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Early and Long-Term Impaired T Lymphocyte Immune Reconstitution after Cord Blood Transplantation with Antithymocyte Globulin

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A B S T R A C T

Immune reconstitution is crucial to the success of allogeneic hematopoietic stem cell transplantation. Umbilical cord blood transplantation (UCBT) has been associated with delayed immune reconstitution. We characterized the kinetics and investigated the risk variables affecting recovery of the main lymphocyte subsets in 225 consecutive pediatric and adult patients (males, n = 126; median age, 15; range, .3 to 60; interquartile range, 4 to 35) who underwent myeloablative single UCBT between 2005 and 2015 for malignant and nonmalignant disorders. Low $CD4^+$ and $CD8^+$ T cell counts were observed up to 12 months after UCBT. In contrast, B and natural killer cells recovered rapidly early after transplantation. In a multivariate regression model, factors favoring $CD4^+$ T cell recovery ≥ 200 cells/μL were lower dose antithymocyte globulin (ATG) (hazard ratio [HR], 3.93; 95% confidence interval [CI], 2.3 to 5.83; $P = .001$), negative recipient cytomegalovirus (CMV) serostatus (HR, 3.76; 95% CI, 1.9 to 5.74; $P = .001$), and younger age (HR, 2.61; 95% CI, 1.01 to 3.47; $P = .03$). Factors favoring $CD8^+$ T cell recovery ≥ 200 cells/μL were lower dose ATG (HR, 3.03; 95% CI, 1.4 to 5.1; $P = .03$) and negative recipient CMV serostatus (HR, 1.9; 95% CI, 1.63 to 2.15; $P = .01$). Our results demonstrate the significant negative impact of ATG on lymphocyte recovery. A reduction of the dose or omission of ATG could improve immune reconstitution and perhaps reduce opportunistic infections after UCBT.

INTRODUCTION

Umbilical cord blood transplantation (UCBT) is commonly used for patients with hematological and nonhematological malignancies who require allogeneic (allo) hematopoietic stem cell transplantation (HSCT), when there are no HLA-matched donors available.

A major limitation for the use of UCBT is the relatively small number of infused hematopoietic stem cells that results in delayed engraftment [1,2]. Previous studies have shown that T cell recovery is often delayed after UCBT [3-5]. In contrast, B and natural killer (NK) cell appear to recover rapidly after UCBT [6]. Of note, major outcomes after transplantation improve in patients with a rapid T cell recovery [7,8].

Initial cellular immune reconstitution after transplantation largely depends on thymic-independent peripheral expansion of donor-derived memory T cells. After that, thymic-dependent maturation is important for diversification of the T cell repertoire and consolidating host immune reconstitution against pathogens or recurrence of malignancy [9,10].

Previous studies demonstrated that post-transplantation immune recovery is affected by several factors, including thymic involution associated to patient age, the conditioning regimen, HLA disparity between donor and recipient, occurrence of graft-versus-host disease (GVHD), and drugs used to prevent or treat GVHD, such as antithymocyte globulin (ATG) [11-21].

Various groups evaluated the lymphocyte kinetics after UCBT and confirmed a delay in T cell subsets' recovery up to 6 months after transplantation; however, by 12 months,

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immune recovery is often at least on par with that seen after conventional HSCT. However, despite this, there are limited data on factors involved in lymphocyte subsets' recovery after UCBT [6,9].

Consequently, to understand the key factors influencing lymphocyte recovery after UCBT, we retrospectively explored the main lymphocyte subset kinetics profile and analyzed the predictive factors associated with a prompt lymphocyte recovery in a cohort of 225 pediatric and adult patients diagnosed with neoplastic and non-neoplastic hematological diseases who underwent myeloablative single unit UCBT (sUCBT) using a very consistent selection criteria and conditioning protocol.

MATERIALS AND METHODS

Patient Cohort

A total of 271 patients received an UCBT from January 2005 to April 2015. For the purpose of the study, we excluded patients who received a second allo-HSCT (n = 7), a related UCBT (n = 18), coinfusion of bone marrow with UCBT (n = 6), or UCBT with haploidentical third-party $CD34^+$ selected cells (n = 15). We included all 225 (121 [54%] pediatric and 104 adult) patients who received a first sUCBT in the Hospital Vall d'Hebron (Barcelona), Hospital de Sant Pau (Barcelona), and Hospital Germans Trias i Pujol (Barcelona), consisting of 3 adult and 2 pediatric transplantation programs.

