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Evaluation of post-thaw CFU-GM: clinical utility and role in quality assessment of umbilical cord blood in patients receiving single unit transplant

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Abstract

BACKGROUND: The CFU assay is considered the only in vitro assay that assesses the biologic function of hematopoietic stem and progenitor cells (HSPC).

STUDY DESIGN AND METHODS: To investigate the impact of post-thaw CFU-GM counts on the quality of umbilical cord blood (UCB), we studied transplant outcomes in 269 patients receiving single UCB transplant. We also correlated the post-thaw CFU-GM counts of 1912 units with the pre-freeze and post-thaw graft characteristics, hoping to optimize selection criteria of UCB. Data analysis included: total nucleated cells, viability, CD34+, nucleated red blood cells (NRBC), hematocrit, frozen storage time, and cord blood bank (CBB).

RESULTS: We demonstrated an association between post-thaw CFU-GM dose and the speed of neutrophil and platelet engraftment ($p < 0.01$). Higher post-thaw CFU-GM dose showed an increased benefit for neutrophil and platelet engraftment ($p < 0.01$). Post-thaw CD34+ cell dose and CFU-GM dose were strongly correlated ($r = 0.78$). However, CFU-GM dose showed additional benefit for patients receiving the lowest quartile of CD34+ dose. HLA disparity did not adversely impact either neutrophil or platelet engraftment. Post-thaw CFU-GM/million nucleated cells plated showed moderate correlation with pre-freeze and postthaw CD34+ and weak correlation with other parameters. Post-thaw CFU-GM was not influenced by storage time, but was impacted by the CBB from which the unit is obtained ($p < 0.01$).

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CONCLUSION: Post-thaw CFU-GM is an effective measure of the quality and efficacy of the UCB graft, particularly adding valuable clinical information when the CD34+ cell dose is low. Consideration of pre-freeze CD34+ cell content and CBB as additional selection criteria is warranted.

The first umbilical cord blood (UCB) transplant was performed in 1988. Since then, the use of UCB has dramatically increased as an alternative source of hematopoietic stem and progenitor cells (HSPC). It is considered an important curative therapy for a number of malignant and non-malignant diseases, in both pediatric and adult patients. It offers many clinical advantages over bone marrow and mobilized peripheral blood progenitors, including an expanded donor pool, absence of associated risk to donors, decreased severity of graft-versus-host disease (GVHD), higher proliferative capacity of immature hematopoietic cells and reduced histocompatibility requirements.¹ Despite the remarkable progress that has been made in the past few years with new conditioning protocols, expansion trials, and infection and GVHD prevention regimens,²⁻⁴ UCB graft failure remains a significant problem.⁵

Given the relatively low number of HSPC in an UCB unit, proper selection of the unit to be used in the clinical setting of single unit transplant becomes of paramount importance. Typically, physicians select UCB based predominantly on the HLA match and nucleated cell dose (NC/Kg). It is well established that HLA matching is a crucial factor that contributes to the success of transplantation. An accepted approach is to choose UCB with the closest match 4/6 HLA (HLA -A and -B at antigen level and -DRB1 at allele level typing) or 4/8 (HLA-A, -B, -C, -DRB1 at allele level). Previous studies have demonstrated that a higher NC dose can overcome the negative impact of HLA disparity. The importance of NC dose has been demonstrated in previous studies, with a threshold value of $2.5-7 \times 10^7$ NC/Kg being the value that best predicts engraftment.⁶⁻⁸

Reports have also demonstrated the importance of CD34+ cell dose/kg, with better outcomes in recipients of higher doses.^{8,9} The use of CD34+ cell enumeration as an index of hematopoietic cell graft quality, however, remains somewhat problematic, as variation in CD34+ cell analysis continues with significant inter-laboratory variability.^{10,11} Additionally, it has been demonstrated that the CD34+ stem cell marker detects a very small fraction of stem cells,¹¹⁻¹³ the majority of which are progenitor cells that have no role in engraftment.¹¹

While the clonogenic, or CFU (colony forming unit), assay is the only in vitro assay that assesses the biologic function of HSPC, few studies have documented the influence of the CFU dose on engraftment after UCB transplantation.^{14,15} Most of these studies have been small,¹⁶ and have included only prefreeze CFU data in the analysis.¹ The processes of cryopreservation and subsequent thawing can significantly impact the quality of the unit the patient receives.¹⁵ Thus, assessment of post-thaw CFU would appear to be warranted.

