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Cord Blood Units with High CD3$^+$ Cell Counts Predict Early Lymphocyte Recovery After In Vivo T Cell–Depleted Single Cord Blood Transplantation

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Although high absolute lymphocyte count (ALC) early after transplantation is a simple surrogate for immune reconstitution, few studies to date have established the predictive factors for ALC after umbilical cord blood transplantation (UCBT). We retrospectively studied the factors associated with early lymphocyte recovery and the impact of the ALC on day +42 (ALC42) of $\geq 300 \times 10^6$/L on outcomes in 210 consecutive pediatric and adult patients (112 males; median age, 15 years; range, 0.3 to 60 years; interquartile range, 4 to 36 years) who underwent myeloablative in vivo T cell–depleted single UCBT between 2005 and 2014 for malignant and nonmalignant disorders. In a logistic multivariate regression model, factors favoring a higher ALC42 were higher infused CD3$^+$ cell dose (odds ratio [OR], 2.7; 95% CI, 1.4 to 5.2; $P = .004$), lower antithymocyte globulin dose (OR, 2.3; 95% CI, 1.2 to 4.5; $P = .01$), and better HLA match (OR, 2.1; 95% CI, 1.1 to 4.1; $P = .03$). In multivariate analysis, lower ALC42 was associated with higher nonrelapse mortality (hazard ratio [HR], 1.76; 95% CI, 1.34 to 2.32; $P = .001$), whereas a higher ALC42 was associated with better disease-free survival (HR, 2.03; 95% CI, 1.15 to 3.6; $P < .001$) and overall survival (HR, 2.03; 95% CI, 1.17 to 3.6; $P < .001$). Our study suggests that the selection of better HLA-matched cord blood units containing higher CD3$^+$ cell counts and the use of conditioning regimens with lower ATG doses could improve immune reconstitution after UCBT.

INTRODUCTION

Delays in lymphocyte recovery after umbilical cord blood transplantation (UCBT) are associated with poor outcomes [1,2]. Absolute lymphocyte count (ALC) could be a simple method for monitoring immune reconstitution and already has been identified as a good predictor of outcomes after UCBT [3-5]. Therefore, understanding factors that promote better ALC recovery are of interest for improving outcomes after UCBT.

Early studies have shown a marked delay of lymphocyte recovery after single UCBT [6], especially when low cell counts are administered. These findings have triggered a practice change to protocols using larger or even multiple cord blood units, leading to recent improvements in immune reconstitution [7,8]. Nonetheless, delayed lymphocyte recovery remains a concern, causing high infection-related mortality (30% to 45%) in the first 6 months after transplantation, especially when an in vivo T-cell depletion strategy is used [9,10]. Interestingly, researchers using conditioning protocols omitting T cell depletion [11,12] found decreased infection-related mortality, suggesting that T cell passengers present in the graft and surviving to infusion could contribute to this lymphocyte recovery [13]. Moreover, in a recent registry analysis including a large number of transplants, Pascal et al. [14] confirmed that the use of antithymocyte globulin (ATG) could be detrimental in a reduced-intensity conditioning setting, especially when

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administered at the end of conditioning. This deleterious effect of ATG on outcomes and the risk of infection has not been confirmed in other settings, however [15].

Various pretransplantation variables have been studied in an effort to identify risk factors for lymphocyte recovery after UCBT under a EUROCORD analysis. Niehues et al. [16] described the positive influence of better HLA matching, high infused total nucleated cell (TNC) dose, younger recipient age, recipient-positive cytomegalovirus (CMV) serology, and the omission of in vivo T cell depletion in children undergoing UCBT. More data are needed to confirm the main risk factors for lymphocyte recovery after transplantation.

To confirm the importance of greater lymphocyte recovery on outcomes after UCBT, we retrospectively analyzed the predictive role of ALC on day +42 (ALC42) and investigated pretransplantation factors associated with accelerated lymphocyte recovery in a cohort of 210 pediatric and adult patients diagnosed with a hematologic malignancy or nonmalignancy who underwent myeloablative T cell–depleted single UCBT.

