
ARTÍCULO ESPECIAL

Hematopoietic stem-cell transplantation using umbilical-cord blood cells

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INTRODUCTION

Umbilical-cord blood transplantation (UCBT) has extended the availability of allogeneic hematopoietic stem-cell transplantation (HSCT) to patients who would otherwise not be eligible for this curative approach. Since the first successful UCBT from an HLA-identical sibling in a child with severe Fanconi's anemia reported by Gluckman et al. in 1989,¹ the number of UCB transplants from siblings and unrelated donors has increased dramatically, and between 3,000 to 6,000 patients have undergone UCBT from unrelated donors thus far.²

In Japan, nowadays, approximately 50% of HSCT from unrelated donors are being performed with cord blood cells (T. Takahashi, personal communication). In comparison with other sources of allogeneic HSCT, UCB offers substantial logistic and clinical advantages such as:

1. Significantly faster availability of banked cryopreserved UCB units, with patients receiving UCB transplantation in a median of 25-36 days earlier than those receiving BM.^{3,4}
2. Extension of the donor pool due to tolerance of 1-2 HLA mismatch.
3. Lower incidence and severity of acute graft-versus-host disease (GVHD).
4. Lower risk of transmitting infections by latent viruses, such as cytomegalovirus (CMV) and Epstein-Barr virus (EBV).
5. Lack of donor attrition.

6. Lack of risk to the donor and higher frequency of rare haplotypes compared to bone marrow registries.⁵

The disadvantages of UCBT are:

1. The low number of hematopoietic progenitor cells and HSCs in UCB compared with BM or mobilized PB, that translates in increased risk of graft failure and delayed hematopoietic engraftment.
2. The impossibility of using donor lymphocyte transfusion for immunotherapy.

In this review, we will focus on recent clinical results and new innovative strategies developed to improve outcomes after UCBT and extend the use of this exciting source of HSCs to a larger number of patients in need.

CLINICAL EXPERIENCE WITH UMBILICAL-CORD BLOOD TRANSPLANTATION

Umbilical-cord blood transplantation from related donors

Related UCBT has been performed almost exclusively in children. In an update of the Eurocord experience with a median follow-up of 41 months after related UCBT for children, the survival estimate at 3-years was $47 \pm 5\%$ in patients with malignancies ($n = 96$), $82 \pm 7\%$ in patients with bone marrow fa-

ilure ($n = 33$), 100% in patients with hemoglobinopathies ($n = 52$), and $70 \pm 15\%$ ($n = 10$) in patients with inborn errors of metabolism or primary immunodeficiency [Eurocord unpublished data]. For children with malignancies, 3-year overall survival was $71 \pm 12\%$ for early phase of the disease (first complete remission of leukemia), $45 \pm 7\%$ for those in intermediate phase of the disease (second complete remission) and $24 \pm 7\%$ for those with advanced phase of the disease. A joint study by the Eurocord and International Bone Marrow Transplant Registry⁶ has compared the outcome of 113 children who received UCB from HLA-identical siblings with that of 2,052 children who underwent HLA-identical sibling BM transplantation (BMT). UCB transplants recipient had slower recovery of neutrophils and platelets and lower risk of acute and chronic GVHD. Interestingly relapse-related deaths, mortality rate at 100 days after transplantation and overall survival were not significantly different between two groups.⁷ Although longer follow-up is required, these findings suggest that in the HLA-identical sibling setting UCBT is as useful as BMT in children.

The Eurocord group has also reported specific results of related UCBT for 44 patients with Thalassemia and Sickle cell disease (SCD).⁸ All donors were HLA matched with the patients, except 3 who were HLA-A disparate. No patient died and 36 out of 44 children remain disease-free, with a median follow-up of 24 months (range 3-76). Only one patient with SCD did not have sustained donor engraftment as compared to 7 out of the 33 patients with thalassemia. Three of these 8 patients had sustained donor engraftment after a subsequent allogeneic BMT from the same donor. Four patients experienced grade II acute GVHD and only 2 out of the 36 patients at risk developed limited chronic GVHD. The 2-year probability of disease-free survival is 79% and 90% for patients with thalassemia and SCD, respectively. Use of MTX for GVHD prophylaxis was associated with a greater risk of treatment failure [Hazard ratio: 6.2 (95% Confidence interval: 1.5-26); $p = 0.01$]. Therefore, related UCBT in patients with hemoglobinopathies offer a probability of success comparable to that offered by BMT and is associated with a lower risk of both TRM and chronic GVHD.⁹ Based on these results we recommend to collect and freeze cord blood units in families where a child is affected with genetic or hematological diseases.

