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Post-Thaw Viable CD45$^+$ Cells and Clonogenic Efficiency are Associated with Better Engraftment and Outcomes after Single Cord Blood Transplantation in Adult Patients with Malignant Diseases

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A B S T R A C T

The quantity of cells is widely accepted as the main factor influencing the outcome after umbilical cord blood transplantation (UCBT) however, the quality of the cord blood units (CBUs) has been less studied. In order to determine the impact of qualitative variables in UCBT outcomes, we conducted a multicenter retrospective study in adult patients with hematological malignancies who underwent single UCBT after a common myeloablative conditioning regimen. One hundred and ten patients from 3 institutions [median age, 35 years (range 18-55)] were included. Quantitative (TNC and total CD34+cells) and qualitative variables [viable CD45+ (vCD45+), vCD34+ and clonogenic efficiency [(CLONE), quotient of post-thaw colony-forming units (CFU)] and pre-freeze CD34+ cells predicted engraftment in univariate analysis however, only 2 qualitative variables remained significant in the multivariate analysis. Infusion of more than 2×10^7 post-thaw vCD45+ cells per kilogram was significantly associated with faster neutrophil ($P = .01$), platelet engraftment ($P = .01$), higher disease-free ($P = .01$) and overall survival (0.02). In addition, CLONE $\geq$ 20% predicted a faster neutrophil ($P = .005$), platelet engraftment ($P = .01$) and contributed to decrease the non-relapse mortality ($P = .02$). Our study suggests that the vCD45+ cells dose and CLONE are powerful surrogate markers of graft quality and can potentially help on CBUs selection if tested with representative reference samples.

INTRODUCTION

In addition to the level of HLA matching, total nucleated cells (TNC) and CD34$^+$ cells content are the most important factors influencing engraftment after umbilical cord blood transplantation (UCBT) [1-3]. In spite of well-defined cellular thresholds for cord blood unit (CBU) selection, engraftment delay and graft failure still remain major concerns, suggesting that other "qualitative" factors may also influence CBU engraftment capacity. In this regard, there is evidence that colony-forming unit (CFU) counts are associated with rate and kinetics of engraftment and also with long-term outcomes, suggesting the role of functional assays in determining CBU quality [4,5].

Graft potency, defined as the ability of the hematopoietic progenitor cells (HPC) to engraft, is in general assessed by viability of flow cytometry and/or clonogenic assays. These tests allow the enumeration of viable cells and infused CFU, which are both associated with outcomes after UCBT [5]; however, clear definitive thresholds have not been established. In addition, and based on the correlation between CFU and CD34$^+$ cells, we previously proposed the use of the clonogenic efficiency (CLONE) as a new measure of HPC potency [6]. Herein, we defined the estimated CLONE (ECLONE), measured as the ratio between post-thaw CFU counts and prefreezing CD34$^+$ cell numbers, that could provide insight into the dynamics of the CD34$^+$ function

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from cryopreservation to thawing. Thus, when numbers of recovered post-thaw CFU are below the expected value for the seeded prefreezing $CD34^+$ cells, a loss of HPC function may be anticipated.

In this study, we assessed quantitative CBU characteristics (as conventional TNC and $CD34^+$ cell counts) and qualitative variables (viable $CD45^+$ [$vCD45^+$], viable $CD34^+$ [$vCD34^+$], viable $CD3^+$ [$vCD3^+$] cell counts, CFU, and ECLONE). We hypothesized that qualitative variables may impact outcomes after UCBT when the cell dose threshold is achieved. To identify the qualitative factors affecting outcomes after UCBT, we conducted a retrospective study of adult patients with hematological malignancies who underwent single UCBT.

METHODS

Enrollment Criteria

The study included 110 consecutive adult patients who received unrelated single UCBT in the Hospital Vall d'Hebrón, Sant Pau and Germans Trias i Pujol (Barcelona) between January 2005 and December 2013. All patients with hematological malignancies were eligible for enrollment if they met the following criteria: (1) high-risk hematological malignancy requiring urgent hematopoietic cell transplantation; (2) absence of suitable related or unrelated donor (HLA identical or 1 antigen mismatched); (3) lack of suitable HLA-matched unrelated donor at a reasonable time after the start of the search through international registries, and (4) a suitable umbilical CBU available, as described below. Patients receiving ex vivo–expanded grafts were excluded. Patients gave written informed consent for their inclusion in each transplantation protocol.