MATERIALS AND METHODS

Patients

A total of 253 patients underwent UCBT in the study hospitals between January 2005 and August 2014. For the purpose of this study, patients who received a second UCBT (n = 7), a related UCBT (n = 14), coinfusion of bone marrow with UCBT (n = 5), or UCBT with haploidendical third-party $CD34^+$ selected cells (n = 17) were excluded from the analysis. The study cohort included all 210 patients (110 [52%] pediatric patients and 100 adult patients) who underwent a first single-unit UCBT at Hospital Vall d'Hebron, Hospital de Sant Pau, or Hospital Germans Trias i Pujol, consisting of 3 adult and 2 pediatric transplantation programs.

Enrollment Criteria

All patients with a hematologic or nonhematologic malignancy were eligible for enrollment if there was a lack of a suitable HLA-matched unrelated donor within a reasonable time after a search through international registries and a suitable umbilical cord blood unit (CBU) available, as described below. This research was performed in accordance with local regulatory approval. Patients or their guardians provided written informed consent for inclusion in each transplantation protocol and for data collection in accordance with the Declaration of Helsinki. For all patients included in the analysis, a single UCBT was the first allogeneic hematopoietic cell transplantation received.

Cell Dose Analysis

All CBUs were thawed and washed by centrifugation. An aliquot was removed from the final product for the analysis before the infusion. This sample was processed for measurement of TNC and flow cytometry assessment of graft composition, including $CD34^+$ and $CD3^+$ cells, by single platform and the corresponding viability by 7-aminoactinomycin using a modified International Society for Hematotherapy and Graft Engineering gating system [17]. For functional assessment of the CBUs, colony-forming unit assays were performed after thawing. All cell processing tests were performed as described previously [18].

Transplantation Procedure and CBU Selection

All patients received myeloablative conditioning before UCBT. The most commonly used protocol, published previously [19], was based on thiotepa (10 mg/kg i.v), fludarabine (150 mg/m^2 i.v), busulfan (9.6 mg/m^2 i.v), and in vivo T cell depletion with ATG 6 to 10 mg/kg i.v. ATG was administered on different schedules starting on day -5 or -4, depending on the overall dose administered, and continuing to day -2.

Graft-versus-host disease (GVHD) prophylaxis was based on cyclosporine (CsA) 1.5 mg mg/kg/12 hours i.v., followed by 3 to 5 mg/kg/12 hours orally when oral intake was possible, with slow tapering starting between day +90 and day +180 if feasible. CsA was combined with a short course of steroids (1 mg/kg/day) from day +14 to +28 or with mycophenolate mofetil (15 mg/kg/day from day -1 to day +30). As supportive care, all patients received post-transplantation granulocyte colony-stimulating factor from day +7 until neutrophil recovery.

For adult patients, the minimum recommended pre-cryopreserved cell counts were TNC $\geq 1.5 \times 10^7$/kg and $CD34^+$ cells $\geq 0.6 \times 10^5$/kg. A degree of HLA matching between CBU and the recipient of at least 4 of 6 (considering HLA-A and -B at the antigen level and -DRB1 at the allele level) was required. For pediatric patients with malignant diseases, the minimum pre-cryopreserved cell counts recommended for selection were TNC $\geq 3 \times 10^7$/kg and $CD34^+$ $\geq 1.5 \times 10^5$/kg for a 4 to 6 of 6 HLA mismatch. For children with nonmalignant diseases, the minimum pre-cryopreserved cell dose recommended was TNC $\geq 5 \times 10^7$/kg and $CD34^+$ $\geq 2 \times 10^5$/kg for a 5 to 6 of 6 HLA mismatch.

Definitions

Myeloid engraftment

Neutrophil engraftment was defined as the first of 3 consecutive days with an ANC $\geq 0.5 \times 10^9$/L and transfusion-independent platelets $\geq 20 \times 10^9$/L for 7 consecutive days, respectively.

Sustained donor engraftment

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 the AmpFlSTR Identifiler Plus PCR Amplification Kit (Applied Biosystems, Carlsbad, CA). Based on a previous report [20], 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 loss of the engraftment.

Assessment of GVHD, nonrelapse mortality, opportunistic infection–related mortality, relapse, disease-free survival, overall survival, and disease status

Recipients were evaluated weekly for development and grading of acute GVHD (aGVHD). aGVHD and chronic GVHD (cGVHD) were diagnosed and graded according to standard criteria [21-24]. Patients dying before +100 day were not considered for cGVHD analysis. Nonrelapse mortality (NRM) was defined as death from any cause without evidence of relapse. Opportunistic infection (OI)-related mortality was defined as death from a viral, fungal, or protozoal infection. Disease-free survival (DFS) was defined as survival from the time of transplantation without evidence of disease relapse. Overall survival (OS) was defined as survival from the time of transplantation. Disease status at the time of transplantation was classified as follows: early phase: acute leukemia, myelodysplastic syndrome (MDS), and lymphoma in first complete remission, untreated MDS with <5% blasts, and/or chronic myelogenous leukemia (CML) in the first chronic phase; intermediate phase: acute leukemia, lymphoma, or MDS in a second remission and CML in a second or further chronic or accelerated phase; advanced phase: acute leukemia and lymphoma not in remission, CML in blast crisis, and untreated refractory anemia with excess blasts [25].