Most of the related cord blood units come from local hospitals where patients or families have been fo-

llowed up, however logistics for establishing related cord blood banking, optimal criteria and procedures for collecting and freezing related cord blood units have just begun to be discussed.¹⁰

Umbilical-cord blood transplantation from unrelated donors in children

Several series reported in the literature¹⁰⁻¹⁸ have shown that unrelated donor UCBT in children was able to reconstitute hematopoiesis and achieve sustained engraftment in most cases; was associated with a low incidence of GVHD; and did not result in a higher relapse risk. Almost all pediatric series on UCBT from unrelated donors have demonstrated the profound impact of cell dose, measured as total nucleated cells,^{6,10,11,18,19} colony-forming cells,^{20,21} CD34⁺ cells,^{14,15,19} and nucleated red blood cells²⁰ on engraftment,^{14,15,19,20,22} adverse transplant-related events^{10,20,22} and survival.¹⁴ Although the prognostic importance of HLA disparity was not clearly recognized in earlier series, it became apparent in recent updates.^{14,19}

Recently, results of unrelated UCBT in children with specific diseases have been reported.^{10,17} Eurocord group has reported prognostic factors and outcomes of UCBT from unrelated donors for children with AML.¹⁰ We analyzed 95 children receiving UCBT for AML (20 in CR1, 47 in CR2 and 28 in more advanced stage). Poor prognosis cytogenetic abnormalities were identified in 29 cases. Most patients received a 1 or 2 HLA antigens mismatched transplant. The median number of collected or frozen nucleated cells (NC) was $5.2 \times 10^7/\text{kg}$. Cumulative incidence of neutrophil recovery was $78 \pm 4\%$, acute GVHD (grade II-IV) was $35 \pm 5\%$ and 100-day TRM was $20 \pm 4\%$. In multivariable analysis, a collected NC dose higher than $5.2 \times 10^7/\text{kg}$ (median cell dose) was associated with a lower 100-day TRM. The 2-year relapse rate was $29 \pm 5\%$ and it was associated with disease status. The 2-year Leukemia free survival (LFS) was $42 \pm 5\%$, ($59 \pm 11\%$ in CR1, $50 \pm 8\%$ in CR2, and $21 \pm 9\%$ for children not in CR). Children with poor prognosis cytogenetic had similar LFS compared to other patients ($44 \pm 11\%$ vs. $40 \pm 8\%$). In CR2, LFS was not influenced by the length of CR1 ($53 \pm 11\%$ in CR1 < 9.5 months compared to $50 \pm 12\%$ in later relapses). These encouraging results show that UCBT is a good therapeutic choice for children with very poor prognosis AML and who lack a related donor.

Table 1. Comparison of outcomes after UCBT and UBMT in children.