CBU Selection

The CBUs selected for transplantation should be ≥4/6 HLA–matched with the recipient (considering HLA-A and -B at antigen level and -DRB1 at allele level). Cell dose was considered the most important criterion for unit selection. Those units fulfilling HLA-matching criteria were ranked according to their content of TNCs and $CD34^+$ cells. Minimum cell doses required were TNC $>1.5 \times 10^7$/kg and $CD34^+$cells $>.6 \times 10^5$/kg.

Conditioning Regimen, Graft-versus-Host Disease Prophylaxis, and Supportive Care

All patients received myeloablative conditioning. Two conditioning protocols were used and were described previously [7-9]. In brief, 8 patients received thiotepa (10 mg/kg), busulfan i.v (9.6 mg/kg), and cyclophosphamide (120 mg/m^2), whereas the remaining 102 patients received the same conditioning regimen but cyclophosphamide was replaced by fludarabine (150 mg/m^2). All patients received in vivo T cell depletion with rabbit antithymocyte globulin (Thymoglobulin, Genzyme, Lyon, France) at a total dose, 6 mg/kg to 8 mg/kg.

Graft-versus-host disease (GVHD) prophylaxis included cyclosporine (1.5 mg/kg/12 hours i.v) followed by 3 to 5 mg/kg/12 hours orally when oral intake was possible and slow tapering starting between day +90 and + 180, if feasible. Cyclosporine was combined with a short-course of steroids (1 mg/kg/daily) from day +14 to +28 or mycophenolate mofetil (15 mg/kg/daily from -1 to +30 day).

Granulocyte colony–stimulating factor was administered s.c at 5 μg/kg/daily from day +7 until neutrophil engraftment. Antibacterial and antifungal prophylaxis were given according to each center's protocol.

Cord Blood Banks and Graft Characteristics

CBUs were thawed and washed according to the standard method of Rubinstein et al. [10] or a modified approach using an automated system [11]. Both international and domestic (Spanish) cord blood banks (CBBs) provided CBUs for transplantation. Prefreezing information was obtained from the original CBB provider. Post-thaw characteristics were determined before infusion at Banc de Sang i Teixits cell therapy service, which served as a processing laboratory for all referring transplantation units.

Laboratory Analysis

Automatic cell count

Counting of TNC, RBC, and platelets were performed on each sample by using an automatic cell counter that detects and measures changes in electrical resistance (impedance) when a particle in a conductive liquid passes through the device (Coulter AcT diff, Beckman Coulter, Miami, FL).

Flow cytometry

Post-thaw $CD45^+$ and $CD34^+$ cell enumeration, including viability, was performed following the International Society of Hematology and Graft Engineering protocol but with modified gating for better determination of viable cells, similar to the method applied by Scaradavou et al. [12]. This methodology excludes only erythroblasts and debris to evaluate the number of live and dead cells. All cellular events present were thus counted and therefore, proportions of dead and live cells were more precisely calculated.

Samples were taken after washing and incubated for 15 minutes at 4°C in the dark with antihuman phycoerythrin (PE)–labeled monoclonal antibodies (mAbs) against $CD34^+$and $CD3^+$ combined with FITC-labeled $CD45^+$ at a saturating concentration. After incubation, the samples were lysed in ammonium chloride for 10 minutes and a volume of commercially available fluorescent mycrobeads were added and gently mixed as an internal standard before acquisition, according to the manufacturer's instructions (Perfect Count; Cytognos, Salamanca, Spain) to evaluate their absolute count using cytometric single-platform analysis [13]. The samples were also incubated with 7-aminoactinomycin D for 5 minutes to determine the cell viability/permeability. The cell subsets were then analyzed by their fluorescence profile and forward and side-scatter characteristics, on a Becton-Dickinson FACScalibur flow cytometer with the BD software, Cellquest (Becton-Dickinson, Franklin Lakes, NJ).