Statistical Analysis

Our preliminary exploratory univariate analysis demonstrated a positive association between ALC42 $<300 \times 10^6$/L after transplantation and worse survival; therefore, we used the ALC value at this time point in our univariate and multivariate analyses. The median ALC42 of the cohort was 345×10^6/L (<300 versus ≥300 used in a cutoff model for analysis; range, 0 to 2800; IQR, 200 to 600). We then evaluated the associations of factors predictive of ALC $\geq 300 \times 10^6$/L. Patients diagnosed with primary graft failure (n = 9), relapse (n = 1), or any grade of aGVHD (n = 18), and those who died (n = 14) before day +42 were excluded from the analysis (n = 42), to avoid their influence on ALC42. Binary logistic regression was used for univariate and multivariate analyses to estimate the association of predictive factors of $\geq 300 \times 10^6$/L. Characteristics selected for inclusion in the multivariate model were those with some indication of association in univariate analysis ($P < .10$). Results were expressed as odds ratio (OR) and corresponding 95% confidence interval (CI).

We then estimated the cumulative incidence for engraftment, NRM, GVHD, and relapse, and also calculated the probability of DFS and OS in the whole cohort. Then we analyzed the risk factors for general outcomes, including ALC42 $\geq 300 \times 10^6$/L, using a landmark analysis at day +42 in the whole cohort. Cumulative incidence curves were used in a competing-risks setting to calculate the cumulative incidences of neutrophil and platelet engraftment, aGVHD, cGVHD, relapse, and NRM [26]. A Cox proportional hazard model or the Fine and Gray method for competing events was used for multivariate analysis [27]. Death without engraftment was the competing event for neutrophil and platelet engraftment, and death without relapse was the competing event for relapse. Relapse or death without the development of aGVHD or cGVHD was the competing event for aGVHD and cGVHD, respectively. Survival probability was calculated using the Kaplan-Meier estimate [28], and comparisons were made using the log-rank test. Baseline characteristics are reported as median, range, and interquartile range (IQR) for quantitative variables and as frequency and percentages for categorical variables. Characteristics selected for inclusion in

the multivariate model were those with some indication of association in univariate analysis ($P < .10$). All statistical tests were conducted using SPSS version 20.0 (IBM, Armonk, NY). Cumulative incidence with competing risks were analyzed using R version 3.1.1 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Patient and CBU Characteristics

Characteristics of the 210 study patients are summarized in Table 1. During the study period, 210 consecutive patients underwent myeloablative single UCBT from an unrelated donor. The median age at transplantation was 15 years (range, 0.3 to 60 years; IQR, 4 to 36 years). The majority of the patients had acute leukemia (n = 152; 72%). Among the patients diagnosed with a malignant disease (n = 183; 87%), 102 (56%) were in an early disease stage before UCBT, 49 (27%) were in an intermediate disease stage, and 32 (17%) were in an advanced stage. Regarding HLA compatibility, matching was 6 of 6 in 35 patients (17%), 5 of 6 in 90 patients (43%), and 4 of 6 in 85 patients (40%). The median follow-up was 48.3 months (range, 7 to 108 months). According to the foregoing enrollment criteria, a total of 168 patients were evaluable in this study. The median infused TNC, $CD34^+$, and $CD3^+$ cell counts were 3.4×10^7/kg (range, 0.2 to 26.3×10^7/kg; IQR, 2.4 to 6.5×10^7/kg), 1.3×10^5/kg (range, 0.1 to 22.7×10^5/kg; IQR, 0.9 to 2.4×10^5/kg), and 4.2×10^6/kg (range, 0.5 to 89.6×10^6/kg; IQR, 3.4 to 11×10^6/kg), respectively. Ninety patients (43%) received CBUs with a major ABO blood mismatch.