	Barker (24)		Barker (24)		Rocha [†] (23)			Dalle (4)	
	UCBT	UBMT	UCBT	T-UBMT	UCBT	UBMT	T-UBMT	UCBT	UBMT
Number of patients	26	26	31	31	99	262	180	36	28
Methods	Retrospective-matched pair analysis (single center)*		Retrospective-matched pair analysis (single center)*		Retrospective-statistical adjustment (multicentric) ^o			Retrospective-strategy based comparison (single center)	
Year of transplantation	1994-99	1991-99	1994-99	1991-99	1994-98			1996-2002	
Diagnosis others	Malignancies others	Malignancies others	Malignancies others	Malignancies others	ALL/AML	ALL/AML	ALL/AML	Malignancies others	Malignancies others
Median age (range)	4.5 (< 18)	4.7 (< 18)	5.8 (< 18)	6.8 (< 18)	6.0 (< 15)	8.0 (< 16)	8.0 (< 16)	7.5 (< 20)	6.8 (< 21)
HLA-matched (6/6)	19%	100%	13%	100% ¹¹	8%	80.5%	54%	6%	100%
5/6	46%		71%		43%	17.6%	34%	50%	-
4/6	31%		16%		41%	0.4%	6%	44%	-
3 and 2/6	4%				7%		1%		
Median NC infused x 10 ⁸ /kg	0.3	2.0	0.3	0.5	0.38	4.2	3.8	0.43	4.3
MTX for GVHD prophylaxis	No	Yes	No	No	18%	83%	34%	77%	100%
Probability of engraftment	88% (at day 45)	96%	85% (at day 45)	90%	80% (at day 60)	96%	90%	61% (at day 30)	82%
Median days ANC recovery	29	22	27	14	32	18	16	28	24
Median days PT recovery	66	30	61	59	81	29	29		
Acute GVHD of grade ≥ II	42%	35%	36%	35%	35%	58%	20%	13%	4%
1 or 2 years chronic GVHD	5%	20%	7%	13%	25%	46%	12%	-	
Day 100 TRM	27%	15%	23%	16%	39%	19%	14%	27%	11%
2 or 3 years overall survival	53%	41%	52%	56%	35%	49%	41%	59%	57%
2 years disease-free survival	-	-			31%	43%	37%		
Results of UCBT compared to T cell depleted (T-UBMT) or unmanipulated (UBMT)	Delayed time to engraftment, however probability was not different. Similar acute and chronic GVHD. Similar TRM and Survival.	Delayed time to engraftment, however probability was not different. Similar acute and chronic GVHD. Similar TRM and Survival.	Delayed time to engraftment, however probability was not different. Similar acute and chronic GVHD. Similar TRM and Survival.	Delayed time to and probability of engraftment, decreased acute and chronic GVHD and higher early TRM compared to UBMT. Relapse rate, long term LFS and survival similar to UBMT.	Delayed time to and probability of engraftment. Similar acute GVHD. Similar TRM and Survival.				

Footnote: *Matched for age, diagnosis and disease risk.^o Significant differences in age, diagnosis, and disease risk.[†] Differences between groups not reported UCBT: unrelated donor umbilical cord blood transplantation; UBMT: unrelated donor bone-marrow transplantation; TUBMT: T-cell-depleted unrelated donor bone marrow transplantation; HLA: human leukocyte antigen; NC: nucleated cells; UCB: umbilical cord blood; MTX: methotrexate; GVHD: graft-versus-host disease; ANC: absolute neutrophil count; PT: platelets; TRM: transplant-related mortality.

More recently, the group of Duke University has reported outcomes of 20 children with Hurler's syndrome given UCBT.¹⁷ Median time from starting the search process and conditioning was only 41 days. The median nucleated cell dose infused was $6.77 \times 10^7/\text{kg}$ and all except one patient received an HLA mismatched CB graft. All available patients engrafted, five patients had acute GVHD grade II or III, and none had chronic GVHD. Event-free survival was 85% with improvement of neurocognitive performance and decreased somatic features of Hurler's syndrome.¹⁷ These results showed that UCBT should be considered in young children with genetic and metabolic diseases in which time from diagnosis to definitive treatment may represent a crucial period in which to prevent further progression of the disease a readily available source of stem-cells is extremely desirable.

Umbilical-cord blood transplantation compared to bone marrow from unrelated donors in children

The comparison of the results of UCBT and BMT from unrelated donors in children is of paramount relevance, because for many patients the search process will identify both UCB units and UBM donors. Three published studies: two single-center studies and Eurocord registry series, have reported retrospective analyses comparing outcomes after UCBT and UBMT in children^{4,23,24} (Table 1). Briefly, in these 3 studies, recipients of UCBT were transplanted in a shorter time compared to children given an UBMT, neutrophil and platelet recovery were delayed, acute GVHD decreased and overall survival was not significantly different after UCBT compared to UBMT. The Eurocord group has reported higher early TRM probably due to infections related to delayed engraftment. It is important to remark that all patients in the Eurocord series were transplanted before 1998, period in which UCBT was still considered as a last option for leukemia treatment. These data¹ strongly suggest that UCB is an acceptable alternative to matched unrelated BM in children, and² support the start of a simultaneous search for BM and UCB unrelated donors. The final selection of unrelated donor BM versus UCB should be based on the urgency of the transplant, and the characteristics of the BM and UCB unrelated donor such as cell dose and HLA compatibility.