Pre-freeze UCB data are provided by the cord blood bank (CBB) that supplies the unit for transplantation. Our UCB units are obtained from many CBBs, and uniform post-thaw testing is conducted at our facility using samples from the unit after thawing and washing.

To determine the potential impact of “post-thaw” CFU-granulocyte, macrophage (CFU-GM) on transplant outcomes, we retrospectively reviewed clinical data in patients transplanted

with a single UCB unit and evaluated factors that might influence the number of post-thaw CFU-GM.

MATERIALS AND METHODS

We retrospectively reviewed data from 269 patients with malignant and non-malignant diseases, who received a single unrelated UCB unit between 1/1/2000-12/31/2017. All patients were treated in a University of Minnesota Institutional Review Board-approved protocol.

UCB units were obtained from 24 CBBs. The unit with the best HLA match (minimum of 4/6 loci match) and the highest nucleated cell dose ($>3 \times 10^7$ NC/Kg) was selected for transplantation. Graft characteristics of transplanted units are tabulated (Table 1).

Neutrophil and platelet engraftment data were available for all 269 patients, and the rates and speed of engraftment were determined. Neutrophil engraftment was defined as an absolute neutrophil count of 500/pl, counted as the first day of three consecutive measurements. Platelet engraftment was defined as platelet count of 20,000/ μ l, counted as the first day of three consecutive measurements without transfusion support for seven consecutive days. We studied the impact of UCB testing and characteristics (post-thaw CFU-GM, post-thaw CD34+ and HLA) on clinical outcomes (i.e., engraftment). We analyzed these two primary endpoints separately by conditioning regimen (myeloablative versus reduced intensity conditioning [RIC]) due to the difference in kinetics of engraftment.

An additional goal of our study was to analyze the prefreeze and post-thaw characteristics and QC testing of 1912 UCB units that were transplanted during the study period from January 1, 2000 to December 31, 2017, for correlation to postthaw CFU-GM testing. Only 14% of the 1912 units had cell doses that were sufficient for use as a single unit graft; the remaining units were used as double unit grafts. Data analysis included TNC, viability, CD34+ cell enumeration, nucleated red blood cells (NRBC), post-thaw hematocrit, frozen storage time and CBB. Our hope was to optimize selection criteria for UCB units.

Post thaw samples for QC testing were obtained at the time of unit thaw and wash. Standard institutional procedures were used for our routine testing, which includes: TNC, viability by fluorescence microscopy (acridine orange/ propidium iodide [AOPI]), flow cytometry (7-AAD viability, CD34, CD3), ABO typing, microbial culture, and CFU-GM.

Description of the post-thaw CFU-GM assay

Enumeration of CFU-GM was performed by cultivating mononuclear cells in a semisolid Methocult[™] GF H4534 Complete Methylcellulose Media containing Recombinant Cytokines without Erythropoietin. The UCB was diluted with an in-house progenitor assay media (supplemented with 2% fetal bovine serum) to an optimal cell concentration of 0.3×10^5 , with an acceptable range of dilution of $0.2-0.4 \times 10^5$ NC/ml. The cell suspension was added to the thawed Methocult media. One mL of the media/cell culture (20,000-40,000 cells) was placed into each of two wells of a tissue culture plate, and the remaining wells were filled with sterile water for humidification. The plate was put in a 37°C/5% CO₂

incubator for 14-16 days and colonies were counted by morphology using a dissecting microscope at 2-2.3X. A CFU-GM colony was defined as a cluster of at least 40 cells and wells containing more than 50 colonies were reported as >50 “colonies.”