Risk Factors Associated with Lymphocyte Recovery

For the whole cohort, factors predictive of ALC42 $\geq 300 \times 10^6$/L in univariate analysis were nonmalignant disease, early disease status, negative recipient CMV serostatus, neutrophil engraftment at day 42, lower ATG dose, greater HLA mismatch, and higher infused TNC, $CD34^+$, and $CD3^+$ cell counts (Table 2). In multivariate analysis, higher infused $CD3^+$ cell counts (OR, 2.7; 95% CI, 1.4 to 5.2; $P = .004$), lower ATG dose (OR, 2.3; 95% CI, 1.2 to 4.5; $P = .01$), and greater HLA mismatch (OR, 2.1; 95% CI, 1.1 to 4.1; $P = .03$) remained as factors predictive of ALC42 $\geq 300 \times 10^6$/L (Table 3). The variables that did not remain significant in multivariate analysis were recipient age (OR, 1.9; 95% CI, 0.4 to 1.71; $P = .26$), sex (OR, 1.8; 95% CI, 0.6 to 1.9; $P = .23$), underlying disease (OR, 2.2; 95% CI, 0.5 to 3.2; $P = .32$), disease status before transplantation (OR, 2.1; 95% CI, 0.42 to 2.4; $P = .34$), previous autologous stem cell transplantation (ASCT) (OR, 3.3; 95% CI, 0.28 to 6.2; $P = .19$), recipient CMV status (OR, 2.5; 95% CI, 0.1 to 4.2; $P = .80$), ABO group incompatibility (OR, 1.9; 95% CI, 0.6 to 3.4; $P = .26$), ANC $\geq 500 \times 10^6$/L (OR, 2.06; 95% CI, 0.1 to 4.8; $P = .20$), GVHD prophylaxis (OR, 2.12; 95% CI, 0.5 to 3.8; $P = .20$), TNC dose (OR, 2.43; 95% CI, 0.12 to 3.7; $P = .30$), and $CD34^+$ cell dose (OR, 3.1; 95% CI, 0.2 to 4.6; $P = .10$).

General Outcomes and Risk Factors

The results of the multivariate analyses for the various transplantation outcomes are summarized in Table 4. The results of the univariate analyses are provided in Supplementary Data.

Engraftment

Fourteen patients (7%) died before engraftment (median time, 17 days; range, 13 to 25 days) and were not evaluable for this event. Nine of 196 (5%) patients had graft failure. The

Table 1
Patient and Graft Characteristics (n = 210)

Characteristic	Value*
Year of transplantation, n (%)	
2005-2009	105 (50)
2010-2014	105 (50)
Sex, n (%)	
Male	112 (53)
Age at transplantation, yr	
Median (range, IQR)	15 (0.3-60, 4-36)
Time from initial diagnosis to transplantation, mo	
Median (range, IQR)	11.5 (0.5-181, 8-14)
Recipient CMV serostatus, n (%)	
Positive	144 (69)
Negative	66 (31)
Conditioning regimen, n (%)	
TT-Flu-Bu-ATG	123 (59)
TT-Flu-Cy-ATG	6 (3)
VP16-Cy-TBI-ATG	34 (16)
Cy-TBI-ATG	3 (1)
Bu-Cy-ATG	12 (6)
Bu-Cy-Mel-ATG	6 (3)
Flu-Mel-ATG	5 (2)
Flu-Cy-ATG	6 (3)
Bu-Cy-Mel-ATG	6 (3)
FLAG-IDA-Mel-ATG	5 (2)
Treosulfan-Cy-ATG	4 (2)
Underlying disease, n (%)	
Malignant	180 (86)
Nonmalignant	30 (14)
Underlying disease, n (%)	
AML	69 (33)
ALL	83 (40)
HL	9 (4)
NHL	5 (2)
MDS	5 (2)
CML	8 (4)
CMML	4 (2)
Immunodeficiency	21 (10)
Metabolic disorders	6 (3)
Disease phase, n (%)†	
Early	102 (56)
Intermediate	49 (27)
Advanced	32 (17)
GVHD prophylaxis, n (%)	
CsA-PDN	120 (57)
CsA-MMF	90 (43)
HLA match, n (%)‡	
4/6	85 (40)
5/6	90 (43)
6/6	35 (17)
ABO group incompatibility, n (%)	
Major	90 (43)
Minor	50 (24)
None	70 (33)
ATG dose, mg/kg, n (%)	
6	118 (56)
7.5	42 (20)
8	34 (16)
10	16 (8)
Post-thawing TNC dose	
Median (range, IQR)	3.4 (0.2-26.3, 2.4-6.5)
Post-thawing $CD34^+$ dose	
Median (range, IQR)	1.3 (0.1-22.7, 0.9-2.1)
Post-thawing $CD3^+$ dose	
Median (range, IQR)	4.2 (0.5-89.6, 3.4-11)