Umbilical-cord blood transplantation from unrelated donors in adults

Recent reviews focusing on the clinical results of unrelated donor UCBT in adults are available.²⁵⁻²⁷ To date, more than 1000 UCBT have been performed in adults with a unit coming from the Netcord organization,² however the available information in this setting is still limited to small series of patients.²⁸⁻³⁶ As expected from retrospective and multicentric studies, the series were heterogeneous in terms of recipients and disease-related characteristics, such as type and status of the disease at transplant.^{28,32,33} However, single center reports more homogeneous series of patients and diseases with standard conditioning regimen and GVHD prophylaxis.²⁹⁻³¹ For example, in the Japanese series,^{30,31,34} the analysis are from a single center, reporting patients with MDS or AML, with homogeneous conditioning regimen (without ATG) and use of methotrexate in combination with CsA as GVHD prophylaxis. Another important difference is that in 4 of the 6 series, the median number of nucleated cells infused per kilogram of the recipient's weight was below $2 \times 10^7/\text{kg}$, and several patients received less than 1.5×10^7 nucleated cells/kg, figures that are below recent recommendations.^{14,19} However, in the Japanese series, very few patients received a cord blood cell dose inferior to $2 \times 10^7/\text{NC/kg}$. Granulocyte colony-stimulating factor was commonly used after UCBT in all series. The myeloid engraftment rate at 60 days ranged from 80-100% and probability of platelet engraftment at 180 days was 65-90%. Median time to achieve a neutrophil count above $0.5 \times 10^9/\text{L}$ varied from 22 days to 32 days. There were large variations of acute and chronic GVHD, TRM at 100 days (0 to 54%) and disease free survival (15% to 76%). It is difficult to explain the reason of such differences since factors such as patients and cord blood graft selection, disease and disease status, center effect and period of transplant, may be involved. Moreover studies of prognostic factors with larger series of adults given an UCBT are still missing and any attempt to explain the different outcomes among these series is still premature.

Results of unrelated cord blood transplants compared to unrelated bone marrow transplants in adults with hematological malignancies

Table 2 lists three retrospective studies recently published comparing results of UCBT with UBMT in

adults.³⁴⁻³⁶ Investigators from a single center in Japan have compared the outcomes of 113 adult patients with hematological malignancies who received unrelated UBMT ($n = 45$) or unrelated UCBT ($n = 68$). In this single center analysis time from donor search to transplantation was significantly shorter among UCBT recipients (median 2 months) compared to 11 months in UBMT. Neutrophil and platelet recovery were delayed in UCBT recipients. UCBT recipients experienced a rapid tapering of immunosuppressive drugs after transplantation and treatment of acute GVHD with steroids was less frequent. Moreover no UCBT recipient died of GVHD, in spite of the high degree of HLA mismatching. TRM and DFS after UCBT were superior when compared to UBMT (Table 2). In this study, all but 4 patients received a cord blood cell dose superior to $2 \times 10^7/\text{kg}$.

The Acute Leukemia Working Party of EBMT in collaboration with Eurocord has performed a retrospective comparison of 98 adults with acute leukemia given an UCBT and with 584 unrelated bone marrow transplants (UBMT) performed between 1998 to 2002.³⁵ Outcomes were compared using multivariate analysis to adjust for confounding clinical factors. Recipients of UCBT were younger (median 24.5 versus 32 years, $p < 0.001$), weighed less (median 58 versus 68 kg, $p < 0.001$), had more advanced disease at transplant (52 % versus 33%, $p < 0.001$). All UBMT were HLA-matched whereas 96% of UCBT were HLA-incompatible ($p < 0.001$). The median number of nucleated cord blood cells infused was $0.23 \times 10^8/\text{kg}$ compared with $2.9 \times 10^8/\text{kg}$ nucleated bone marrow cells ($p < 0.001$). Multivariate analysis demonstrated lower risks of grade II-IV acute graft versus-host disease (GvHD) (Relative Risk (RR) = 0.57, 95% Confidence Interval (95CI) = 0.37-0.87; $p = 0.01$) after UCBT, however neutrophil recovery was significantly delayed (RR = 0.49, 95CI = 0.41-0.58, $p < 0.001$). Transplantation-related mortality, relapse, chronic GvHD, and leukemia-free survival were not significantly different between UCBT and UBMT recipients.