Enrollment Criteria

All patients with hematological malignancies and nonhematological diseases were eligible for enrollment if there were a lack of a suitable HLA-matched unrelated donor within a reasonable time after the search through international registries and there was a suitable umbilical cord blood unit (CBU) available, as described below. Patients or their guardians gave written informed consent for their inclusion in each transplantation protocol. For all patients included in the analysis, the sUCBT was the first allo-HSCT received.

Transplantation Procedure and CBU Selection

All patients received myeloablative conditioning. The most commonly used protocol has been previously published [22] and 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 (Thymoglobulin, Sangstat/Genzyme, Lyon, France). ATG was administered in different schedules starting on day –5 or –4 to –2 day depending on the overall dose administered.

GVHD prophylaxis was based on cyclosporine, 1.5 mg 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/day from –1 to +30 day). As supportive care, all patients received post-transplantation granulocyte colony–stimulating factor from day +7 until neutrophil recovery.

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

Definitions

Assessment of GVHD, nonrelapse mortality, relapse, disease-free survival, overall survival, and disease status

Recipients were evaluated weekly for development and grading of acute GVHD (aGVHD). Acute and chronic GVHD (cGVHD) were diagnosed and graded according to the standard criteria [23,24]. 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. *Disease-free survival* was defined as survival from the time of transplantation without evidence of disease relapse. *Overall survival* was defined as survival from the time of transplantation. Disease status at the time of transplantation was classified as follows: (1) early phase, including acute leukemia, myelodysplastic syndrome (MDS) and lymphoma on the first complete remission, untreated MDS with < 5% blasts and/or chronic myeloid leukemia (CML) in the first chronic phase; (2) intermediate phase, including acute leukemia, lymphoma, or MDS in a second remission and CML in a second or further chronic or accelerated phase; and (3) advanced phase, including acute leukemia and lymphoma not in remission, CML in blast crisis, and untreated refractory anemia with excess blasts [25].

Flow cytometry analysis of peripheral blood

Immunophenotyping was performed on whole-blood samples generally obtained at 3, 6, 12, 18, and 24 months after transplantation. Quantification of the following subsets was performed: absolute number of T cells ($CD3^+$), helper T cells ($CD3^+CD4^+$), cytotoxic T cells ($CD3^+CD8^+$), B cells ($CD19^+$), and NK cells ($CD3^-CD16^+CD56^+$) and were determined using 4-color immunofluorescence and fluorescence-activated cell sorting analysis. Briefly, a volume of 10 μL of CD3-FITC, CD45-PerCP, CD19-APC or CD3-FITC, CD8-PE, CD45-PerCP, CD4-APC reagent (PerfecT count, Cytognos, Salamanca, Spain) was added to a tube containing a known quantity of beads, followed by 25 μL of EDTA-treated whole blood and incubated for 15 minutes at room temperature. Red blood cells were subsequently lysed for 15 minutes with 450 μL of FACS Lysing Solution (Cytognos). Samples were acquired using FACSCalibur and analyzed with Multiset software (Becton-Dickinson, Franklin Lakes, NJ).

Kinetics of lymphocyte recovery and risk factors assessment

Lymphocyte recovery kinetics were studied calculating the median and range of $CD3^+$ T cell, $CD4^+$ T cell, $CD8^+$ T cell, B, and NK cells measured at 3, 6, 12, 18, and 24 months after UCBT by age (<20 and ≥20 years) and in the whole population and we compared the results with our laboratory reference value. We also calculated the median time to reach several lymphocyte endpoints because of the clinical significance based on a previous report [26], as follows: time to reach $CD3^+$ T cell ≥ 500 cells/μL, $CD3^+$ T cell ≥ 1500 cell/μL, $CD4^+$ T cells ≥ 50 cell/μL, $CD4^+$ T cells ≥ 200. To evaluate the potential effect of ATG on T lymphocyte recovery in the post-transplantation period, we calculated the median and range of $CD4^+$ and $CD8^+$ T cell at 3 and 6 months after CBT in the patients who did not receive ATG in the conditioning regimen.

Pretransplantation variables studied for their potential impact on the lymphocyte endpoints were year of transplantation, recipient age, disease type, disease phase, autologous stem cell transplantation before UCBT, pretransplantation recipient cytomegalovirus (CMV) serology, HLA match at antigenic and allelic level, infused TNC dose, infused $CD34^+$ cell dose, infused $CD3^+$ cell dose, infused colony-forming unit, pretransplantation ATG dose, and GVHD prophylaxis.