TT indicates thiotepa; Flu, fludarabine; Bu, busulfan; VP16, etoposide; Cy, cyclophosphamide; TBI, total body irradiation; Mel, melphalan; FLAG, fludarabine-AraC-G-CSF; IDA, idarrubicine; AML, acute myelogenous leukemia; ALL, acute lymphoblastic leukemia; HL, Hodgkin lymphoma; NHL, non-Hodgkin lymphoma; CMML, chronic myelomonocytic leukemia; CsA, cyclosporine; PDN, prednisone; MMF, mycophenolate mofetil.

* Percentages might not sum to 100 because of rounding.

† Considering only patients diagnosed with a hematologic malignancy.

‡ Considering HLA-A and -B at the antigen level and -DRB1 at the allele level.

Table 2
Univariate Analysis of Risk Factors Associated with Lymphocyte Recovery According to Patient and Graft Characteristics

Characteristic	OR (95% CI)	*P* Value
Age		
<20 yr*		
>20 yr	1.44 (0.82-2.53)	.20
Sex		
Male*		
Female	1.12 (0.1-2.18)	.69
Underlying disease		
Nonmalignant	2.4 (0.98-5.9)	.06
Malignant*		
Disease status before transplantation		
Early	1.63 (1.2-2.39)	.04
Intermediate-advanced*		
Previous ASCT		
Yes*	1.12 (1.01-1.22)	.778
No		
Recipient CMV serostatus		
Positive		
Negative*	1.8 (1.49-2.62)	.02
ABO group incompatibility		
None*		
Major	0.93 (0.46-1.94)	.48
Minor	1.37 (0.55-3.42)	
ANC >500 × 10^6/L		
<42 d post-UCBT*	1.87 (1.2-2.2)	.07
>42 d post-UCBT		
ATG dose		
≤6 mg/kg	2.9 (1.57-5.03)	<.001
>6 mg/kg*		
GVHD prophylaxis		
CsA-PDN*		
CsA-MMF	1.47 (0.51-1.61)	.74
HLA mismatch		
4/6*		
5-6/6	2.8 (2.36-3.24)	<.001
Number of post-thawing TNC doses		
<2.5		
>2.5*	2.42 (1.9-2.92)	.004
Number of post-thawing $CD34^+$ doses		
<1*		
≥1	1.67 (0.71-0.96)	.073
Number of post-thawing $CD3^+$ doses		
<4*		
≥4	3.5 (3.1-3.85)	<.001

* Reference category.

remaining 187 patients achieved neutrophil and platelet engraftment at a median of 23 days (95% CI, 20 to 26 days) and 51 days (95% CI, 41 to 61 days), respectively. Indeed, for evaluation of risk factors for neutrophil engraftment, univariate binary logistic regression analysis demonstrated a trend toward an association between ANC and ALC42 ≥300 × 10^6/L (OR, 1.87; 95% CI, 1.2 to 2.2; $P = .07$), although in multivariate analysis the ANC did not remain associated with ALC42 ≥300 × 10^6/L (HR, 1.81; 95% CI, 0.87 to 1.74; $P = .20$). The median number of infused $CD3^+$ cells in patients who engrafted within the first 42 days after UCBT was 5.7 × 10^6/kg (range, 1.8 to 53.9 × 10^6/kg; IQR, 3.5 to 11.4 × 10^6/kg), and that for patients without evidence of neutrophil engraftment within the first 42 days after UCBT was 2.3 × 10^6/kg (range, 1.3 to 46.3 × 10^6/kg; IQR, 2.6 to 10.9 × 10^6/kg).

Table 3
Multivariate Analysis of Risk Factors Associated with Lymphocyte Recovery according Patient and Graft Characteristics

Characteristic	OR (95% CI)	*P* Value
HLA mismatch		
4/6*		
5-6/6	2.1 (1.1-4.1)	.03
ATG dose		
≤6 mg/kg	2.3 (1.2-4.5)	.01
>6 mg/kg*		
Number of post-thawing $CD3^+$ doses		
<4*		
≥4	2.7 (1.4-5.2)	.004

* Reference category.