In another registry-based analysis, Laughlin, et al³⁶ found inferior outcomes for patients with leukemia given an UCBT, compared to HLA matched UBMT, however similar outcomes were found when UCBT was compared to 1 HLA mismatched UBMT (Table 2).

The results of these 3 comparative studies gathered together, in spite of their different results, and although definitive conclusions will require larger and homogeneous series of patients with longer follow-up, showed that:

1. UCBT is feasible in adults when a cord blood unit contain a higher number of cells and should be considered an option as an allogeneic stem-cell source for patients lacking an HLA matched bone marrow donor.
2. Despite increased HLA disparity, UCB from unrelated donors offers comparable results to matched UBM in adults with hematological malignancies leading to the conclusion, as in children, that the donor search process for BM and UCB from unrelated donors should be started simultaneously especially in patients with acute leukemia where the time factor is very important.

FUTURE PERSPECTIVES OF UNRELATED DONOR CORD BLOOD TRANSPLANTATION

To improve the results of unrelated donor UCBT, the major aim of future research should focus on reducing the time to hematopoietic recovery and transplant related toxicity.

Accelerating engraftment by increasing the cell dose of umbilical-cord blood units

As shown above, cell dose of the UCB graft is of capital importance in myeloid engraftment and survival after UCBT from unrelated donors. The nucleated cell dose infused seems also to have a direct relationship with T-cell recovery after transplant.³⁷ Thus, to increase cell dose is a major subject of current research. Optimization of the process of UCB collection, establishment of high-quality UCB banks, and expansion of the pool of donors, which is particularly relevant for ethnic and racial minorities, will prove most valuable.^{4,38}

Other innovative research strategies to augment the dose of hematopoietic progenitor cells are *ex vivo* expansion and transplantation of multiple UCB units.

Two phase-1 clinical trials using expanded cord blood cells have been reported.^{39,40} Both studies have demonstrated the feasibility of *ex vivo* expansion but there is a need for more efficient expansion protocols and gene marking of expanded cells to evaluate their capacity of engrafting. Other questions frequently raised in the field of HSC expansion are the potential of long term engraftment of expanded cells, the need of adding other accessory cells such as T-cells or mesenchymal cells, the best population of cells to be expanded and the best combination of growth fac-

Table 2. Comparison of outcomes after UCBT and UBMT in adults.

	Rocha (35)		Laughlin (36)			Takahashi (34)	
	UCBT	HLA matched UBMT	UCBT	1 HLA mismatched dUBMT	HLA matched UMT	UCBT	UBMT
Number of patients	98	584	150	83	367	68	45
Methods	Retrospective-statistical adjustment (multicentric)			Retrospective-statistical adjustment (multicentric)			Retrospective-strategy based comparison (single center)*
Year of transplantation	1998-2002			1996-2001			1997-2003
Diagnosis	AML/ALL			AML/ALL/MDS/CML			ALL/AML/CML/NHL
Median age (years) (range) or frequency > 40 years	24.5 (15-55)	32 (15-59)	26%	29%	38%	26 (16-50)	36 (16-53)
HLA-matched (6/6)	6%	100%	0%	0%	100%	0%	87%
5/6	51%		23%	100%	-	21%	13%
4/6	39%		77%	-	-	54%	-
3 and 2/6	4%	-	-	-	-	25%	-
Median NC infused x 10 ⁸ /kg	0.23	2.9	0.22	2.2	2.4	0.25	3.3
MTX for GVHD prophylaxis	9%	89%	-	-	-	96%	98%
Probability of engraftment	75%	95%	69%	77%	90%	92% (day 42)	100%
Median days ANC recovery	26	19	18	20	27	22	18
Median days PT recovery	-	-	-	-	-	40 (13-99)	25 (10-172)
Cumulative incidence of acute GVHD of grade ≥ II	26%	39%	-	-	-	50%	66%
Chronic GVHD (limited and extensive)	30% (24mo)	46% (24mo)	33%	71%	52%	77%	74%
TRM	44% (24mo)	38% (24mo)	-	-	-	9% (12mo)	29% (12mo)
Overall survival	36% (24mo)	42% (24mo)	-	-	-	-	-
Disease-free survival	33% (24mo)	38% (24mo)	26% (36mo)	20% (36mo)	35% (36mo)	74% (24mo)	44% (24mo)
Results of UCBT compared to UBMT	Delayed time to engraftment Decreased acute GVHD Similar chronic GVHD, relapse, TRM LFS and Survival			Delayed time to engraftment Compared to UBMT similar acute and chronic GVHD decreased survival. Compared to 1 HLA mismatched UBMT: similar TRM and survival			Delayed time to engraftment Decreased acute GVHD and time to stop immunosuppressors Similar chronic GVHD and relapse. Decreased TRM, better DFS