Clonogenic assays

Progenitor cell assays were performed after thawing and washing with methylcellulose-based H4434 medium. In detail, the cells were seeded directly onto methylcellulose, mixed gently and then plated onto petri dishes and incubated at 37°C in a humidified atmosphere of 5% CO_2 in air. Granulocyte macrophage colony–forming units, burst-forming units-erythroid, and CFU-mix colonies were identified and counted using standard criteria after 10 days in culture [14]. For CFU counting, only colonies with over 50 cells were enumerated. *Total CFU*, defined as the total count of colonies, regardless of their lineage, was used for analyzing the clonogenic potential of the product.

Definitions

Variables for CBU specifications

Quantitative variables considered for the study were prefreezing TNC and $CD34^+$ cells and post-thaw TNC enumerated at the processing laboratory. Qualitative variables considered for the study were post-thaw 7-aminoactinomycin D cells counts (infused $vCD45^+$, $vCD34^+$, and $vCD3^+$), CFU, and ECLONE. The latter was calculated using the ratio between post-thaw CFU (obtained in the processing laboratory) and prefreezing $CD34^+$cells (reported by the CBB of origin). Provided that CFU and $CD34^+$ have a statistically significant correlation in standard conditions [6,15] for a given prefreeze $CD34^+$ count, a post-thaw CFU value is expected. Decrease of ECLONE below that which is expected could be a surrogate of loss of graft potency.

Donor engraftment

Myeloid engraftment was defined as the first of 3 consecutive days with an absolute neutrophil count equal or higher to $.5 \times 10^9$/L and t*ransfusion-independent platelet engraftment* was defined as the first of 3 consecutive days with $\geq 20 \times 10^9$/L without transfusion support for 7 consecutive days. *Sustained donor engraftment* was defined as sustained donor derived count recovery with full donor chimerism (>95% donor hematopoiesis). Full donor chimerism was determined by quantitative polymerase chain reaction of informative polymorphic short tandem repeat regions of DNA from donor and recipient using AmpFlSTR Identifiler PlusPCR Amplification kit (Applied Biosystems, Carlsbad, CA). Patients who survived >42 days after transplantation and who failed to achieve myeloid engraftment were considered to have *primary graft failure*. *Secondary graft failure* was defined as the loss of the engraftment in the absence of relapse.

GVHD, nonrelapse mortality, relapse, disease-free survival, and overall survival

GVHD was defined and graded according to standard criteria [16,17]. Patients dying before +100 day were not considered for chronic GVHD analysis. *Nonrelapse mortality* (NRM) was defined as death from any cause without evidence of relapse. *Disease-free survival* (DFS) was defined as survival from the time of transplantation without evidence of disease relapse/progression. *Overall survival* (OS) was defined as survival from the time of transplantation.

Baseline Disease Status

Disease status at the time of transplantation was classified as follows: (1) early phase: acute leukemia, myelodysplastic syndrome (MDS) or

lymphoma in first complete remission, untreated MDS with <5% blasts, and/or chronic myeloid leukemia (CML) in the first chronic phase; (2) intermediate phase: acute leukemia, lymphoma or MDS in a second remission, and CML in a second or further chronic or accelerated phase; and (3) advanced phase: acute leukemia and lymphoma not in remission, CML in blast crisis, and untreated refractory anemia with excess blasts [18].