Statistical analysis

Patient and graft characteristics were compared across conditioning regimens by the Kruskal-Wallis test for continuous factors and by the chi-square test for categorical factors. The primary endpoints of neutrophil and platelet engraftment were estimated using cumulative incidence treating non-event death as a competing risk.¹⁷ Univariate comparisons were completed by Gray’s test. To evaluate the independent effect of CFU-GM/Kg (both as continuous factor as well as split into quartiles) on engraftment, we used Fine and Gray regression to model its effect.¹⁸ In addition to CFU-GM, the pre-specified factors of age (treated as a continuous factor), HLA disparity (6/6 vs. 5/6 vs. 4/6) and diagnosis (malignant vs. non-malignant) were included in regression models regardless of statistical significance due to their potential clinical impact and confounding on engraftment. In the regression analysis among RIC, CFU-GM/Kg was categorized above and below the median due to the small sample size. Spearman’s correlation coefficient was used to estimate correlation between CFU-GM/Kg and CD34+/Kg. In the analysis of post-thaw CFU-GM count by center, we used the Kruskal-Wallis test to look for any overall differences and the Dwass, Steel, Critchlow-Fligner method to check for any pairwise differences between centers controlling the familywise error rate.

RESULTS

Evaluation of clinical outcomes in the 269 patients receiving single UCB transplant

Patient characteristics—Of the 269 patients enrolled in this study, 57% were males. Their ages ranged from <1 to 66 years with a median of 7 years. Their median body mass index (BMI) was 18 (16-21). A total of 139 patients (52%) were being treated for malignancies and 130 patients (48%) had non-malignant conditions. Eighty-four percent of patients received myeloablative conditioning and 16% received RIC (Table 1).

Correlation between post-thaw CFU-GM/kg and postthaw CD34+/kg—The median dose of post thaw CFU-GM $\times 10^4/\text{kg}$ in the infused products was 6 (Range, 3.3-11.4) (Table 1). Postthaw CD34+ cell dose and post-thaw CFU-GM dose were strongly correlated ($r = 0.78$). The correlation coefficient (r value) for patients receiving myeloablative and RIC was 0.76 and 0.52, respectively.

Overall incidence of neutrophil and platelet engraftment—The overall cumulative incidence of neutrophil engraftment by post-transplant Day 42 was 96% (95% confidence interval [CI], 93-98%), with a median time to engraftment of 19 days (Inter-quartile range [IQR], 14-24 days). Incidence of platelet engraftment >20 K by Day 180 post-transplant was 83% (95% CI, 76-91%) with a median time of 47 days (IQR, 38-73 days).

Comparing engraftment by intensity of the conditioning regimen, showed an increased benefit in neutrophil engraftment among RIC patients compared to myeloablative patients

(Hazard ratio [HR] = 3.9; 95% CI, 1.0-2.0, $p = p < 0.01$). RIC also showed a benefit in the same direction for platelet engraftment (HR = 1.4; 95% CI, 0.9-2.1, $p = 0.09$). Due to these statistical differences as well as clinical differences, we chose to assess engraftment separately for myeloablative and RIC.

Neutrophil and platelet engraftment in patients receiving myeloablative conditioning—The cumulative incidence and speed of neutrophil and platelet recovery are tabulated (Table 2).

Shorter time to engraftment of both neutrophils and platelets was observed with increased CFU-GM dose ($p < 0.01$). Speed of neutrophil and platelet recovery in myeloablative patients with the four quartiles of CFU-GM/kg doses are shown in Figs. 1A and 1B. Plots for correlation of CFU-GM/kg to time to engraftment are presented in Figs. 1C and 1D.

CD34 + count also significantly predicted neutrophil engraftment ($p < 0.01$). Although CD34+ and CFU-GM were heavily confounded in their effects on engraftment, increased CFU-GM dose did show improved engraftment in the 1st quartile of CD34+ ($p = 0.02$). HLA did not adversely impact either neutrophil or platelet engraftment ($p > 0.05$). Various graft parameters influencing engraftment of both neutrophils and platelets in myeloablative patients are summarized in Table 2. In univariate analysis, there was no statistically significant association between engraftment and transplantation year or BMI.

Given the collinearity between CFU-GM and CD34+, other than interactions, CD34+ was excluded in favor of CFU-GM since this was our factor of interest. In a regression model of CFU-GM and the clinically pre-specified factors of HLA, age, and diagnosis among patients receiving myeloablative conditioning, the engraftment of both neutrophils and platelets increased sequentially by increasing quartiles of CFU-GM ($p < 0.01$) (Table 3).

Results were similar when evaluating CFU-GM as a continuous factor ($p < 0.01$).