In almost of all the three studies significant statistical differences between the groups were status of the disease, cell dose, HLA disparities and GVHD prophylaxis. *Statistical difference in the donor search process.

Abbreviations: UCBT: unrelated donor umbilical-cord blood transplantation; UBMT: unrelated donor bone-marrow transplantation; T-UBMT: T-cell-depleted unrelated donor bone-marrow transplantation; HLA: human leukocyte antigen; NC: nucleated cells; UCB: umbilical-cord blood; MTX: methotrexate; GVHD: graft-versus-host disease; ANC: absolute neutrophil count; PT: platelets; TRM: transplant-related mortality.

tors.⁴¹ Other compounds to expand CD34 have been investigated. Recently, Peled, et al have demonstrated that linear polyamine copper chelator augments long-term ex vivo expansion of cord blood derived CD34+ cells and increases their engraftment potential in NOD/SCID mice.⁴² Phase 1 clinical trials using expanded CB cells with copper chelator have already started.

Transplantation of UCB from two^{43,44} or more⁴⁵ partially HLA-matched unrelated donors is another method of increasing the cell dose. Experience with three or more CB grafts have shown high rate of graft failure,⁴⁵ however results with double cords, although preliminary, support the safety of the procedure in terms of cross immunological rejection. Other approach to reduce the neutropenia period after UCBT is the cotransplantation of an UCB unit with highly purified CD34+ cells from haploidentical family donors.⁴⁶ Larger series of patients are clearly required to determine the effect of double cords or co-transplantation of haplo CD34+ cells on neutrophil engraftment and TRM.

Other possible approaches to improve engraftment of cord blood cells are the co transplantation with mesenchymal cells from an allogeneic donor,⁴⁷ or the intra bone infusion of cord blood cells.^{48,49} Recently, it has been suggested in mice that intrabone infusion of CD34 CB cells has an engraftment advantage 15 times higher than intravenous infusion, probably decreasing cell loss during the circulation of the cells before homing.⁴⁹ This approach seems attractive and it has been shown feasible in patients undergoing bone marrow transplantation.⁵⁰

Choosing the best umbilical-cord blood unit

It has been suggested that cell dose and number of HLA mismatches interact mutually on engraftment and on other outcomes. Thus, a higher cell dose in the graft could partially overcome the negative impact of HLA for each level of HLA disparity; however this hypothesis has not been demonstrated. In order to better understand the impact of HLA disparities and cell dose and to establish guidelines for cord blood donor choice based on these two factors, the Eurocord group has analyzed 550 UCBT recipients with hematological malignancies.¹⁹ Nucleated cell dose (NC) at freezing was used as a surrogate marker of the number of progenitors in the graft, because this measure is well-standardized, likely to be reproducible between laboratories, and mainly because this information is readily available during

