lymphocytes + %monocytes + %blasts) $\times$ TNCs].

CD34+—Enumeration of CD34+ cells was determined by flow cytometry (ProCOUNT, BD Biosciences, San Jose, CA). ProCOUNT reagent (20 μL) was added to 12×75 test tubes followed by 50 μL of CB sample. Samples were diluted with phosphate-buffered saline-bovine serum albumin wash (Gibco, Invitrogen, Carlsbad, CA) as needed for a concentration of fewer than 4×10^7 cells/mL. Tubes were mixed gently and incubated in the dark at room temperature for 15 minutes. Red blood cells (RBCs) were further lysed using 450 μL of a 1:10 dilution of lysing solution (FACS, BD Biosciences) for samples and incubated in the dark at room temperature for 15 minutes. A two-laser, four-color flow cytometer was used

to analyze samples. Results were analyzed by computer software (CellQuest Pro, BD Biosciences) using gating strategies (ProCOUNT, BD Biosciences).

Precryopreservation CFU assay—CFU progenitor cells including subsets were enumerated on precryopreservation samples of CB. Initial samples of 2.5×10^5 CB cells were removed for testing and diluted in 0.5 mL of Iscove's modified Dulbecco's medium (IMDM) plus 2% fetal bovine serum (FBS; StemCell Technologies, Vancouver, British Columbia, Canada). Cells were further diluted by adding 5×10^4 cells in a total of 0.5 mL of IMDM plus 2% FBS to achieve a final cell count of 2.5×10^4 /mL when mixed with 1.5 mL of MethoCult medium 4434 (StemCell Technologies). Cells were plated in triplicate (0.5 mL/well) at 1.25×10^4 cells/well in 24-well tissue culture plates and incubated in a humidified 37°C 5% CO₂ incubator for 11 to 14 days. Colony growth was scored by trained personnel using an inverted phase contrast microscope and reported as the mean colony count $\times 10^5$ nucleated cells. These numbers were used to calculate the number of progenitors in the entire graft by using the precryopreservation TNC count.

Postthaw CFU assay—CFU progenitors were enumerated on postthaw samples of CB and a total of all CFU progenitors were calculated for each graft infused. Samples of thawed CB containing 4×10^5 cells were diluted in IMDM plus 2% FBS. A total of 4×10^4 cells in 0.1 mL of IMDM plus 2% FBS were mixed with 0.5 mL of MethoCult medium 4434 (StemCell Technologies). Cells were plated in duplicate (0.3 mL/well) at 2×10^4 cells/well in 24-well tissue culture plates and incubated in a humidified 37°C 5% CO₂ incubator for 11 to 14 days. Colony growth was reported as the mean colony count $\times 10^5$ nucleated cells. These numbers were then used to calculate the number of progenitors in the entire graft by using the postthaw TNC count.

Statistical analysis

Neutrophil engraftment was defined as the first day of 3 consecutive days of an absolute neutrophil count of at least 500 confirmed donor cells $\times 10^6$ /L. PLT engraftment was defined as the first day of achieving an untransfused count of at least 50×10^9 PLTs/L for 7 days. Confirmation of donor cell chimerism was performed using fluorescence in situ hybridization or restriction fragment length polymorphism. Nonengraftment was defined as not achieving neutrophil engraftment with donor cells due to death, relapse, autologous recovery, or primary graft failure. Time to engraftment in patients surviving past Day 14 was estimated using the cumulative-incidence-function method, with competing risks of death, relapse, autologous recovery, and graft failure.²² Associations between graft or patient characteristics and neutrophil or PLT engraftment were evaluated univariately by Gray's K-sample test.²³ Overall survival was estimated as the time to death or censored at the last date of contact and estimated by the Kaplan-Meier method.²⁴

To better define a quality CBU, the CBA was developed and validated through the following steps using neutrophil engraftment data.

1. The overall database was randomly divided into two subsets: a training set (n = 299, 68%) and a testing set (n = 136, 32%). The training set was used to further develop the CBA and the testing set was used to validate. Continuous variables were compared between the training and testing set using the Wilcoxon rank sum test while the chi-square test was used for categorical covariates.
2. Gray's K-sample tests were used to identify graft characteristics significantly associated with the time to neutrophil engraftment. To develop the scoring systems, four precryopreservation and four postthaw graft characteristics were selected and weighted based on the magnitude of the HR from the univariate analysis using the

training data set as well as the importance in clinical practice. Using the training set to define the limits of the quartiles (Table 1), each quartile of the selected graft characteristics was assigned weighted points.