Neutrophil and platelet engraftment in patients receiving RIC—The cumulative incidence and speed of neutrophil and platelet recovery are summarized in Table 4. There was a shorter time to neutrophil engraftment with increased CFU-GM dose ($p = 0.048$).

Speed of neutrophil and platelet recovery in patients receiving RIC with the four quartiles of CFU-GM/kg doses are shown in Figs. 2A and 2B. Plots for correlation of CFU-GM/kg to time to engraftment are presented in Figs. 2C and 2D.

In both regression analysis and univariate analysis, HLA mismatch did not adversely impact engraftment ($p > 0.05$).

In a regression model of CFU-GM comparing doses above and below the median and pre-specified factors of HLA, age and diagnosis among patients receiving RIC conditioning, CFU-GM had a positive association with neutrophil engraftment (HR = 2.1; 95% CL, 1.0-4.3, $p = 0.04$) (Table 5). The effect was similar when treating CFU-GM as a continuous variable. In evaluating the effect among the first quartile of CD34+ cell dose, the post thaw CFU-GM dose provided additional information to the quality of the graft. The hazard ratio

for neutrophil engraftment was 1.02 for every $1 \times 10^4/\text{kg}$ increase in CFU-GM ($p = 0.01$) and 1.02 for platelet engraftment ($p < 0.01$). There was no additional benefit from increased CFU-GM cell dose in patients receiving a high CD34+ cell dose ($>3.2 \times 10^5/\text{kg}$). Other pre-specified factors included in the regression models were HLA disparity, type of disease and conditioning intensity (Table 5).

Day +100 survival—The overall survival in patients receiving myeloablative and RIC was 89% (95% CI, 84%–892%) and 79% (95% CI, 64-89%), respectively. In both regression and univariate analysis, neither clinical nor graft parameters were predictors of Day +100 survival.

Evaluation of graft characteristics of the 1912 UCB units

The mean concentration of the post-thaw CFU-GM/million nucleated cells plated of the 1912 UCB units was 1267.9 ± 786.5 , with a median of 1150 (Range, 0-7100). Evaluation of the units revealed moderate correlation between the post-thaw CFU-GM/million nucleated cells plated and CD34 + cells in both the pre-freeze ($r = 0.35$) and post-thaw data sets ($r = 0.41$ and 0.46 , respectively for CD34+ cells and CD34 + %). Pre-freeze total CFU counts weakly correlated with post-thaw CFU-GM counts ($r = 0.11$).

Post-thaw CFU-GM demonstrated weak correlation with other pre-freeze and post-thaw graft parameters (TNC, NRBC and viability), as well as post-thaw hematocrit.

Storage time of the unit did not influence the post-thaw CFU-GM ($r = -0.04$). In our analysis, post-thaw CFU-GM was significantly impacted by the CBB ($p < 0.01$). Multiple Pairwise Comparisons among the 24 CBB demonstrated that bank number 6 had significantly lower post-thaw CFU-GM counts compared to other CBB ($p < 0.01$) (except bank numbers 4, 12, and 17) (Fig. 3).

DISCUSSION

To investigate the impact of post-thaw CFU-GM counts on the quality and efficacy of UCB, we studied the transplant outcomes in a cohort of patients receiving single UCB transplant, evaluating rates and speed of engraftment. We also correlated the post-thaw CFU-GM counts of 1912 units with the pre-freeze and post-thaw graft characteristics, hoping to optimize selection criteria of UCB.

Compared to other studies,^{14,15,19} our overall cumulative incidence of neutrophil and platelet recovery was quite high. Rates of neutrophil and platelet engraftment as reported in large cohort studies ranged from 76.9% to 87% and 55% to 71%, respectively.^{14,15,19} Previous studies reportedly demonstrated longer median times to neutrophil and platelet engraftment of 22 to 29 days and 84 to 134 days, respectively.^{1,14,15} The high rates of engraftment demonstrated in our study may be related to the high CFU-GM counts/Kg, with a median of $6 \times 10^4/\text{kg}$. Another factor that may have contributed to our higher engraftment rates is the improvement of conditioning protocols over time. In the present study, 42 of the 43 patients (98%) who received RIC achieved neutrophil engraftment at a median time of 16 days ($p < 0.01$). Of note, the early recovery stages in transplanted patients receiving RIC

result in a state of mixed chimerism, where both recipient and donor cells are present in the bone marrow. This allows donor T cells to eradicate the remaining recipient stem cells by graft versus tumor effect.