Definition of infections

Severe infections starting from the day of progenitors infusion (day 0) to 24 months after transplantation were collected from all participating centers, according to predefined criteria [27].

Statistical analysis

Baseline characteristics were described as median, range, and interquartile range (IQR) for quantitative variables and frequency and percentages for categorical variables. Lymphocyte kinetics was described as median and range for each lymphocyte subset ($CD4^+$ T cell, $CD8^+$ T cell, B cell, and NK cell) at different time-points (3, 6, 12, 18, and 24 months) after transplantation by age (<20 and ≥20 years) and in the whole population. Additionally, we calculated the median and range of time to reach the different lymphocyte endpoints mentioned above in those patients at risk at the time of the analysis (those alive patients showing sustained engraftment).

We conducted a univariate analysis to assess the factors influencing lymphocyte recovery. Characteristics selected for inclusion in the multivariate model were those with $P < .10$ in univariate analysis. Cumulative incidence curves were used in a competing risk setting to calculate the cumulative incidence of neutrophil and platelet engraftment, aGVHD, cGVHD, relapse, and NRM for the entire population [28]. Death without engraftment was the competing event for neutrophil and platelet engraftment. Death without relapse was the competing event of relapse. Relapse or death without developing aGVHD or cGVHD were the competing events for aGVHD and cGVHD, respectively. Survival probability was calculated using Kaplan-Meier estimation in whole population [29]. A Cox proportional hazard model or the Fine and Gray method for competing events were used for multivariate analysis [30]. All statistical tests were conducted using SPSS statistical software (SPSS version 20.0, Chicago, IL). Cumulative incidence with competing risks was conducted in R software, version 3.1.1 (The CRAN project).

RESULTS

Patient and CBU Characteristics

A total of 225 patients were included in this study. Clinical characteristics are summarized in Table 1. During the study period, 225 consecutive patients underwent

Table 1
Patients, Cord Blood Grafts and Transplantation Characteristics (N = 225)

Variable	Value[a]
Age	
≤20 years	122
>20 years	103
Median age at transplantation, yr (range, IQR)	15 (0.3-60, 4-35)
Median weight at transplantation, kg (range, IQR)	48 (3-117, 16-70)
Gender, n (%)	
Male	126 (56)
Year of transplantation, n (%)	
2005-2009	104
2010-2015	121
Median time from initial diagnosis to transplant, months (range, IQR)	12 (0.3-181, 6-30)
Previous ASCT	29 (23)
Diagnosis, n (%)	
Acute lymphoblastic leukemia	87 (39)
Acute myeloid leukemia	70 (31)
Chronic myeloid leukemia	8 (4)
Myelodysplastic syndrome	7 (3)
Non-Hodgking lymphoma	6 (2)
Hodgking lymphoma	9 (4)
Myeloprolypherative disease	3 (1)
Severe aplastic anemia	4 (2)
Metabolic disorder	8 (4)
Immunodeficiency	23 (10)
Disease phase[b], n (%)	
Early	104 (55)
Intermediate	54 (28)
Advanced	34 (17)
Conditioning regimen, n (%)	
TT-FLU-BU-ATG	144 (64)
VP16-CY-TBI-ATG	36 (16)
CY-TBI-ATG	6 (3)
BU-CY-ATG	13 (6)
Other[c]	26 (11)
GVHD prophylaxis, n (%)	
CsA-PDN	138 (61)
CsA-MMF	87 (39)
Recipient CMV seropositive, n (%)	150 (67)
Recipient EBV seropositive, n (%)	166 (74)
Recipient ancestry, n (%)	
European	165 (73)
Non-european	60 (27)
ATG dose, n (%)	
Non-ATG	14 (6)
6 mg/kg	115 (51)
7.5 mg/kg	43 (19)
8 mg/kg	33 (15)
10 mg/kg	20 (9)
HLA-A, -B antigen, -DRB1 allele match to patient, n (%)	
4/6	86 (38)
5/6	98 (44)
6/6	41 (18)
Sex mismatch, n (%)	80 (35)
Post-thaw TNC dose, $\times 10^7$/kg, median (range, IQR)	3.47 (0.5-26.3, 2.46-6.5)
Post-thaw $CD34^+$ dose, $\times 10^5$/kg, median (range, IQR)	1.35 (0.1-22.7, 0.9-2.26)
Post-thaw $CD3^+$ dose, $\times 10^4$/kg, median (range, IQR)	5.2 (0.5-89, 3.4-11)
Major ABO mismatch, n (%)	
Major	48 (21)
Minor	50 (22)
None	127 (57)