Table 4
Fine and Gray Multivariate Analysis for Outcomes

Outcome	HR	95% CI	*P* Value
aGVHD			
Older patients	1.82	1.27-2.63	.01
Previous ASCT	1.76	1.25-2.33	.01
cGVHD			
Male	1.84	1.16-2.77	.02
NRM			
Advanced disease	1.72	1.51-1.81	.006
ALC42 <300 × 10^6/L	1.76	1.34-2.32	.001
OI-related mortality			
4/6 HLA match	1.61	1.2-2.03	.01
ALC42 <300 × 10^6/L	1.96	1.2-4.81	<.001
Relapse			
Advanced disease	1.54	1.24-1.91	.008
DFS			
Early-intermediate disease	1.6	1.2-2.16	.01
ALC42 >300 × 10^6/L	2.03	1.15-3.6	<.001
OS			
Early-intermediate disease	1.6	1.2-2.06	.008
ALC42 >300 × 10^6/L	2.03	1.17-3.6	<.001

ALC42 indicates absolute lymphocyte count at day +42.

aGVHD and cGVHD

The cumulative incidence of grade II to IV aGVHD at day +180 was 26% (95% CI, 23.7% to 29.1%), and that for grade III to IV aGVHD was 5.8% (95% CI, 4.8% to 6.5%) at a median time of 23 days (range, 13 to 151 days) and 44 days (range, 13 to 104 days), respectively. Fourteen (12%) of 116 evaluable patients developed cGVHD. The median time to the development of cGVHD was 126 days (range, 119 to 827 days). The cumulative incidence of cGVHD at 5 years was 8.3% (95% CI, 8.1% to 8.5%) and was mild in 8 patients, moderate in 4 patients, and severe in 2 patients. Multivariate analysis showed a higher incidence of aGVHD in patients age ≥20 years (HR, 1.82; 95% CI, 1.27 to 2.63;

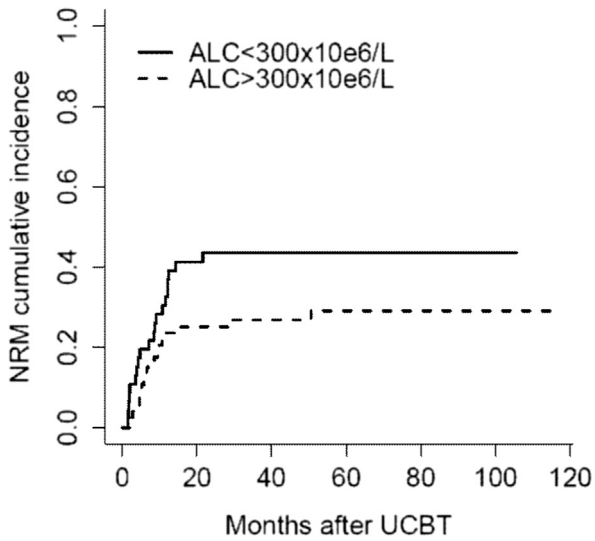

Figure 1. Cumulative incidence of NRM based on ALC42. Impact of ALC42 ≤300 or >300 × 10^6/L.

$P = .01$) and who underwent previous ASCT (HR, 1.76; 95% CI, 1.25 to 2.33; $P = .01$), whereas the incidence of cGVHD was higher in males (HR, 1.84; 95% CI, 1.16 to 2.77; $P = .02$). ALC42 had no impact on aGVHD ($P = .50$) or cGVHD ($P = .80$) events.

Relapse

Among the patients diagnosed with a hematologic malignancy (n = 183), 35 (19%) relapsed after UCBT, with a 5-year cumulative incidence of relapse of 26% (95% CI, 24.2% to 28.1%). The median time to relapse was 13.1 months (range, 0.8 to 61 months). Multivariate analysis showed an association between the risk of relapse and advanced disease status before UCBT (HR, 1.54; 95% CI, 1.24 to 1.91; $P = .008$). ALC42 was not a risk factor associated with relapse ($P = .20$).