3. For each transplanted CBU, points were assigned for each of four precryopreservation (CFUs, TNCs, CD34+, and volume collected) and four postthaw graft characteristics (CFUs, MNCs, CD34+, and TNCs) based on the cell doses per kg (volume collected expressed in milliliters) that the CBU provided and their relationship to the training model. For example, a CBU that provided a precryopreservation CFU dose in the highest quartile (as defined by the training data set) would be assigned four points compared to a CBU with CFU doses in the lowest quartile, which would be assigned one point. Likewise, a high and low precryopreservation TNC count ($\times 10^7/\text{kg}$) would be assigned 2 and 0.5 points, respectively.
4. Using the points assigned for the components of the CBA, three scores were then assigned to each transplanted CBU: a PCS as the summation of assigned points of precryopreservation graft factors only, a PTS as the summation of assigned points of postthaw graft factors only, and a CS as the summation of points for both precryopreservation and postthaw graft factors (Table 2). Possible PCSs, PTSs, and CSs ranged from 2.25 to 9 for PCS, 3 to 12 for PTS, and 5.25 to 21 for CS. The probability of neutrophil engraftment on Day 42 was estimated for each quartile using the cumulative-incidence-function.
5. In the validation testing set, the performance of the scoring system developed from the training set was assessed, by testing its ability in discriminating patients belonging to the different score quartiles. Engraftment probability was computed at 42 days, to make the results comparable with those obtained in the training sample.

The CBA was then applied to the combined testing and training set to explore the impact of the CBA on the overall study population. Multivariable analysis based on overall data set (i.e., combined training + testing set) was performed for each CS with patient characteristics and matching status, using the model of Fine and Gray²⁵ with backward selection. Specifically, patient characteristics included age, sex, disease type, recipient and unit ethnicity, recipient weight, and cytomegalovirus (CMV) status. Matching status of recipient to unit included sex, ABO, HLA match, and ethnicity. Continuous variables were categorized by corresponding medians or quartiles. The predictive power of the individual and combined graft characteristics on neutrophil engraftment was compared to the CBA using the overall data set. A p value of 0.05 or less was considered significant. All analyses were completed using computer software (SAS, Version 9.2, SAS Institute, Cary, NC; and R, Version 2.7, <http://www.r-project.org/>).

RESULTS

Patient characteristics

A data set, retrospectively created from the results of 435 consecutive, myeloablative, single UCBTs performed from 2000 to 2008, was used to develop the scoring system. The median age of the overall group was 5.3 years (range, 3 weeks–55.8 years) including 43 adults (>18 years of age; 9.8%). The median patient weight at time of transplant was 19.8 kg (range, 2.7–118.4 kg), 58.2% had malignancies, 61.6% were males, 70.3% were Caucasian, and 37.7% were CMV seropositive (before transplant). Patients and donors were matched for ABO, race/ethnicity, and sex in 53.1, 79.1, and 50.3% of cases, respectively. Patients and CBUs were matched at six of six (7.6%), five of six (33.6%), four of six (55.9%), or three of six or fewer (3.0%) HLA loci. Slightly more patients were prepared for transplantation with

non-total body irradiation-containing regimens ($n = 245$, 56.4%) and most received cyclosporine based graft-versus-host disease (GVHD) prophylaxis ($n = 330$, 75.9%). Characteristics of the overall, training, and testing sets are presented in Table 3. Patient characteristics of the training and testing sets were similar with the exception of a higher median age among patients randomly assigned to the testing set (Table 3).

In the overall study group, neutrophil engraftment occurred in a median of 26 days (range, 7–104 days) after transplant with a cumulative incidence of 76.9% at 42 days (95% confidence interval [CI], 71.3%–82.5%). PLT engraftment of at least 50×10^9 PLTs/L occurred at a median 134 days (range, 22–471 days after transplant) with a cumulative incidence at 180 days of 55.0% (95% CI, 49.3%–60.7%). The probability of overall survival in the study group was 58.9% (95% CI, 54.2%–63.6%) at 1 year. There were 221 patient deaths with primary cause of progressive disease (28.5%), graft failure (5.9%), GVHD (9.1%), infection (31.8%), organ failure (18.1%), or other causes, including secondary malignancy or hemorrhage (6.6%). No differences were seen between the training and testing sets with respect to neutrophil engraftment, PLT engraftment or overall survival (data not shown).