Statistical Analysis

Clinical, demographic, and graft potency variables considered for prognostic factors analysis were year of transplantation, gender, age, disease status at transplantation, diagnosis, recipient cytomegalovirus (CMV) serology, patient's body weight, HLA match, GVHD prophylaxis, ABO blood group compatibility, prefreezing TNCs, prefreezing total $CD34^+$ cells, post-thaw TNCs, post-thaw $vCD45^+$ cells, post-thaw $vCD34^+$ cells, post-thaw $vCD3^+$, CFUs, and ECLONE. Continuous variables were dichotomized at the most discriminative cut-off for each outcome. Baseline characteristics were described as median and interquartile range for quantitative variables and frequency and percentages for categorical variables. Correlation between variables was analyzed by Spearman's correlation test. Cumulative incidence curves were used in a competing risk setting to calculate the cumulative incidence of neutrophil and platelet engraftment, NRM, and relapse [19]. Death without engraftment was the competing event for neutrophil and platelet engraftment. Relapse was considered the competing event for NRM. Death without relapse was the competing event for relapse. Unadjusted time to event analysis were performed by Kaplan-Maier estimate and for comparisons, the log-rank test was used [20]. Characteristics selected for inclusion in multivariate model were established at $P < .10$ in univariate analysis. Cox proportional hazard regression method and Fine and Gray method for competing events were used for multivariate analysis [21]. Only cases with complete data (n = 105) were considered in multivariate model. Two-sided P values < .05 were considered statistically significant in the multivariate model. Statistical analysis was conducted using the SPSS statistical software (SPSS version 20.0, Chicago, IL) and R version 2.15.2 (The CRAN project) with packages survival v2.37-7, cmprsk v2.2-7, splines v3.1.7 and prodlim v1.4.5.

RESULTS

Patient, Graft, and Transplantation Characteristics

Table 1 shows the main characteristics of the 110 patients included in the study. Median age was 35 years (range, 18 to 55), 81 patients (74%) had acute leukemia, 51 (46%) received transplantation in the early phase of the disease, and 71 (65%) received CBUs mismatched at 2 loci.

Cell Doses and Potency Factors of the Umbilical CBU

Table 2 summarizes cell dose and qualitative characteristics contained in CBU prefreezing and at the time of infusion (post-thaw). The R correlation coefficient of post-thaw $vCD45^+$ and prefreezing TNC was .59 ($P < .01$) and correlation of post-thaw $vCD34^+$ and prefreezing total $CD34^+$ cell numbers was .73 ($P < .01$). Interestingly, ECLONE showed a very high linear correlation ($R = .082$, $P < .01$).

Factors Predictive of Outcome

Results of multivariate analyses are summarized in Table 3. The data from univariate analysis are presented in Supplementary Material.

Neutrophil engraftment

Nine patients (8%) died before day 42 after transplantation without engraftment (median time, 15 days; range, 8 to 38) and were considered not evaluable for engraftment. Eleven of 101 patients (11%) had primary graft failure and the remaining 90 patients (82%) achieved neutrophil engraftment at a median of 23 days (range, 8 to 50). The cumulative incidence of neutrophil engraftment at day 28, 42, and 60 after transplantation was 57% (95% confidence interval [CI], 48% to 67%), 75% (95% CI, 67% to 84%), and 82% (95% CI, 76% to 90%). Multivariate analysis indicated that higher post-thaw $vCD45^+$ cells ($P = .01$), higher post-thaw $vCD34^+$ cells ($P = .01$), higher ECLONE ($P = .005$), and recipient CMV negative status ($P = .02$) were independently associated with higher cumulative incidence of engraftment. Figures 1A and 1B show the effect of post-thaw $vCD45^+$ cell dose and ECLONE on neutrophil engraftment in univariate

Table 1
Patient and Transplantation Characteristics

Characteristics (N = 110)	No. of Patients (%)*
Age, median (range, IQR), yr	35 (18-55, 26-46)
Year of transplantation	
2005-2009	46 (42)
2010-2013	64 (58)
Sex	
Female	56 (51)
Male	54 (49)
Recipient's body weight, median, (range, IQR), kg	70 (40-113, 60-77)
CMV serologic status	
Positive	77 (70)
Negative	33 (30)
Diagnosis	
AML	55 (50)
ALL	26 (24)
Lymphoma (non-Hodgkin and Hodgkin)	17 (16)
CML	6 (5)
Other	6 (5)
Disease status	
Early	51 (46)
Intermediate	28 (25)
Advanced	31 (28)
Previous autologous transplantation	
Yes	20 (18)
No	90 (82)
GVHD prophylaxis	
CsA-PDN	78 (71)
CsA-MMF	32 (29)
Conditioning regimen	
TT + FLU + BU + ATG	102 (93)
TT + CY + BU + ATG	8 (7)
HLA compatibility†	
4 out of 6	71 (65)
5 out of 6	32 (29)
6 out of 6	7 (6)
ABO blood group incompatibility	
Major	24 (22)
Minor	27 (24)
None	59 (54)
Donor-recipient sex match, no. (%)	
Male-male	34 (31)
Male-female	25 (23)
Female-male	23 (21)
Female-female	28 (25)