IQR indicates interquartile range; ASCT, autologous stem cell transplantation; TT, thiotepa; FLU, fludarabine; BU, busulfan; ATG, antithymocyte globulin; HLA, histocompatibility leukocyte antigen; TNC, total nucleated cell dose; VP16, etoposide; CY, cyclophosphamide; TBI, total body irradiation; MEL, melphalan; FLAG, fludarabine-AraC-G-CSF; IDA, idarrubicine, GVHD, graft versus host disease; CsA, ciclosporine; PDN, prednisone; MMF, mycophenolate mophetil; CMV, citomegalivirus; EBV, Ebstein-Barr virus.

[a] Percentatges may not sum to 100 because of rounding. [b] Only considered patients diagnosed with hematological malignancies. [c] Other conditioning include BU-CY-MEL-ATG (n = 7), FLAG-IDA-MEL-ATG (n = 7), FLU-BU-ATG (n = 8), Treosulfan-CY-ATG (n = 4). CBU were originally selected on basis of cell dose and HLA matching for HLA-A and –B antigens and for HLA-DRB1 alleles without consideration of HLA-C and –DQ in the matching algorithm

myeloablative sUCBT from an unrelated donor. Overall, the median age at transplantation was 15 years (range, .3 to 60; IQR, 4 to 35). Most patients had acute leukemia (n = 157, 70%). Among patients diagnosed with malignant diseases (n = 190, 84%), 104 (55%) of them were in early, 54 (28%) were in intermediate, and 32 (17%) were in advanced disease status before UCBT. Regarding HLA compatibility, the units were 4/6 (n = 86, 38%), 5/6 (n = 98, 44%), and 6/6 (n = 41, 18%) matched. Patients received different ATG doses: none (n = 14, 6%), 6 mg/kg (n = 115, 51%), 7.5 mg/kg (n = 43, 19%), 8 mg/kg (n = 33, 15%), and 10 mg/kg (n = 20, 9%) based on type of underlying disease (malignant or nonmalignant) and risk of relapse. Subsequently, this variable was dichotomized in ATG dose ≤ 6 mg/kg and > 6 mg/kg to conduct the analysis of predictive factors for lymphocyte reconstitution.

Median follow-up among survivors was 49.3 months (range, 12.1 to 67). The median of infused TNC, $CD34^+$, and $CD3^+$ cell counts were 3.47×10^7/kg (range, .5 to 26.3; IQR, 2.46 to 6.5), 1.35×10^5/kg (range, .1 to 22.7; IQR, .9 to 2.26), and 5.2×10^6/kg (range, .5 to 89; IQR, 4 to 11), respectively. Forty-eight (21%) patients received UCB units with major ABO blood mismatch.

Transplantation Outcomes

The cumulative incidence of sustained donor engraftment by day 42 was 83% (95% confidence incidence [CI], 83% to 85%), and 20 (9%) patients experienced primary graft failure. The median times to neutrophil and platelet ≥ 20×10^9/L engraftment were 22 days (range, 12 to 58 days) and 39 days (range, 15 to 182 days), respectively. The cumulative incidence of grades II to IV aGVHD at day 180 was 19.6% (95% CI, 14.2% to 22.4%). At day 180 after UCBT, 24 of 158 evaluable patients (15%) had active late or an overlap syndrome. The 5-year cumulative incidence of cGVHD was 8.1% (95% CI, 7.6% to 9.1%). The 5-year cumulative incidence of NRM was 32% (95% CI, 30.8% to 33.6%). The 5-year probabilities of overall survival and disease-free survival of the entire cohort were 49% (95% CI, 40% to 58%) and 53% (95% CI, 44% to 62%), respectively. The primary causes of death were infections (n = 60), relapse (n = 26), aGVHD (n = 6), primary graft failure (n = 3), and others (n = 8).

Lymphocyte Recovery Kinetics

The median times to reach the different lymphocyte endpoints are summarized in Table 2. Interestingly, the median absolute lymphocyte count of 674 cells/μL (range, 200 to 2300 cells/μL) was below the laboratory reference range value (1200 to 3400 cells/μL) by month 3 after transplantation.