Graft characteristics

Donor CBUs were obtained from 16 public CB banks in the United States including the CCBP at Duke (46%, $n = 199$), the National Cord Blood Program (22%, $n = 97$), or 14 other public banks who distribute CBUs through the National Marrow Donor Program (32%, $n = 139$). Eighty-three (19%) units were banked through the Cord Blood Transplantation Study (COBLT) funded by the National Heart, Lung, and Blood Institute. The majority of CBUs were volume reduced and RBC depleted before cryopreservation; 10 (2%) contained RBCs as they were processed via plasma depletion alone. Characteristics of the CBUs in the overall, training, and testing data sets are presented in Table 4.

Development of the CBA score

The major goal of selection of a CBU for transplantation is to choose the CBU most likely to engraft in the shortest time frame, for example, the graft with the highest potency. To this end, we developed a scoring system that we named the *Cord Blood Apgar (CBA)*. For the purpose of developing and validating the scoring system, the overall study population was randomly divided into two groups, a *training set* ($n = 299$, 68%) and a *testing set* ($n = 136$, 32%). Based on HRs from the univariate analyses of neutrophil engraftment in the training data set, a weight was assigned to each component of the CBA as described under Materials and Methods (Table 2). After a weighted assignment, based on quartiles of TNCs, CD34+, CFUs, MNCs, and volume collected was developed, each transplanted CBU was assigned three scores, a PCS using precryopreservation characteristics, a PTS based on postthaw values, and a CS based on combined precryopreservation and postthaw graft characteristics. Since not all patients had complete graft data, among the 430 patients who survived more than 14 days after transplant, 229 patients had CSs available (156 and 73 in the training and testing sets, respectively), while 253 patients had PCSs available (173 and 80 in the training and testing sets, respectively) and 381 patients had PTSs available (257 and 124 in the training and testing sets, respectively).

Validating the CBA on the testing data set

The CBA scoring algorithm was then applied to the testing data set to determine if it could predict neutrophil engraftment. As shown in Table S1 (available as supporting information in the online version of this paper), all patients transplanted with CBUs with PCS above the 75th percentile engrafted neutrophils before Day 42 compared to a cumulative incidence of 78.5% (95% CI, 68.3%–88.7%) for patients receiving CBUs that scored below the 75th

percentile. Looking at it another way, patients transplanted with CBUs scoring above the 75th percentile were 3.5× more likely to engraft earlier (95% CI, 1.94–6.34; $p < 0.0001$) than those receiving lower scoring units (Table S1).

Applying the PTS and the CS to the testing set, we identified two scores that were predictive of neutrophil engraftment, the 75th percentile and the median, with the 75th percentile showing higher sensitivity (Table S1). In the testing data set, patients transplanted with a CBU assigned a PTS above the 75th percentile were 3.16× more likely to engraft earlier (95% CI, 1.75–3.12; $p < 0.0001$) than patients receiving lower scoring units. In this population using the PTS, the cumulative incidence of neutrophil engraftment on Day 42 for patients transplanted with units scoring above and below the 75th percentile was 96.7% (95% CI, 88.5%–100%) and 70.7% (95% CI, 61.3%–80.1%), respectively. Applying the CS to the testing data set, patients receiving grafts with CSs above the 75th percentile were 4.01× more likely to engraft earlier than those scoring below the 75th percentile (95% CI, 2.23–7.01; $p < 0.0001$). All patients transplanted with grafts scoring in at least the 75th percentile had engrafted neutrophils by Day 42, compared to a cumulative incidence of 78.2% (95% CI, 67.0%–89.4%) for patients receiving grafts scoring below the 75th percentile.

Comparison of individual and combined graft characteristics

We then evaluated the predictive power of the individual graft characteristics and combined graft characteristics to determine if using an individual component or two graft variables from the CBA scoring system would adequately predict engraftment compared to using the four characteristics as described. In the overall data set, the combined precryopreservation CFUs + CD34+ (HR 2.49 [95% CI, 1.80–3.44], $p = 0.0002$) and the combined postthaw CFUs + TNCs (HR 2.63 [95% CI, 1.93–3.59], $p < 0.0001$) performed the best among the precryopreservation and postthaw graft characteristics, respectively. However, the CBA performed better than either the individual or the two characteristics combined (Fig. 1).