IQR indicates interquartile range; AML, acute myeloid leukemia; ALL, acute lymphoblastic leukemia; CML, chronic myeloid leukemia; CsA, cysclosporine; PDN, prednisone; MMF, mycophenolate mofetil; TT, thiotepa; FLU, fludarabine; BU, busulfan; ATG, antithymocyte globulin; CY, cyclophosphamide.

* Percentatges may not add to 100 because of rounding.

† By considering HLA-A and -B at antigen level and -DRB1 at allele level.

Table 2
Cell Content of the Cord Blood Units

Cell	Median (Range, IQR)
At time of freezing	
TNCs × 10^7 per kg	3 (1.5-6.7, 2.28-3.85)
$CD34^+$ cells × 10^5 per kg	1.73 (.6-9.2, 1.24-3.12)
At time of infusion	
TNCs × 10^7 per kg	2.6 (1-5.3, 2.02-3.2)
Viable $CD45^+$ × 10^7 per kg	2.4 (.5-4.8, 1.7-3)
Viable $CD34^+$ cells × 10^5 per kg	1 (.2-4.2, 0.67-1.84)
Viable $CD3^+$ cells × 10^7 per kg	4.1 (.8-38, 2.6-6.2)
CFU × 10^4 per kg	3.7 (.1-16, 2.04-6.65)
ECLONE, (%)	22 (.6-64, 11.3-36.2)

Table 3
Factors Affecting the Outcome after UCBT in Multivariate Analysis

Variables	HR	95% CI	*P* Value
Neutrophil engraftment			
Post-thaw viable CD45$^+$ $\geq 2 \times 10^7$/kg	1.9	1.07-3.3	.01
Post-thaw viable CD34$^+$ $\geq 1 \times 10^5$/kg	1.2	1.09-1.4	.01
ECLONE $\geq$20%	1.7	1.2-2.9	.005
Recipient CMV negative status	1.31	1.19-2.03	.02
Platelet engraftment			
Post-thaw viable CD45$^+$ $\geq 2 \times 10^7$/kg	1.19	1.08-1.33	.01
ECLONE $\geq$20%	1.53	1.09-2.1	.01
Recipient CMV status negative	1.47	1.12-2.22	.02
NRM			
Early disease status	.37	.09-.7	.01
ECLONE $\geq$20%	.68	.38-.8	.02
Relapse risk			
Advanced disease status	1.9	1.6-2.7	.02
Age	1.1	1.07-1.3	.01
DFS			
Early disease status	1.9	1.1-3.5	.02
Post-thaw viable CD45$^+$ $\geq 2 \times 10^7$/kg	2	1.4-2.7	.01
OS			
Early disease status	1.8	1.1-3.5	.02
Post-thaw viable CD45$^+$ $\geq 2 \times 10^7$/kg	2.1	1.4-2.7	.02

HR indicates hazard ratio.

analysis. The medians of neutrophil engraftment when post-thaw vCD45$^+$ was $>$ or $<2 \times 10^7$/kg were 21 (range, 9 to 49) and 25 (range, 8 to 50) days, respectively. When ECLONE was above the median (20%), the medians of neutrophil engraftment were 22 (range, 8 to 49) and 26 (range, 17 to 51) days, respectively. Among patients who presented primary graft failure, 9 (82%) of them showed vCD45$^+$ $<2 \times 10^7$/kg and 8 (73%) showed ECLONE $<$ 20%. In these patients, the median vCD45$^+$ and ECLONE values were 1.65×10^7/kg (range, .9 to 2.12) and 12% (range, 4 to 57), respectively.