As expected, higher post-thaw CFU-GM counts were predictive of favorable transplantation outcomes. In myeloablative patients, higher post-thaw CFU-GM was strongly predictive of neutrophil and platelet engraftment $p < 0.01$. We demonstrated an association between postthaw CFU-GM/Kg dose and the speed of engraftment of both neutrophils and platelets $p < 0.01$. Median times to neutrophil and platelet engraftment were 26 and 59 days for the lowest post-thaw CFU-GM dose, compared to 14 and 41 days with the highest post-thaw CFU-GM dose, respectively. We observed a strong correlation between the postthaw CD34+ dose/kg and post-thaw CFU-GM count/kg in patients receiving both myeloablative and RIC regimens. Regression analysis, however, revealed an interaction in which post-thaw CFU-GM showed additional benefit for patients receiving the lowest quartile of CD34+ cell dose.

Because of the high CFU-GM counts of our transplanted single units and the associated high engraftment rates, no threshold value for CFU-GM/Kg dose (above which successful engraftment was likely) could be determined in our cohort of patients.

HLA disparity did not adversely impact either neutrophil or platelet engraftment. Similar results were observed previously following single UCB transplant in patients with AML.²⁰ It has been suggested that variation in conditioning and higher cell dose can further counter the negative impact of HLA mismatch.^{6,21}

The value of CFU was described initially in a study conducted in 2000 by the New York Blood Center, in which the pre-freeze CFU showed greater correlation with the engraftment of both neutrophils and platelets, as compared with TNC.¹ More recent studies demonstrated the importance of post-thaw CFU on engraftment.^{14,16} Page and colleagues described a significant correlation between engraftment and CFU measured either pre-freeze or post-thaw.¹⁵ Different findings regarding the influence of CFU counts on clinical outcomes following bone marrow and peripheral blood progenitor cell transplant have been reported previously.^{22–27}

The CFU assay has its difficulties—it requires technical expertise, special materials and reagents, results are delayed, and it is not easily standardized.^{27,28} In the current study, postthaw CFU-GM was significantly impacted by the CBB ($p < 0.01$). Interlaboratory variability was previously reported and was thought to be related to individual colony interpretation, sample preparation and plating.^{29–34} Additionally, different labs utilize different media for their assay and use different techniques for cell isolation, mixing and plating. Lack of standardization with thaw methods such as wash versus dilution, the use of different thawing reagents and methods can also contribute to testing variability. The lack of correlation of our post-thaw CFU-GM/million cells plated with the pre-freeze CFU counts provided by various shipping banks supports this variation in bank practice. This weak correlation may also be explained by cell loss (decreased viability and low recovery) during cryopreservation, thawing, and washing.¹⁵

Evaluation of the 1912 units showed that despite potential CD34+ testing standardization issues (i.e., pre-freeze testing at bank, then post-thaw testing at transplant center), post-thaw CFU-GM/million showed moderate correlation with CD34 + cell content measured both pre-freeze and post-thaw.

Other graft parameters revealed weak correlation with the post-thaw CFU-GM/million. It has been widely reported that thawing and washing can impact the number of TNC.³⁵ TNC includes NRBC which are significantly variable and susceptible to hemolysis during freezing,³⁶ though they correlate with progenitor cell numbers. Additionally, numbers of nucleated cells, particularly neutrophils are decreased during cryopreservation and thawing due to cell loss and clumping of damaged cells.^{37,38} In the current study, cell viability measured by different centers weakly correlated with our post-thaw CFU-GM/million cells. This finding highlights the need for standardization of this assay. Furthermore, most centers use trypan blue dye exclusion, which while simple and rapid, may not be the optimal assay for hematopoietic viability/potency.^{39,40} Another factor that may possibly influence the reliability of this assay is that cell death as assessed by TNC viability presumably reflects the loss of differentiated cells which have lesser role in engraftment as compared to progenitor/stem cells.⁸