the search process. We found that both NC at freezing and number of HLA disparities were associated with the probability of myeloid engraftment (more than 2 HLA disparities decreased the probability of engraftment), while CD34+ cell dose and HLA disparities were jointly associated with the probability of acute GvHD grade III-IV (but not with acute GvHD grade II-IV). Disease relapse was higher in matched transplants showing a graft-versus-leukemia effect increased in HLA mismatched transplants. Overall 3-year survival was 34.4%. Prognostic factors for survival were recipient age, gender and disease status. A center and period effect were found to be associated with outcomes and were used in a multivariate model to adjust for clinical factors. Other objective of this study was to delineate an algorithm to help clinicians to choose the best cord blood unit also taking into consideration other patient and disease-related factors. This would require establishing thresholds of number of nucleated cord blood cells before freezing and the maximal number of HLA disparities allowed in the cord blood donor selection. In fact, we found that cell dose at freezing and number of HLA mismatches, were log-linearly related to the hazard of neutrophil engraftment. Thus, we observed that the higher the number of cord blood cells, the lower the number of HLA disparities and the higher was the probability of engraftment. Accordingly, no definitive threshold for cell dose and HLA disparities could be defined, however based on previous data^{11,14} and Eurocord data,¹⁹ we recommend a cord blood graft with no more than 2 HLA disparities and more than 2×10^7 /kg nucleated cells at cryopreservation. Studies are needed to determine the impact of HLA high resolution typing for class I and II on outcomes after UCBT.

Reducing conditioning-related mortality

Encouraging results regarding engraftment and TRM have recently been reported with the use of reduced-intensity conditioning regimens.⁵¹ In the largest experience to date investigators from the University of Minnesota have reported the preliminary results of UCBT from mismatched unrelated donors after non-myeloablative (NMA) conditioning in 43 adult patients (median age 49.5 years) with advanced or high-risk hematological malignancies.⁵¹ The median cryopreserved cell dose, 3.7×10^7 nucleated cells/kg, was higher than those usually reported in series of adults undergoing UCBT after a myeloablative conditioning. In this series, some pa-

tients received two cord blood units and two types of non myeloablative conditioning regimen: fludarabine 200 mg/m², busulfan 8 mg/kg and TBI 200 cGY (Flu/Bu/TBI) for the initial 21 patients and fludarabine 200 mg/m², cyclophosphamide 50mg/kg and TBI 200 cGY (Flu/Cy/TBI). All patients received CsA and MMF as GVHD prophylaxis. The median time to neutrophil recovery was 26 days (range 12-30 days) with a cumulative incidence of engraftment of 76% for the Flu/Bu/TBI recipients and only 9.5 days (range 5-28 days) with a cumulative incidence of engraftment of 94% for the Flu/Cy/TBI recipients. Despite the use of 1 or 2 HLA antigens mismatched grafts in 93% of the recipients, the cumulative incidence of acute GVHD grade II-IV and III-IV was 44% and 9% respectively. TRM at day 100 was 48% for Bu/Flu/TBI recipients and 28% for Flu/Cy/TBI. Causes of death during 100 days were predominantly organ failure and infections. DFS at one year of these high-risk patients was 24% for Flu/Bu/TBI recipients and 41% for Flu/Cy/TBI recipients. A similar approach has also been reported by investigators at Duke University.⁵² A total of 10 patients with hematological malignancies (n = 9) and one patient with metastatic melanoma received fludarabine 120mg/m², cyclophosphamide 2 g/m² and ATG as a NMA conditioning. The median age was 51 years,¹⁹⁻⁶² the median number of cells infused was 2.1 x 10⁷/kg and the majority had 2 HLA disparate grafts. Six patients had donor chimerism between 4 weeks and 6 months after transplantation, however only 3 became full donor. Among these 3 patients, one had acute GVHD grade III. Five patients died, 3 from disease progression or relapse, 1 of fungal infection and one of cerebral infarction. The estimated OS and DFS at 2 years were 36% and 27%, respectively. Using this NMA conditioning no treatment-related mortality was observed within the first 100 days.⁵²

The experience of NMA conditioning in children given UCBT is still limited. Recently, Del Toro, et al reported, in a pilot study, the experience of NMA in 14 children with malignant and non-malignant diseases given a 1 or 2 HLA mismatched UCBT. The median number of nucleated cells infused was 4.3 x 10⁷/kg. The median time to neutrophil recovery was 18 days and 3 out of 14 patients did not engraft. Full chimerism (> 94% of donor cells) was observed in 10 patients and one patient died at day + 79 with 55% of donor cells. Eleven out of 14 patients are alive and 6 without disease. In spite of the small number of patients and heterogeneity of diseases it seems that NMA for children given a UCBT is feasible.⁵³