Platelet engraftment

Platelet recovery was achieved in 90 patients at a median of 36 days after UCB transplantation (range, 11 to 131). The cumulative incidence of platelet engraftment at 120 days was 76% (95% CI, 68% to 86%). Multivariate analysis indicated that a higher post-thaw vCD45$^+$ cells ($P = .01$), a higher ECLONE ($P = .01$), and recipient CMV negative status ($P = .02$) were independently associated with improved cumulative incidence of platelet recovery. The impact of vCD45$^+$ cells and ECLONE on platelet engraftment is drawn in Figures 2A and 2B.

NRM and relapse risk

After a median follow-up of 49 months (range, 12 to 114), 63 (57%) patients died, 47 (43%) of them from transplantation-related causes. The cumulative incidence at 5 years of NRM was 39% (95% CI, 29% to 49%). Multivariate analysis showed that early disease status ($P = .01$) and a higher value ECLONE threshold ($P = .02$) were associated with a lower NRM. The effect of ECLONE on NRM is shown in Figure 3A. Sixteen patients relapsed after UCB transplantation, with a cumulative incidence of relapse at 5 years of 19% (95% CI, 12% to 28%). The 5-year cumulative incidence of relapse risk was higher in patients in advanced disease status ($P = .02$) and in older patients ($P = .01$).

DFS and OS

The 5-year DFS and OS of the whole cohort were 40% (95% CI, 31% to 51%) and 41% (95% CI, 32% to 52%), respectively. In multivariate analysis, variables associated with improved DFS and OS were a higher number of post-thaw vCD45$^+$ cell dose ($P = .01$) and early disease status ($P = .02$ and $P = .02$). The effect of post-thaw vCD45$^+$ cell dose on DFS and OS is represented in Figures 3B and 3C.

DISCUSSION

This study highlights the ability of qualitative variables, in particular vCD45$^+$ cell dose and ECLONE, to predict engraftment and NRM after myeloablative single UCBT in adult patients with malignant diseases. Additionally, vCD45$^+$ events also correlated with DFS and OS.

Main factors to ensure engraftment after UCBT are the use of well-matched HLA and high cell dose CBUs [1-3,7,22]. Nevertheless, despite the use of an adequate cell dose, delayed engraftment or graft failure still remain concerns in many UCBT series. Thus, the possibility arises that engraftment may be influenced by additional quality factors.

Relevance of qualitative factors could derive from the fact that cord blood undergoes several processing steps at different times from collection to cryopreservation that can trigger cell death by apoptosis [15]. With the current methods used in clinical practice for determining cell viability, apoptosis is not well assessed and ECLONE could be more specific for this purpose.

Interestingly, in our study, post-thaw vCD45$^+$ cell dose appears to be a better predictor than TNCs of most important transplantation outcomes. First, post-thaw vCD45$^+$ cell dose

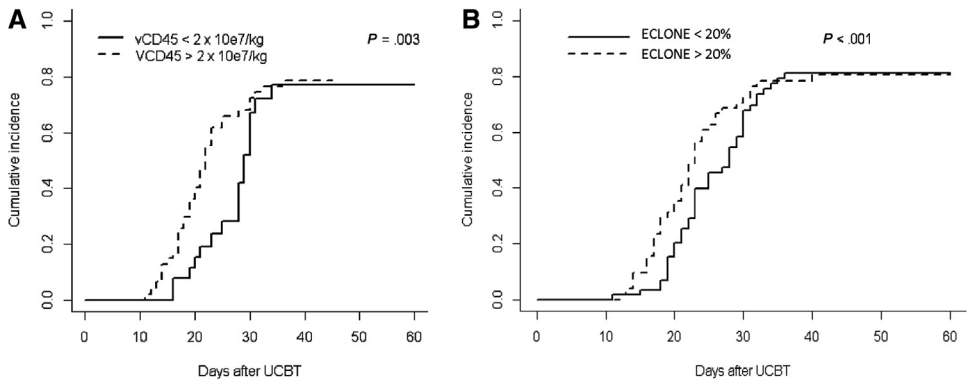

Figure 1. Unadjusted cumulative incidence of neutrophil engraftment according to post-thaw vCD45$^+$ $\times 10^7$/kg (A) and according to ECLONE threshold (B). For patients who received CBU with post-thaw vCD45$^+$ above and below 2×10^7/kg, the cumulative incidence at 60 days and the median time to neutrophil recovery were 82% and 21 days and 67% and 25 days, respectively ($P = .003$). For patients who received CBU with ECLONE above and below 20%, the cumulative incidence at 60 days and the median time to neutrophil recovery were 82% and 22 days and 80% and 26 days, respectively ($P < .001$).