Table 2
Median Times to Lymphocyte Subset Recoveries after UCBT

Lymphocyte Subset Endpoint	No. of Patients at Risk	Median Time, mo.	Range (IQR), mo.
ALC ≥ 300 cells/μL	188	6.7	1.9-19.6 (5.3-8.1)
$CD3^+$ T cell ≥ 500 cells/μL	141	5.8	.5-23.5 (7.2-15.4)
$CD3^+$ T cell ≥ 1500 cells/μL	106	13.3	3.3-53.2 (15.8-36.4)
$CD4^+$ T cell ≥ 50 cells/μL	172	4.6	.9-6.1 (2.7-11.8)
$CD4^+$ T cell ≥ 200 cells/μL	144	10.9	3.7-14.5 (4.9-13.3)
$CD4^+$ T cell ≥ 500 cells/μL	146	14.3	4.6-35.6 (6.1-16.7)
$CD8^+$ T cell ≥ 200 cells/μL	135	18.2	2.1-26 (3.8-22)
B cell ≥ 200 cells/μL	157	2.5	1.2-6.6 (1.4-5.9)
NK cell ≥ 100 cells/μL	179	.9	.8-6.1 (1.3-5.8)

ALC indicates absolute lymphocyte count.

Reconstitution of T cells

The median $CD3^+$ T cell counts of 151 cells/μL (range, 0 to 621 cells/μL) at 6 months and 358 (range, 0 to 1781cells/μL) at 12 months after transplantation were under the reference range value (900 to 4500 cells/μL). Of note, the median $CD4^+$ T cell counts of 59 cells/μL (range, 0 to 399 cells/μL) and 115 cells/μL (range, 0 to 1266 cells/μL) were < 200 cells/μL by month 6 and 12 after transplantation. Additionally, median, range, and IQR of times to reach $CD4^+$ T cell ≥ 200 cells/μL and ≥ 500 cells/μL were 10.9 months (range, 3.7 to 14.5; IQR, 4.9 to 13.3) and 14.3 months (range, 4.6 to 35.6; IQR, 6.1 to 16.7), respectively. Similarly, the median $CD8^+$ T lymphocyte count of 74 cells/μL (range, 0 to 890 cells/μL) by month 12 was under the normal range value (.3 to 1.6 cells/μL). Median time, range, and IQR to reach $CD8^+ \geq 200$ cells/μL was 18.2 months (range, 2.1 to 26; IQR, 3.8 to 22). The $CD4^+$ and $CD8^+$ T lymphocyte kinetics in the first 24 months after UCBT by age and in whole population are shown in Figures 1 and 2, respectively. We observed better lymphocyte recovery for all cell populations in children than in adults. T lymphocyte immune recovery increased considerably in both adult and pediatric patients by 12 months, especially $CD4^+$ T cells after transplantation remaining higher in pediatric population.

Interestingly, for the 14 patients who did not receive ATG in the conditioning regimen, the median $CD4^+$ T cell counts were 83 cells/μL (range, 63 to 98 cells/μL) and 249 cells/μL (range, 168 to 398 cells/μL) by months 3 and 6 after UCBT, respectively. In those patients, the median $CD8^+$ T cell counts were 79 cells/μL (range, 22 to 105 cells/μL) and 161 cells/μL (range, 101 to 189 cells/μL) at 3 and 6 months after UCBT, respectively.

Reconstitution of B and NK cells

In contrast to T lymphocyte recovery, B cell recovery was faster. The median count of 314 cells/μL (range, 0 to 1722; IQR, 0 to 754) was within the normal range value (200 to 2100 cells/μL) by 3 months and remained high and progressively increased by 24 months after transplantation. The median number of NK cell count of 288 cells/μL (range, 0 to 1552; IQR, 221 to 776) by 3 months, also was within the normal range value (100 to 1000 cells/μL), and remaining constantly high by 3 months and raising at 6 months. B and NK