Our findings are in accordance with those reported by Purtill et al.,⁴¹ which revealed that CD34+ cell count, whether measured pre-freeze or post-thaw, was a more reliable measure of unit quality than TNC. The authors also stated that at their center they prioritize pre-freeze CD34+ cell count over TNC among units with TNC and CD34+ values of greater than $1.5 \times 10^7/\text{kg}$ and $0.7 \times 10^5/\text{kg}$, respectively. Similar findings have been reported in previous studies.^{42,43}

In this study, storage time of the unit did not impact post-thaw CFU-GM counts. Our results are consistent with those obtained in a previous study which demonstrated no significant difference in hematopoietic stem cell viability or proliferation for up to 14.5 years after cryo-preservation.⁴⁴ Post-thaw hematocrit did not impact the post-thaw CFU-GM counts. Our results contrast with those reported by Assmus et al.,⁴⁵ which revealed that the number of contaminated RBCs in bone marrow has been found to impact viability and CFU capacity. However, with standard UCB manufacturing methods, red cell content is much lower than that of marrow.

In the present study, we observed significant variability in the quality of units among various banks, which indicates the importance of qualifying CBBs. It has been demonstrated that the quality of UCB can be different from unit to unit^{46,47} and that initial unit selection criteria should include the CBB from which the unit is obtained.^{48,49}

At our facility, we have established a qualification process for CBBs to ensure the quality of units and to meet FDA vendor qualification requirements. This approach allows our team to track and trend the quality indicators of UCB units, and when quality issues are noted, the designated CBB is notified and potentially disqualified.⁴⁹

To select the unit that best predicts engraftment, a cord blood Apgar scoring system has been previously suggested. This scoring system assesses the pre-freeze and post-thaw graft

parameters, including CFU, and arrives at a composite score.^{11,50} Our data further underscores the potential clinical utility of post-thaw CFU-GM assay as an important quality control index of UCB as determined by the hazard ratio and speed of engraftment of both neutrophils and platelets.

The in vitro analysis of post-thaw CFU-GM, however, showed considerably weaker correlation with pre-freeze CFU counts than that observed with pre-freeze and post-thaw CD34+. This may indicate that CD34+ cell enumeration is more standardized among CBB. Accordingly, we suggest that pre-freeze CD34+ cell counts provided by the shipping bank can help in the primary selection of the appropriate unit to be used in the setting of single UCB transplant.

Currently, at our facility we perform a CFU-GM assay on the post-thaw sample at the time of unit infusion with results available 2 weeks post-transplant. The clinical team is notified of poor results. Optimally, a post-thaw CFU result would be available prior to transplant. Perhaps CBB could test CFU (and other QC parameters) post-cryopreservation (e.g., 1 year out) to assist transplant centers with selection of the optimal unit(s) for their patients. Standardization and validation of testing with a segment, however, would be required to systematically implement this practice for UCB testing.⁵¹

Certainly, while post-thaw CFU-GM/ million nucleated cells plated provides a valuable assessment of the biologic activity and potency of the unit as a whole, the number of cells contained in a unit and the dose it provides are also crucial in identifying an appropriate graft.

Our data underscores the importance of assay standardization as a means to help improve the validity and consistency of UCB criteria. Previously our group published on the standardization of the CFU-GM assay, showing that improved control over cell count seeding of culture wells decreased assay variability by 70-73%.³¹ Over the years at our center, much effort has been and continues to be dedicated to standardization of the CFU assay. In the era of evolving technology, faster and improved methods for CFU enumeration or other potency assessment are currently being investigated.⁵² This may help to resolve the assay limitations, ultimately achieving best practices in the field of UCB QC testing.

CONCLUSION

CFU-GM measured at the time of unit thaw is an effective measure of the quality and potency of UCB, particularly adding valuable clinical information when the post-thaw CD34+ cell dose is low. It can also help in the assessment and qualification of CBBs, ultimately ensuring the greatest potential for efficacy of the final patient product.

Our analysis suggests that both pre-freeze and postthaw CD 34+ cell enumeration are reflective of unit potency. Hence, CD34+ cell enumeration provided by banks could be considered as an additional selection criterion, particularly when HLA match and nucleated cell dose are equivalent among available options.