NRM

A total of 74 patients (35%) died of a transplantation-related cause. Infectious complications were the most frequent main cause of death (n = 58; 78%) followed by aGVHD (n = 7; 9%), autoimmune cytopenias (n = 7; 9%), and thrombotic microangiopathy associated with transplantation (n = 2; 3%). The 5-year cumulative incidence of NRM was 33% (95% CI, 32% to 34%). In multivariate analysis, factors associated with a significantly increased risk of NRM were ALC42 $<300 \times 10^6$/L (HR, 1.76; 95% CI, 1.34 to 2.32; $P = .001$) and advanced disease status (HR, 1.72; 95% CI, 1.51 to 1.81; $P = .006$). The impact of ALC42 on NRM is shown in Figure 1. The 5-year cumulative incidence of OI-related mortality was 22% (95% CI, 18.1% to 26.2%). In multivariate analysis, factors associated with a significantly increased risk of OI-related mortality were ALC42 $<300 \times 10^6$/L (HR, 1.96; 95% CI, 1.2 to 4.81; $P < .001$) and greater HLA mismatch (HR, 1.61; 95% CI, 1.2 to 2.03; $P = .01$).

Survival

Of the 210 patients, 110 (52%) were alive at the end of the study, and 108 were disease-free. The 5-year probability of OS and DFS of the entire cohort was 48% (95% CI, 40% to 58%) and 52% (95% CI, 44% to 62%), respectively. Factors associated with improved DFS and OS in multivariate analysis were a higher ALC42 (for DFS: HR, 2.03; 95% CI, 1.15 to 3.6; $P < .001$; for OS: HR, 2.03; 95% CI, 1.17 to 3.6; $P < .001$) and nonadvanced disease status (for DFS: HR, 1.6; 95% CI, 1.2 to 2.16; $P = .01$; for OS: HR, 1.6; 95% CI, 1.2 to 2.06; $P = .008$). The effects of ALC42 on DFS and OS are shown in Figure 2.

DISCUSSION

Our findings identify the main factors associated with a higher ALC42 after UCBT as infused $CD3^+$ cell dose, ATG dose, and the degree of HLA matching, demonstrating the impact of ALC42 on NRM and survival. We chose to evaluate ALC42 based on previous studies that established a diagnosis of graft failure in patients who had not achieved neutrophil recovery by day +42. Interestingly, we analyzed ALC at days +30 and +60 and found similar results. Although a number of reports have confirmed the influence of ALC on outcomes after UCBT [2,6,29-31], there are minimal published data on predictive factors for lymphocyte recovery after transplantation [16].

The relevance of infused $CD3^+$ cell dose further confirms the importance of the post-thymic passenger T cells contained in grafts for immune reconstitution in the UCBT setting. This may be because administering an excess of lymphocytes, especially when in vivo T cell depletion is used, would facilitate the persistence of some lymphocyte subsets that accelerate lymphocyte recovery.

Although we did not study the effect of lymphocyte subsets in the grafts, our data are in agreement with other reports of an association between T cell $CD8^+$ cell counts in the graft and long-term outcomes after UCBT [32,33]. A comparative analysis of cell subsets in cord blood and adult peripheral blood has revealed phenotypic and functional peculiarities of cord blood cells. This finding focuses attention on cord blood T cell specificities as a key type of cell in UCBT. In this regard, Mold et al. [34] suggested that fetal and adult T cells arise from different populations of hematopoietic stem cells that are present at various stages of development; therefore, cord blood lymphocytes appear to be derived largely from ontogeny, which could confer different properties on transplant biology. Thus, they also may be responsible for some of the differences in clinical features observed after UCBT [35]. We recently evaluated the impact of viable $CD45^+$ cell counts on outcomes after UCBT, and attributed the potential effects of infused leukocytes in that effect [18]. Our present data identify T cell fraction as the main mediator of such effects; therefore, strategies that preserve viable T cells linked to the use of better HLA-matched CBUs could be a good balance for accelerating immune reconstitution without increasing the risk of GVHD.