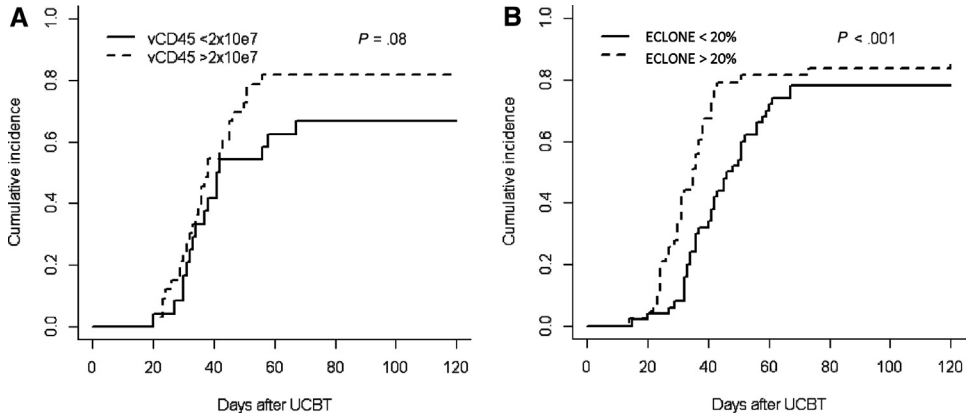

Figure 2. Unadjusted cumulative incidence of platelet engraftment according to post-thaw vCD45$^+$ $\times$ 10^7 per kg (A) and according to ECLONE threshold (B). For patients who received CBU with vCD45$^+$ above and below 2 $\times$ 10^7/kg, the cumulative incidence at 60 days and the median time to platelet recovery were 82% and 34 day and 76% and 36 days, respectively ($P = .08$). For patients who received CBU with ECLONE above and below 20%, the cumulative incidence of platelet recovery at 60 days and the median time to platelet recovery were 86% and 32 days and 78% and 37 days, respectively ($P < .001$).

affects early outcomes, such as neutrophil and platelet engraftment. Assessment of vCD45$^+$ cell dose at the time of infusion can help to establish the quality status of CBUs as cord blood manufacturing combines multiple processing steps and prolonged time from collection to cryopreservation that might result in decreased CD45$^+$ cells viability after thawing. Further, we observed a favorable effect of post-thaw vCD45$^+$ cell dose on DFS and OS, compared with that of TNCs.

The impact of post-thaw vCD45$^+$ cell dose on the outcome after UCBT has not been widely studied. Barlogis et al. [23] reported an improvement of lymphocyte subset reconstitution in children after UCBT receiving a higher post-thaw vCD45$^+$ cell dose/kg threshold than collected or infused TNC and CD34$^+$ cells. The hypotheses to explain this observation is that TNC counts include other cells, such as nonviable cells and mature erythroblasts, that are not involved in engraftment. To the contrary, vCD45$^+$ may represent the group of cells actually delivering the biological function such as lymphocytes, monocytes, and other accessory cells contained in CBUs. The effect of non-HPC in CB engraftment has been proposed by other groups, suggesting the role of specific subsets, such as infused CD8$^+$ in clinical outcomes after UCBT [24].

Contrary to data from other groups, our data support the value of CD34$^+$ as a significant factor affecting engraftment [3,25], although interestingly, we found a statistically significant association between post-thaw vCD34$^+$ and myeloid engraftment but neither total prefreezing nor total infused CD34$^+$ cell counts were predictive. These data reinforce the importance of determining quality variables on CBUs and the use of viable cell dose to better assess the potency. Considering the latter, our data are in accordance with other studies that explored the role of post-thaw CD34$^+$ cells viability, concluding that CBUs with poor viability were unlikely to engraft [12].