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Fig. 1. Neutrophil (ANC) and platelet (PLT) recovery in the myeloablative patients. Analysis of the four quartile CFU-GM/kg doses (A) and (B). Correlation of CFU-GM/kg to time to engraftment (C) and (D) (Triangles indicate engraftment and circles indicate graft failure)

Fig. 2. Neutrophil (ANC) and platelet (PLT) recovery in patients receiving reduced intensity conditioning. Analysis of the four quartile CFU-GM/kg doses (A) and (B). Correlation of CFU-GM /kg to time to engraftment (C) and (D) (Triangles indicate engraftment and circles indicate graft failure)

Fig. 3. Medians of post-thaw CFU-GM/million nucleated cells plated in units collected by 24 cord blood banks. [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 1.
Patients' demographics and graft characteristics of the transplanted single cord blood units

Variable	Conditioning			p value
	All patients	Myeloablative	Reduced Intensity	
Number of patients	269	226	43	
Age	7 (<1-66), (2-15)	6 (<1-55), (1-12)	32 (<1-66), (9-59)	<0.01
Gender: Male	154 (57%)	135 (60%)	19 (44%)	0.06
Diagnosis				
Aplastic anemia	22 (8%)	22 (10%)	0	
Immune deficiency	9 (3%)	9 (4%)	0	
Metabolic disorder	94 (35%)	81 (36%)	13 (30%)	
Epidermolysis bullosa	5 (2%)	3 (1%)	2 (5%)	
Acute lymphoblastic leukemia	45 (17%)	44 (19%)	1 (2%)	
Acute myeloid leukemia	48 (18%)	34 (15%)	14 (33%)	
Chronic myeloid leukemia	3 (1%)	3 (1%)	0	
Other leukemia	5 (2%)	5 (2%)	0	
Myelodysplasia	10 (4%)	9 (4%)	1 (2%)	
Non-Hodgkin's lymphoma	22 (8%)	16 (7%)	6 (14%)	
Hodgkin's lymphoma	4 (1%)	0	4 (9%)	
Myeloproliferative disorders	1 (<1%)	0	1 (2%)	
Chronic lymphoid leukemia	1 (<1%)	0	1 (2%)	
Pre-freeze TNC ($\times 10^9$) as reported by CBB median range (IQR)	1.8 (0.4-18.5) (1.3-2.4)	1.7 (0.6-18.5) (1.3-2.3)	2.2 (0.4-4.1) (1.8-2.9)	<0.01
Pre-freeze CD34+ ($\times 10^6$) as reported by CBB median range (IQR)	6.0 (0.7-4.9) (3.2-9.1)	5.8 (0.7-4.9) (3.2-9.0)	7.1 (13.8-35.5) (3.5-12.2)	<0.01
Post-thaw CD34+ ($\times 10^6$ /kg) median range (IQR)	0.6 (0.1-9.7) (0.3-1.2)	0.6 (0.1-9.7) (0.3-1.0)	0.5 (0.1-7.5) (0.4-1.3)	0.11
Post-thaw CFU/Kg ($\times 10^4$ /kg) median range (IQR)	6.0 (1.1-152.7) (3.3-11.4)	6.2 (0.1-132.2) (3.5-12.0)	5.5 (0.4-152.7) (2.7-7.3)	<0.01
HLA match				0.33
6/6	72 (27%)	61 (27%)	11 (26%)	
5/6	144 (54%)	124 (55%)	20 (47%)	
4/6	53 (20%)	41 (18%)	12 (28%)	

TABLE 2.

Univariate analysis of graft parameters affecting both neutrophil and platelet engraftment in patients receiving myeloablative conditioning

Variable	Number of patients	Cumulative incidence			Time to engraftment (Days)		p value	
		Neutrophil engraftment	Platelet engraftment		Neutrophil engraftment	Platelet engraftment	Neutrophil engraftment	Platelet engraftment
All Patients	226	96% (92-98%)	(83%) 75-91%		19 (14-25)	47 (38-72)	<0.01	<0.01
Post-thaw CFU/Kg ($\times 10^4$)								
1st Quartile (<3.5)	57	93%	79%		26	59		
2nd Quartile (3.5-6.2)	56	95%	79%		21	54		
3rd Quartile (6.2-12.0)	56	96%	89%		17	42		
4th Quartile (>12.0)	57	98%	84%		14	41		
Post-thaw CD34/kg ($\times 10^6$)								
1st Quartile (<0.35)	56	93%	80%		25	54	<0.01	0.06
2nd Quartile (0.4-0.61)	57	100%	86%		22	48		
3rd Quartile (0.61-1.3)	56	95%	84%		16	43		
4th Quartile (>1.3)	57	95%	81%		14	43		
Post-thaw CFU/Kg ($\times 10^4$) among lowest quartile of Post-thaw CD34/kg ($\times 10^6$)							0.01	0.02
1st Quartile	14	79%	64%		21	61		
2nd Quartile	14	93%	86%		30	51		
3rd Quartile	14	100%	71%		23	57		
4th Quartile	14	100%	100%		28	47		