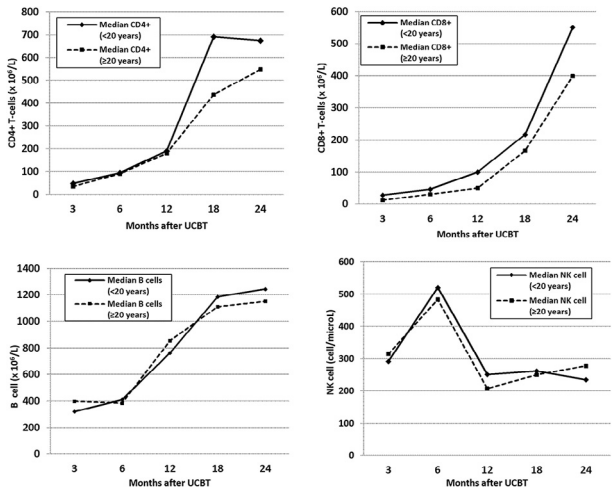

Figure 1. Median $CD4^+$ T-cell recovery (A), $CD8^+$ T-cell recovery (B), B cell recovery (C) and NK cell (D) after single-UCBT according to age.

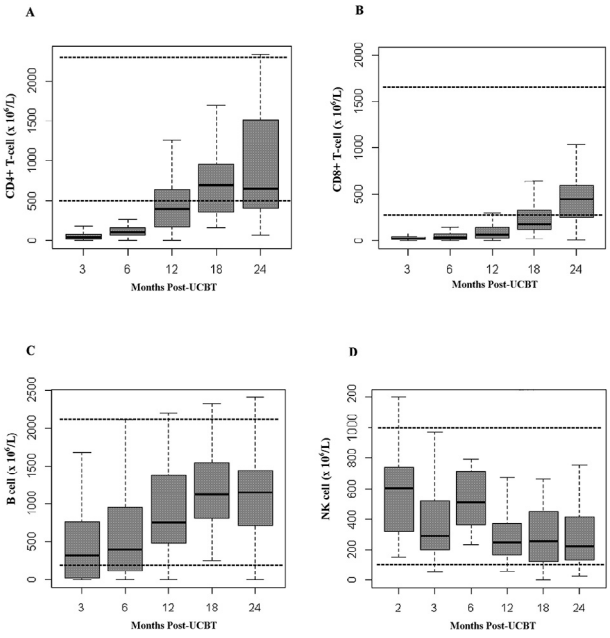

Figure 2. CD4⁺ T cell recovery (A), CD8⁺ T cell recovery (B), B cell recovery (C), and NK cell recovery (D) after single-unit UCBT using ATG in conditioning regimen in whole population. The boxes represent the interquartile range and the solid lines within the boxes represent the median value. The horizontal lines represent the normal ranges.

cell lymphocyte kinetics were similar in both pediatric and adult population. Recovery of B cell and NK cell by age and in whole population are shown in Figures 1 and 2.

Risk Factors for Immune Reconstitution

Regarding the whole cohort, factors associated with reaching the different lymphocyte endpoints in the univariate analysis are provided in Supplementary Data. In multivariate analysis (Table 3), the time to reach CD3⁺ T cell ≥ 500 cells/μL was favorably affected by lower ATG dose (hazard ratio [HR], 2.8; 95% CI, 1.81 to 5.02; $P = .02$) and negative recipient CMV serostatus (HR, 2.1; 95% CI, 1.31 to 4.87; $P = .01$). The time needed to reach CD4⁺ T cell ≥ 200 cells/μL was favorably affected by lower ATG dose (HR, 3.93; 95% CI, 2.3 to 5.83; $P = .001$), negative CMV recipient serostatus (HR, 3.76; 95% CI, 1.9 to 5.74; $P = .001$), and recipient's age (HR, 2.6; 95% CI, 1.01 to 3.47; $P = .03$). Lower ATG dose and negative CMV serostatus were in favor of CD8⁺ T cell ≥ 200 cells/μL (HR, 3.03; 95% CI, 1.4 to 5.1; $P = .03$ and HR, 1.9; 95% CI, 1.63 to 2.15; $P = .01$, respectively). A favorable risk factor to reach B cell ≥ 200 cells/μL was negative recipient CMV serostatus (HR, 3.47; 95% CI, 1.3 to 4.25; $P = .03$). NK cell ≥ 100 cells/μL was favorably affected by negative recipient CMV serostatus (HR, 2.3; 95% CI, 1.03 to 3.7; $P = .03$) and higher infused CD3⁺ dose (HR, 1.8; 95% CI, 1.1 to 2.8; $P = .04$.

Infectious Complications

The incidence of serious infections by type and time period is summarized in Figure 3. One hundred eighty-six patients (83%) had 1 or more infections in the first 30 days after UCBT, 67% of patients had 1 or more infections between days 31 and 100, 54 % had 1 or more infections between days 101 and 180, 38% had 1 more infections between days 181 and 365, 33% had 1 or more infections between days 366 and 540, and 15% had 1 or more infections between days 541 and 730.