Applying the CBA to the overall data set

We then applied the CBA to the overall (combined training + testing) data set for the purpose of gaining more information about the CBA system using a larger sample. The following analyses were not included in the CBA validation.

Neutrophil engraftment and the PCS

In this population using the PCS, the cumulative incidences of neutrophil engraftment on Day 42 for patients transplanted with units scoring above and below the 75th percentile were 93.1% (95% CI, 86.0%–100%) and 74.7% (95% CI, 68.6%–80.8%; Fig. 2A), respectively. Patients transplanted with CBUs scoring above the 75th percentile were 4.67× more likely to engraft earlier (95% CI, 1.15–18.9; $p < 0.0001$) with a median time to engraftment of 19 days compared to 28 days for patients scoring below. The risk of graft failure was 6.34× higher for patients below the 75th percentile (95% CI, 1.48–27.2; $p = 0.01$). In multivariate analysis that included clinical characteristics, a PCS above the 75th percentile was the strongest predictor of faster engraftment ($p < 0.0001$) followed by negative recipient CMV serostatus ($p = 0.0005$) and male donor ($p = 0.01$; Table 5).

Neutrophil engraftment and the PTS

When we applied the PTS to the overall data set, CBUs assigned scores above the 25th percentile, median, and 75th percentile were all predictive of neutrophil engraftment, although the 75th percentile performed the best. Applying the PTS to the overall data set, patients receiving high scoring CBUs (above 75th percentile) were 2.33× (95% CI, 1.75–

3.12; $p < 0.0001$) more likely to engraft neutrophils earlier with a median time to engraftment of 19 days compared to 30 days for patients scoring below. The cumulative incidences of neutrophil engraftment for patients transplanted with CBUs scoring above and below the 75th percentile were 92.4% (95% CI, 87.1%–97.7%) and 71.2% (95% CI, 65.7%–76.7%; Fig. 2B), respectively. In multivariate analysis that included clinical characteristics, a PTS above the 75th percentile was the strongest predictor ($p < 0.0001$), followed by metabolic diagnosis ($p < 0.0001$) and male donor ($p = 0.0006$; Table 5).

Neutrophil engraftment and the CS

When we applied the CS to the overall data set, the median and the 75th percentile again were predictive of neutrophil engraftment, although the latter had a higher discriminant ability. Comparing grafts scoring above and below the 75th percentile, the cumulative incidences of neutrophil engraftment by Day 42 were 96.6% (95% CI, 90.9%–100%) and 73.7% (95% CI, 67.0%–80.4%), respectively (Fig. 2C). The risk of nonengraftment for grafts scoring below the 75th percentile is 3.13× higher than grafts scoring above (95% CI, 2.10–4.66; $p < 0.0001$). In multivariate analysis of the CS and clinical characteristics (Table 5), a CS above the 75th percentile was the strongest predictor of time to neutrophil engraftment ($p < 0.0001$) compared to unit sex ($p = 0.004$), recipient CMV ($p = 0.006$), or diagnosis ($p = 0.007$).

Predictive value of the PCS and CS for patient subgroups

An analysis of the CBA was performed for specific patient subgroups based on weight (<20 kg) and engraftment status (rapid engrafters defined as <20 days vs. nonengrafters). CBUs transplanted into patients weighing less than 20 kg had higher median PCSs and CSs compared to patients weighing more than 70 kg (PCS 6.75 vs. 2.88, $p < 0.0001$; CS 16.13 vs. 6.00, $p < 0.0001$), which would be expected given the inherent differences in cell dosing in small versus large patients. Within the subset of patients less than 20 kg, patients who engrafted had significantly higher PCSs and CSs ($p = 0.01$ and $p = 0.03$, respectively) than patients who did not engraft. In the total patient data set, patients who rapidly engrafted had significantly higher PCSs and CSs ($p = 0.0001$ and $p < 0.0001$, respectively) compared to patients who did not engraft.