In an effort to improve engraftment and limit GVHD, some groups have used in vivo T cell depletion in the conditioning regimen, which is likely associated with delayed T cell recovery [1,14] and could contribute to an increased incidence of OI because of the skewed and limited TCR

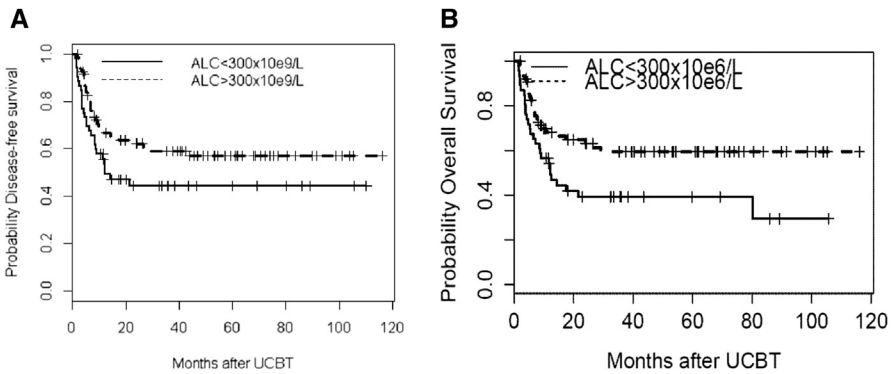

Figure 2. OS (A) and DFS (B) by ALC42. Impact of ALC42 ≤300 or >300 × 10^6/L.

repertoire and an inversion of the normal $CD4^+$:$CD8^+$ ratio. As a direct consequence of delayed lymphocyte recovery, those patients are at high risk for NRM, owing mainly to infections, the main cause of death in the present study. This could explain our results identifying ALC as a risk factor for OI-related morality. To improve immune reconstitution, omitting ATG from the conditioning regimen has been proposed [11,13]. In this setting, faster lymphocyte recovery was observed, with early expansion of T cells in peripheral blood that resulted in low infection-related mortality and only a moderate increase in the incidence of GVHD. This combination promoted a remarkably high survival [13]. In contrast to other studies, in our cohort the incidence of aGVHD was lower than that seen in recipients of double UCBT [36], a fact that could be explained by a lower infused cell dose and by the effect of ATG in our series. Moreover, the latter factor would explain why we did not observe an association between ALC and aGVHD, in disagreement with previous studies [37]. In myeloablative adult unrelated donor transplantation for hematologic malignancies, ATG has been associated with a lower incidence and severity of cGVHD and reduced late mortality risk. Lindemans et al. [37] reported the results of ATG inclusion in recipients of myeloablative or nonmyeloablative conditioning for malignant and nonmalignant diseases and found that although the probability of severe aGVHD was lower in the ATG-conditioned group, immune reconstitution was delayed and viral reactivation was greater in this group. Despite these results, that study and other studies [38,39] found similar survival in recipients of ATG-containing regimens and recipients of non–ATG-containing regimens. Unfortunately, our series included only patients receiving ATG, and thus we are not able to present data on the impact of the omission of T cell depletion.

We found an association between the degree of HLA matching and lymphocyte recovery that is in concordance with previously published data. Komandura et al. [6] reported a delayed immune reconstitution related to HLA disparity that is common in UCBT. Other groups have shown the independent effect of high-resolution HLA typing on outcomes after UCBT [40], and recent studies have reported better outcomes in UCBT with improved allele-level matching for HLA loci (A-, B-, C-, and -DRB1). Those results suggest the prudence of avoiding UCBT with $\geq$3 allele-level mismatches owing to an unacceptable NRM and inferior survival. Therefore, our data further emphasize the need for better HLA-matched CBUs, similar to other groups [41,42].

Even though our study includes a relatively high number of patients treated with common clinical practices and biological assessment, it still has limitations inherent to retrospective analyses. Despite the wide diversity of underlying diseases and patient age, our results conclusively identify the leading factors affecting lymphocyte recovery after UCBT. Another limitation of this study is the unavailability of lymphocyte subsets, although ALC is clinically easy to determine in a very general way to measure immune reconstitution. Our data confirm the impact of low ALC42 on the major outcomes after T cell–depleted single UCBT. This finding might be of value for therapies with adoptive transfer using lymphocytes based on ex vivo expanded T cord blood immune cells, like specific T cells or donor lymphocyte infusion.

In conclusion, infusion of a high number of $CD3^+$ cells, administration of low ATG dose in the conditioning regimen, and the use of better HLA-matched CBUs were the main factors contributing to higher ALC42 values, which affected both NRM and OS. These findings are of interest in CBU selection, emphasizing the positive interaction between cell counts, especially lymphocytes, and HLA matching in UCBT. Refining CBU selection and the use of new strategies for GVHD prophylaxis that reduce, omit, or modify the timing of ATG infusion are central to improve early immune reconstitution and outcomes after UCBT.

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SUPPLEMENTARY DATA

Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.bbmt.2016.03.009.

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