TABLE 3.
Regression analysis on neutrophil and in patients receiving myeloablative conditioning

Characteristic	Number	Hazard ratio 95% CI		p value	
		Neutrophil engraftment	Platelet engraftment	Neutrophil engraftment	Platelet engraftment
Post-thaw CFU/Kg ($\times 10^4$)					
1st Quartile	57	1.0 (reference)	1.0 (reference)	<0.01	<0.01
2nd Quartile	56	1.6 (1.1-2.4)	1.1 (0.7-1.5)	0.01	0.83
3rd Quartile	56	2.8 (1.9-4.3)	2.1 (1.4-3.2)	<0.01	<0.01
4th Quartile	57	6.5 (4.1-10.2)	2.5 (1.6-3.9)	<0.01	<0.01
HLA matching					
6/6	61	1.0 (reference)	1.0 (reference)	NS	NS
5/6	124	1.2 (0.9-1.6)	1.0 (0.7-1.4)		
4/6	41	0.9 (0.6-1.4)	0.7 (0.5-1.2)		
Age (continuous) HR/decade					
Diagnosis					
Malignancy	109	1.0 (reference)	1.0 (reference)	NS	NS
Non-malignancy	107	0.8 (0.6-1.1)	0.5 (0.4-0.7)	0.1	<0.01

Univariate analysis of graft parameters affecting both neutrophil and platelet engraftment in patients receiving reduced intensity conditioning

TABLE 4.

Variable	Number of patients	Cumulative incidence (%) 95% CI		Time to engraftment (Days) Median (IQR)		p value	
		Neutrophil engraftment	Platelet engraftment	Neutrophil engraftment	Platelet engraftment	Neutrophil engraftment	Platelet engraftment
All Patients	42	100%	(86%) 69-100%	16 (7-23)	41 (34-52)	0.048	0.89
Post-thaw CFU/Kg ($\times 10^4$)							
1st Quartile (<3.5)	10	91%	82%	16	40		
2nd Quartile (3.5-6.2)	10	100%	90%	23	40		
3rd Quartile (6.2-12.0)	11	100%	82%	9	44		
4th Quartile (>12.0)	11	100%	91	11	42		
Post-thaw CD34/kg ($\times 10^6$)						0.78	0.86
1st Quartile (<0.35)	10	100%	90%	17	43		
2nd Quartile (0.4-0.61)	11	100%	91%	20	45		
3rd Quartile (0.61-1.3)	10	100%	80%	16.5	40		
4th Quartile (>1.3)	12	92%	83%	9	41		

Regression analysis on neutrophil and platelet engraftment in patients receiving reduced intensity Conditioning

TABLE 5.

Characteristic	N	Hazard ratio 95% CI		p value	
		Neutrophil engraftment	Platelet engraftment	Neutrophil engraftment	Platelet engraftment
Post-thaw CFU/Kg ($\times 10^4$)				0.04	0.51
<Median	21	1.0 (reference)	1.0 (reference)		
Median	22	2.1 (1.0-4.3)	1.2 (0.7-2.3)		
HLA matching				NS	NS
6/6	11	1.0 (reference)	1.0 (reference)		
5/6	20	1.2 (0.5-2.6)	1.0 (0.5-2.1)		
4/6	12	2.5 (0.8-7.3)	0.7 (0.2-2.0)		
Age (continuous) HR/decade		1.1 (0.8-1.4)	1.1 (0.8-1.4)	NS	NS
Diagnosis				NS	NS
Malignancy	28	1.0 (reference)	1.0 (reference)		
Non-malignancy	15	0.9 (0.2-4.0)	1.9 (0.4-9.2)		