CCBB inventory

We took advantage of the large inventory available through the CCBB ($n = 22,559$ CBUs), a 12-year-old public CB banking facility at Duke, and examined how this inventory would score in clinical practice considering potential UCBT recipients of varying weights. We applied the PCS using various patient weights to determine the availability of high-scoring CBUs and the prevalence of low-scoring CBUs that are less likely to engraft. For a 10-kg patient, 50.8% of the CCBB inventory would provide a high-scoring unit (>75th percentile PCS) with 90.2% of the units scoring above the median (Fig. 3). Likewise, only 2.5% of the inventory would be at high risk for graft failure. Conversely, for large patients, less than 0.3% of the CCBB inventory meeting National Cord Blood Inventory criteria provides a high-scoring unit for patients more than 40 kg, while 5.3% would score above the median.

DISCUSSION

Graft failure and engraftment delays, likely due to decreased CBU graft potency, continue to be a major barrier to the overall success of UCBT. Current selection criteria using TNCs and/or CD34+ do not adequately assess potency and fail to discriminate CBUs at increased risk for graft failure. We hypothesized that a combination of graft characteristics would better predict engraftment than any single graft characteristic. To address this issue, we developed and retrospectively validated the CBA score, which is based on a combination of

graft characteristics measured on samples from the precryopreservation and the postthaw CBUs. A CS based on these variables best predicts the potential of an individual CBU to engraft.

The CBA has three components: the PCS, the PTS, and a CS. Results of the univariate analysis of neutrophil engraftment in the training data set were used to create the CBA weighted scoring system that can be used to score an individual CBU on a search report. For example, a CBU of interest that could deliver cell doses (or volume collected) in the highest quartile for all of the four precryopreservation or four postthaw components would be assigned a PCS and PTS of 9 and 12, respectively. Similarly, a CBU that provided cell doses in the highest quartile for all eight CBA components would be assigned a CS of 21. For the purpose of the analyses, scores were grouped according to quartiles (as defined by the training data set). In univariate and multivariate analysis of the training, testing, and combined data sets, the CBA was strongly predictive of neutrophil engraftment. Recipients of high-scoring units had significantly higher cumulative incidences of engraftment and more rapid engraftment compared to those receiving lower-scoring units. In multivariate analysis that considered clinical characteristics along with the CBA scores, the PCS, PTS, and CS were the strongest predictors of neutrophil engraftment in their respective models. We envision that the PCS could be utilized for initial selection of CBU grafts to undergo confirmatory HLA typing and potency testing performed on an attached segment. The PTS and CS could be used for final graft selection after postthaw characteristics are enumerated on a segment attached to the cryopreserved CBU allowing for a PTS score to be assigned. Validation of the segment-based assay would be required to implement and generalize this practice.

Numerous studies have identified correlations of individual graft characteristics with engraftment and survival. Early reports of UCBT outcomes described associations between higher TNC dose and faster and/or higher rates of neutrophil engraftment.^{26–28} These findings have been replicated in subsequent studies to varying degrees.^{2,4,9,29} In a smaller number of studies, CD34+ cell dose has been shown to predict engraftment and survival,^{5–9} but the use of CD34+ is limited by standardization issues and CD34+ enumeration is not available for a significant portion of the existing banking inventory. In addition, the precryopreservation TNC and/or CD34+ content do not necessarily reflect the overall potency of the CBU after cryopreservation, storage, and thawing.

Improved correlations with engraftment are seen when precryopreservation or postthaw CFUs are used. The CFU assay is a functional assay requiring viable cells to produce colonies in culture over 2 weeks. When measured on a CB sample either before cryopreservation or after thawing, total CFU is more representative of the potency of an individual CBU than other measurements routinely performed. The importance of CFU content was initially described in a report from the New York Blood Center¹⁰ who observed that the precryopreservation CFU content not only correlated with neutrophil and PLT engraftment, but displaced TNCs from the multivariate models. More recently, two smaller series have subsequently described the importance of postthaw CFUs on engraftment.^{11,12} We recently reported a strong correlation between CFUs measured either before cryopreservation or after thawing and neutrophil and PLT engraftment after UCBT utilizing the same data set from which we derived the CBA score.¹³ In this study population, all transplants were performed at Duke and almost half of the CBUs transplanted were provided by our local public CB bank. With consistent personnel performing almost half of the precryopreservation CFU and all of the postthaw CFU assays, we examined whether there was a “bank effect” influencing these findings. Considering bank as a covariate in multivariate analysis along with clinical and graft characteristics, the bank providing the CBU was not predictive of neutrophil engraftment after UCBT.