Page et al. [25] and other groups reported infused CFUs dose over TNCs or CD34$^+$ cells as the best predictive factor of outcomes after transplantation [4,26]. Unlike these studies, our results did not find an association between total CFUs infused and results after UCBT. It is likely that the use of a high cut-off number of CD34$^+$ for CBU selection minimizes this factor. Noteably, our study reveals that ECLONE is an independent factor on early outcome after UCBT because of its association with neutrophil, platelet engraftment, and NRM, adding a relevant role for a qualitative assessment of clonogenic assays. We previously hypothesized that a decrease in ECLONE can predict relevant losses in HPC functionality [6] but its role has never been explored in a patient cohort. Our study suggests that ECLONE may be a dynamic surrogate of potency, revealing the apoptotic status of CBUs. A potential application of this finding is the assessment of ECLONE for CB selection, providing CD34$^+$ cells and CFU contents are currently required by international CBB standards (ie, FACT-NETCORD) and is widely available. Rodriguez et al. and others [27,28] suggested that

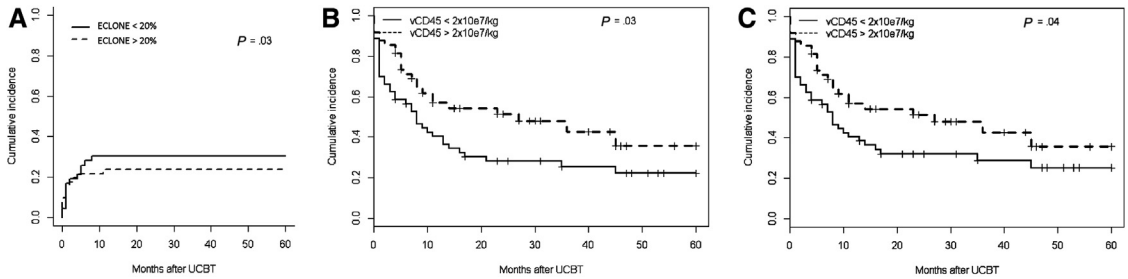

Figure 3. Unadjusted cumulative incidence of NRM (A). For patients who received CBU with ECLONE above 20% the cumulative incidences of NRM at 100 days, 1 year, and 5 years were 20%, 24%, and 30% whereas it was 24%, 38%, and 42% for the threshold below 20% ($P = .03$). DFS and OS (B and C) for patients with post–thaw vCD45$^+$ cell dose above and below 2 $\times$ 10^7/kg were 42% and 41%, respectively, compared with 25% and 24%, respectively, for patients who received a lower dose of post-thaw vCD45$^+$ cell dose ($P = .03$ and $P = .04$ respectively).

potency of CBUs can be tested in a contiguous segment to establish the quality before release of the CBUs because of the high correlation with the bag. In this sense, if the contiguous segments are predictive of graft quality, ECLONE value could be anticipated to identify CBUs with a decreased probability of delayed engraftment or graft failure.

To our knowledge, this is the first evidence that post-thaw vCD45$^+$ cell dose is associated either with main early and long-term outcomes after UCBT and this study also shows the importance of assessing CFUs not exclusively quantitatively but with referral to the clonogenic potential of CD34$^+$ cells in the graft. Taking into account our results, we emphasize the importance of the use of these variables, and we recommend that they are added to the current CBU selection criteria. There are not good assays for quality control of laboratories for graft potency; however, this study suggests the use by CBB of these simple assays (CD45$^+$ viability and ECLONE) to asses cord blood function using a validated contiguous segment. These data can be used to predict potential problems in the CBU and to prevent using a suboptimal units. Nevertheless, it should be noted that despite including patients receiving a relatively homogeneous conditioning regimen and supportive care, this study presents limitations because of the heterogeneous baseline diseases affecting the patients and its limited sample size.

In conclusion, this study demonstrates that early and long-term outcomes after single UCBT in adults with hematological malignancies improves when CBUs contain a higher amount of post-thaw vCD45$^+$ cell dose and a higher ECLONE value, suggesting their clinical implications. Furthermore, the assessment of these variables should be considered and may be assessed in a representative reference sample in the routine selection strategies both at the CBB and at transplantation centers.

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SUPPLEMENTARY DATA

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bbmt.2015.08.016.

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