Table 3
Fine and gray multivariate analysis for lymphocyte recoveries

Outcome	HR	95% CI	*P*-value
CD3⁺ T cell ≥ 500 cells/μL			
ATG dose			
≤ 6 mg/kg	2.8	1.81-5.02	
> 6 mg/kg	1		.02
CMV recipient serostatus			
Positive	1		
Negative	2.1	1.31-4.87	.01
CD3⁺ T cell ≥ 1500 cells/μL			
CMV recipient serostatus			
Positive	1		.01
Negative	2.3	1.81-3.01	
Recipient age			
≤ 20 years	2.02	1.01-3.41	.03
> 20 years	1		
CD4⁺ T cell ≥ 200 cells/μL			
CMV recipient serostatus			
Positive	1		.001
Negative	3.76	1.9-5.74	
Recipient age			
≤ 20 years	2.61	1.01-3.47	.03
> 20 years	1		
ATG dose			
≤ 6 mg/kg	3.93	2.3-5.83	.001
> 6 mg/kg	1		
CD4⁺ T cell ≥ 500 cells/μL			
CMV recipient serostatus			
Positive	1		.04
Negative	1.87	1.1-2.8	
CD8⁺ T-cell ≥ 200 cells/μL			
ATG dose			
≤ 6 mg/kg	3.03	1.4-5.1	
> 6 mg/kg	1		.03
CMV negative			
Positive	1		
Negative	1.9	1.63-2.15	.01
B cell ≥ 200 cells/μL			
CMV recipient serostatus			
Positive	1		.03
Negative	3.47	1.3-4.25	
NK cell ≥ 100 cells/μL			
CMV recipient serostatus			
Positive	1		.03
Negative	2.3	1.03-3.7	
Total CD3 dose			
$<4 \times 10^6$/kg	1		.04
$\geq 4 \times 10^6$/kg	1.8	1.1-2.8	

HR indicates hazard ratio; CI, confidence interval; CMV, cytomegalovirus; ATG, antithymocyte globulin.

Sixty infections caused death and 6 infections contributed to death, with the primary causes of death in addition to graft failure (n = 2) and GVHD (n = 4). Lethal infections happened during a wide range period from day 13 to 872 after UCBT, 6 of them occurred by the first year after transplantation. Indeed, after day 180, serious infections remained common, mainly caused by viral infections (73%).

DISCUSSION

Our study shows the main factors associated to better immune reconstitution after UCBT, such as lower ATG dose in conditioning regimen, negative recipient CMV serostatus, and a younger recipient age, demonstrating their influence to reach higher T lymphocyte subset recovery counts.

Despite a number of groups have compared the immune recovery by sources [5,10,31-33], there are limited data assessing the predictive factor for lymphocyte subset recovery after transplantation [3]. Commonly, a transient delay in the first few months after UCBT is observed but patients achieve normal levels of CD4⁺ T cell counts and TCR repertoire

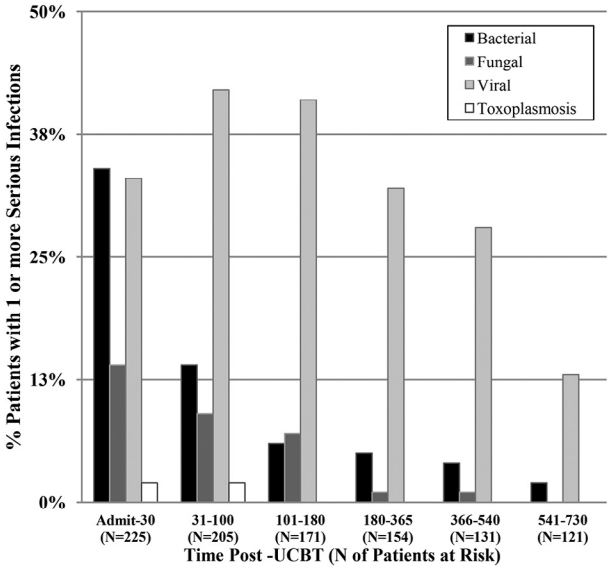

Figure 3. Incidence of serious infections by type and time period in the first 2 years post-CBT.

diversity after 1 year. In concordance, other studies observed a marked delay of immune recovery after sUCBT incorporating ATG in conditioning regimen, where immune reconstitution was reported to occur at a median time of $CD4^+$ T lymphocyte recovery ≥ 200 cells/μL of 9 and 12 months [3,34-37]. These results are in line with ours because we observed a median time of $CD4^+$ T cell counts recovery ≥ 200 cells/μL of 12.9 months after UCBT.