Despite the strong correlation with engraftment, utilization of CFUs for CBU selection has been limited due to the lengthy assay time and issues with interlaboratory standardization.^{30–32} Another focus of ongoing work in our laboratory is to identify an efficient and reproducible segment-based assay that could serve as a surrogate for the CFU assay.³³ The results of these ongoing studies may be incorporated into future versions of the CBA.

During the development of the CBA, we examined other possible models predictive of neutrophil engraftment. Approaches included examining different combinations of graft characteristics (TNC + CD34+, TNC + CFU, CD34+ + CFU) to determine whether these alternative models would allow for better discrimination of CBUs likely to engraft. Interestingly, the precryopreservation CFU + CD34+ components of the PCS was found to be the next best predictor of engraftment. Conversely, TNC-containing models did not as strongly impact engraftment which was also observed in our recent analysis of individual graft characteristics.¹³

We also examined several subgroups of this study population to further define the utility of the CBA in different settings. In the smallest patients receiving high cell doses, the scoring system allowed for better differentiation of cords capable of engrafting compared to the conventional measures currently used for unit selection. Furthermore, the CBA score was significantly different in patients who rapidly engrafted compared to patients who failed to engraft. We also observed that the CBA was predictive of engraftment kinetics.

Given the strong correlation of the CBA dose with engraftment, we asked whether the current inventory in a large public CB bank would provide higher scoring cords for various patient weights. While a sufficient number of potential units appear to be available for smaller patients, there were very few units that would be assigned high scores and therefore be more likely to engraft in larger patients. To overcome this issue, additional emphasis may need to be placed on assessing potency on the postprocessed CBU to expand the inventory to include more potent units. Additionally, this finding indirectly supports the use of double UCBT to expand access to this therapy.

In summary, in this single-center study, we have proposed and retrospectively validated a novel scoring system called the CBA, which is the first such algorithmic tool for graft selection. The CBA was highly predictive of neutrophil engraftment after UCBT and superior to other more traditional methods of CBU selection. Further prospective work is planned including adult populations and clarifying the roles of HLA matching and the CBA in unit selection. While external and prospective validation is warranted, the CBA is a promising tool that has the potential to optimize engraftment after UCBT and significantly decrease engraftment failure.

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ABBREVIATIONS

CB	cord blood
CBA	Cord Blood Apgar

CBU(s)	cord blood unit(s)
CCBB	Carolinas Cord Blood Bank
CS(s)	composite score(s)
HR(s)	hazard ratio(s)
IMDM	Iscove's modified Dulbecco's medium
PCS(s)	precryopreservation score(s)
PTS(s)	postthaw score(s)
TNC(s)	total nucleated cell(s)
UCBT	umbilical cord blood transplantation

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Fig. 1. Risk of neutrophil engraftment comparing individual and combined graft characteristics with the CBA scores. Comparison of the magnitude of the HRs with corresponding 95% CIs indicating the risk of neutrophil engraftment for individual graft characteristics (precryopreservation and postthaw TNCs, CD34⁺, and CFUs; volume collected and postthaw MNCs) and combined graft characteristics (precryopreservation and postthaw TNCs + CD34⁺, TNCs + CFUs, and CFUs + CD34⁺) as compared to the CBA components (PCS, PTS, and CS). Precryopreservation, postthaw, and combined data were available for 253, 381 and 229 patients, respectively.

Fig. 2.

Impact of CBA score on the probability of neutrophil engraftment in the overall data set. Probability plots are shown for scores above and below the 75th percentile. (A) Impact of PCS using the 75th percentile as the cutoff on neutrophil engraftment in the overall data set. (B) Impact of the PTS on neutrophil engraftment in the overall data set using the 75th percentile. (C) Impact of the CS on neutrophil engraftment using the 75th percentile as cutoff.

Fig. 3.

Availability of CBUs, in a large public bank ($n = 22,559$), that contain high CBA scoring units for various patient weights. For various theoretical patient weights, we have presented the percentage of CBUs available in a large, public CB bank (CCBB at Duke). CBUs all met criteria for banking as specified by the National Cord Blood Inventory. The red region depicts CBUs scoring the highest quartile (PCS 7.75–9). The light blue region depicts CBUs scoring in the second quartile (PCS 5.5–7.75). The gray region depicts CBUs scoring in the third quartile (PCS 4.25–5.5), and the cross-hatched region depicts CBUs scoring in the lowest PCS quartile (2.25–4.25).

TABLE 1

Graft characteristics affecting neutrophil engraftment in univariate analysis in the training set (quartiles presented were also used to define quartiles for CBA)

Graft characteristic	HRs (95% CI), p value*
<i>Before cryopreservation</i>	
TNCs ($\times 10^7/\text{kg}$)	
4.1 or less	1.00
>4.1–6.9	1.07 (0.77–1.50)
>6.9–11.1	1.84 (1.31–2.58), p = 0.0004
>11.1	2.12 (1.47–3.06), p < 0.0001
CD34+ ($\times 10^5/\text{kg}$)	
1.2 or less	1.00
>1.2–2.2	1.15 (0.81–1.62)
>2.2–3.7	1.57 (1.08–2.28), p = 0.0180
>3.7	2.40 (1.65–3.50), p < 0.0001
CFUs ($\times 10^4/\text{kg}$)	
11.0 or less	1.00
>11.0–19.0	1.44 (0.96–2.15)
>19.0–36.8	1.48 (0.97–2.26)
>36.8	2.57 (1.66–3.97), p < 0.0001
Volume collected (mL)	
80 or less	1.00
>80–100	0.92 (0.65–1.30)
>100–125	0.94 (0.67–1.31)
>125	1.65 (1.20–2.28), p = 0.0019
<i>After thawing</i>	
TNCs ($\times 10^7/\text{kg}$)	
3.5 or less	1.00
>3.5–5.7	1.31 (0.95–1.81)
>5.7–9.4	1.65 (1.17–2.34), p = 0.0042
>9.4	2.16 (1.51–3.10), p < 0.0001
CD34+ ($\times 10^5/\text{kg}$)	
1.0 or less	1.00
>1.0–1.8	1.51 (1.08–2.10), p = 0.0160
>1.8–3.2	1.57 (1.12–2.22), p = 0.0096
>3.2	2.49 (1.76–3.52), p < 0.0001
CFUs ($\times 10^4/\text{kg}$)	
1.5 or less	1.00
>1.5–3.5	1.65 (1.21–2.24), p = 0.0014
>3.5–7.0	1.85 (1.29–2.63), p = 0.0007
>7.0	3.45 (2.36–5.03), p < 0.0001
MNCs ($\times 10^7/\text{kg}$)	

Graft characteristic	HRs (95% CI), p value [*]
1.3 or less	1.00
>1.3–2.2	1.38 (0.95–2.01)
>2.2–3.6	1.83 (1.30–2.57), p = 0.0005
>3.6	2.50 (1.69–3.69), p < 0.0001

^{*} Significant p values (defined by p = 0.05) are shown.

TABLE 2
The CBA including the PCS, the PTS, and the CS with quartiles and associated points assigned

Quartiles and points assigned						
	Quartile 1 (Highest)	Points	Quartile 2	Points	Quartile 3	Points
					Quartile 4 (Lowest)	Points
Precryopreservation components						
CFUs ($\times 10^4/\text{kg}$)	>36.8	4	>19.0–36.8	3	>1.1–1.9	2
TNCs ($\times 10^7/\text{kg}$)	>11.1	2	>6.9–11.1	1.5	>4.1–6.9	1
CD34+ ($\times 10^5/\text{kg}$)	>3.7	2	>2.2–3.7	1.5	>1.2–2.2	1
Volume collected (mL)	>125	1	>100–125	0.75	>80–100	0.5
Postthaw components						
CFU ($\times 10^4/\text{kg}$)	>7.0	4	>3.5–7.0	3	>1.5–3.5	2
MNC ($\times 10^7/\text{kg}$)	>3.6	4	>2.2–3.6	3	>1.3–2.2	2
CD34+ ($\times 10^5/\text{kg}$)	>3.2	2	>1.8–3.2	1.5	>1.0–1.8	1
TNC ($\times 10^7/\text{kg}$)	>9.4	2	>5.7–9.4	1.5	>3.5–5.7	1
PCS	7.75		>5.5–7.75		>4.25–5.5	4.25
PTS	9.5		>7.5–9.5		>5.5–7.5	5.5
CS	17.75		>13.5–17.75		>9.75–13.5	9.75

TABLE 3

Comparison of patient and transplant characteristics of the overall study (n = 435), training (n = 299), and testing (n = 136) groups