With the aim of improving cellular immune recovery after UCBT, different groups [37,38] adopted alternative strategies, such as the omission of ATG in the conditioning regimen in pediatric and adult patients. For instance, Sauter et al. observed a median time to reach $CD4^+$ T lymphocyte count ≥ 200 cells/μL of 4 months and, consequently, opportunistic infections, especially viral infections, dramatically decreased after double UCBT with the omission of ATG in the conditioning regimen in a cohort of older patients (median age, 36 years). These results are in contrast to ours because incidence of opportunistic infections remained common in the late period after UCBT due to persistent T lymphocyte recovery. Chiesa et al. also omitted ATG in the conditioning regimen in a pediatric cohort and consequently described a pattern of early immune reconstitution after UCBT and observed a rapid increase of T cell counts over the first 2 months after transplantation, especially of the $CD4^+$ compartment, with median $CD4^+$ T cell counts at 30 and 60 days after UCBT of 310 cells/μL and 560 cells/μL, respectively, differing significantly from our results, where we found a median $CD4^+$ T cell count of 41 cells/μL at 3 months after transplantation.

Komaduri et al. [6] observed that absolute $CD8^+$ T lymphocytes counts approached normal values by 1 year after UCBT using ATG in conditioning regimen [6]. However, we found a $CD8^+$ T lymphocyte recovery more delayed: we observed a median $CD8^+$ T lymphocyte counts nearly 200 cells/μL (185 cells/μL) at 18 months after transplantation. Nevertheless, in UCBT with the omission of ATG, $CD8^+$ T lymphocytes show faster recovery compared with other studies [36]. Indeed, studies omitting pretransplantation ATG infusion observed a median time to reach a $CD8^+$ T cell count ≥ 200 cells/μL of 2 months [38].

Admiraal R et al. [39] studied the relationship between an active dose of ATG and immune reconstitution in children after HSCT by measuring ATG exposure and maintaining the ATG dose below the lympholytic concentration using a pharmacokinetic model. Within the cord blood group, they noted decreased immune reconstitution at lowest area under the curve compared with those after grafts of bone marrow or peripheral blood; therefore, immune recovery could be more susceptible to ATG.

Previous studies reveal the impact of CMV serostatus on the immune recovery pattern after transplantation [33,40]. The risk of CMV infection in UCBT recipients is not associated with donor serology, which reflects the maternal exposure history rather than active or latent infection. However, CBU lack CMV-specific memory cells that would confer adoptive immune protection against CMV, and this might have significant implications on the occurrence of CMV infections, especially in CMV-seropositive recipients, who reactivate CMV in nearly 50% of the cases; on the contrary, the incidence of CMV reactivation in CMV-seronegative patients is about 1.3% [41].

We found that age is a significant factor influencing post-transplantation immune recovery in our population, probably because of thymic involution. Klein et al. and other groups [9,42] conducted different studies in pediatric and adult cohorts demonstrating a marked difference in the kinetics of thymic recovery between the 2 groups but did not clarify if the actual cause was related to age or infused TNC dose.

Although our study includes a relatively high number of patients using common clinical practice and biological assessment, it has limitations inherent to retrospective analyses and the wide diversity of underlying diseases and ages of patients. However, the results are conclusive in demonstrating the leading factors affecting the lymphocyte subset recovery after UCBT. Of note, an additional limitation of our study is the unavailability of immunoglobulin value or T-cell excision circles value after transplantation and the inability to study qualitative immune recovery.

In conclusion, the dose of ATG administered in conditioning regimens is the main factor in our population that promotes a serious impact on both early and delayed immune recovery in UCBT recipients. These results highlight the importance of optimizing use of ATG, including new strategies such as change of dosage and timing or omitting its use, with the aim of improving immune reconstitution after UCBT. Other factors, such as age and CMV serostatus, also play a role that may be considered when designing new UCBT protocols to avoid infectious-related mortality.

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SUPPLEMENTARY DATA

Supplementary data related to this article can be found online at doi:10.1016/j.bbmt.2016.11.014.

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