Variables	Overall (%)	Training (%)	Testing (%)
Continuous			
Age (years), median (range)	5.3 (0.1–55.8)	4.8 (0.2–55.8)	6.0 (0.1–51.5) *
Weight (kg), median (range)	19.8 (2.7–118.4)	19.3 (2.7–112.7)	21.4 (4.1–118.4)
Categorical			
Age (years)			
8.5	65.1	68.9	56.6
Sex			
Male	61.6	62.5	59.6
Race/ethnicity			
Caucasian	70.3	70.5	67.6
Diagnosis			
Malignant	58.2	56.5	61.8
Metabolic	30.3	32.1	26.5
Other [†]	11.5	11.4	11.8
Donor sex			
Male	50.1	50.8	48.5
HLA match			
4/6	56.5	57.9	61.1
5/6	33.6	34.4	31.6
6/6	7.6	7.7	7.4
ABO match			
Matched	53.1	53.2	52.9
Recipient-donor sex match			
Matched	50.3	52.2	46.3
Recipient CMV serostatus			
Positive	37.7	39.1	34.6
Negative	61.8	60.9	64.0
Indeterminate	0.2	0	1.4

* Comparison of median age training versus testing, p = 0.02.

[†] Other diagnoses include immunodeficiencies, hemoglobinopathies, or marrow failure syndromes.

TABLE 4Comparison of CBA graft characteristics for the overall study, training, and testing groups^{*}

Characteristics	Overall	Training	Testing
Before cryopreservation [†]			
Number	253	173	80
CFU ($\times 10^4/\text{kg}$)	19.4 (0.3–293.6)	19.0 (0.3–293.6)	21.3 (1.7–124.2)
TNC ($\times 10^7/\text{kg}$)	6.9 (0.9–67.3)	6.9 (1.4–67.3)	6.8 (0.9–38.0)
CD34+ ($\times 10^5/\text{kg}$)	2.3 (0.1–29.3)	2.4 (0.1–29.3)	2.1 (0.1–22.9)
Volume collected (mL)	103 (44–276)	103 (44–276)	104 (50–235)
After thawing [‡]			
Number	381	257	124
CFU ($\times 10^4/\text{kg}$)	3.4 (0–105.3)	3.5 (0–105.3)	2.8 (0–95.5)
MNC ($\times 10^7/\text{kg}$)	2.1 (0.2–15.1)	2.2 (0.4–15.1)	2.0 (0.2–10.7)
TNC ($10^7/\text{kg}$)	5.7 (0.8–50.3)	5.8 (0.9–50.3)	5.6 (0.8–28.1)
CD34+ ($10^5/\text{kg}$)	1.8 (0.1–18.3)	1.8 (0.3–15.2)	1.5 (0.1–18.3)

^{*} No significant differences were observed between the training and testing datasets when comparing the graft characteristics included in the CBA. Patients with CS (n = 229) were a subset of these two populations and the graft characteristics were similar (data not shown).

[†] Precryopreservation characteristics were summarized for patients with PCS assigned (n = 253).

[‡] Postthaw characteristics were summarized for patients with PTS assigned (n = 381).

TABLE 5

Multivariate analysis of neutrophil engraftment including clinical characteristics and the CBA scores

Characteristic	Favorable characteristic	HR (95% CI)	p value*
PCS model			
PCS	75th percentile	2.47 (1.77–3.46)	<0.0001
Recipient CMV	Negative	1.72 (1.27–2.32)	0.0005
Donor sex	Male	1.41 (1.08–1.82)	0.01
PTS model			
PTS	75th percentile	2.26 (1.72, 2.98)	<0.0001
Diagnosis	Metabolic	1.62 (1.27, 2.06)	<0.0001
Donor sex	Male	1.47 (1.18, 1.83)	0.0006
Recipient CMV	Negative	1.31 (1.04–1.67)	0.025
CS model			
CS	75th percentile	2.30 (1.71–3.09)	<0.0001
Donor sex	Male	1.49 (1.14–1.96)	0.004
Recipient CMV	Negative	1.57 (1.14–2.16)	0.006
Diagnosis	Metabolic	1.47 (1.11–1.96)	0.007

* p values correspond to the comparison between the favorable characteristic group (i.e., 75th PCS scoring group) compared to the baseline group (i.e., <75th percentile PCS scoring group).