Improving immune reconstitution and the management of infection

Immune recovery after UCBT is an area of great interest and concern owing to the low number and immaturity of UCB lymphocytes as well as to the degree of HLA mismatching. Despite those facts, several studies have shown that immune reconstitution in children undergoing UCBT is not delayed compared with BMT in terms of number of T-, B- and NK-lymphocyte subsets,³⁷ and T-cell repertoire diversity and thymic function⁵⁸ and recovery of specific immune response toward viruses and fungi.⁵⁴ In contrast, central T-cell recovery and lymphocyte recovery is delayed after UCBT in adults compared to children, especially in the presence of GVHD.^{56,57}

Whether related or not to delayed neutrophil engraftment, GVHD or immune disturbances, infection is the major cause of death after UCBT.¹¹ However, the pattern and type of infectious complications in recipients of UCB transplants has not been studied in detail or in larger series of patients. A large survey by the Eurocord group is currently addressing this question. Hamsa, et al compared myeloid and lymphocyte recovery and incidence and type of infections after 28 UCBT and 23 UBMT adult recipients. Myeloid and lymphocyte recoveries were delayed after UCBT. Overall infection rates, mainly infections from bacterial origin, were higher in UCBT recipients, particularly at early time points (before day + 50) after transplantation.⁵⁷ In another study, the incidence of EBV-associated posttransplantation lymphoproliferative disorders after unrelated donor UCBT was not increased compared with unrelated donor BMT.⁵⁸ In contrast, two other studies have shown that the incidence of HHV-6 (Human herpes virus 6), CMV infection, HSV (Herpes simplex) and VZV (Varicella-zoster) seemed higher after UCBT than after BMT or peripheral blood HSCT.⁵⁹⁻⁶²

CONCLUSIONS

Umbilical-cord blood has emerged as an appealing alternative source of hematopoietic stem-cells for transplantation. Although many issues remain uncertain and greater experience will be required to determine clearly the relative merits of UCBT compared with BMT, all available data suggest that unrelated donor UCBT should be considered as an acceptable option in children and adults with hematological and non hematological malignancies for whom an HLA-matched BM unrelated donor is not readily available. Shorter time to transplant and im-

proved chance of finding a suitable graft are evident advantages of unrelated donor UCBT over unrelated donor BMT. This is particularly relevant to patients requiring urgent transplantation. Prospective randomized studies comparing unrelated donor UCBT to unrelated donor BMT and highly purified CD34+ cells from haploidentical family donors are clearly required to establish the role of UCBT in the alternative donor algorithm for patients requiring HSCT. Hopefully, the current research approaches and the greater experience of transplant centers on UCBT will improve outcomes and will provide successful therapy to a larger number of patients who need an allogeneic HSCT.

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REFERENCES

- Gluckman E, Broxmeyer HE, Auerbach AD, et al. Hematopoietic reconstitution in a patient with Fanconi's anemia by means of umbilical-cord blood from an HLA-identical sibling. *N Engl J Med* 1989; 321: 1174-8.
- Wernet P. The Netcord inventory and use. July 12, 2002. Accessed and available January 11, 2005 at: <http://office.de.netcord.org/inventory.gif>
- Barker JN, Krebski TP, DeFor T, et al. Searching for unrelated donor hematopoietic stem-cell grafts: availability and speed of umbilical-cord blood versus bone marrow. *Biol Blood Marrow Transplant* 2002; 8: 257-60.
- Dalle JH, Duval M, Moghrabi A, et al. Results of an unrelated transplant search strategy using partially HLA-mismatched cord blood as an immediate alternative to HLA-matched bone marrow. *Bone Marrow Transplant* 2004; 33: 605-11.
- Davey S, Armitage S, Rocha V, et al. The London Cord Blood Bank: analysis of banking and transplantation outcome. *Br J Haematol* 2004; 125: 358-65.
- Gluckman E, Rocha V, Boyer-Chammard A, et al. Outcome of cord blood transplantation from related and unrelated donors. Eurocord Transplant Group and the European Blood and Marrow Transplantation Group. *N Engl J Med* 1997; 337: 373-81.
- Rocha V, Wagner JE, Sobocinski KA, et al. Graft-versus-host disease in children who have received a cord blood or bone marrow transplant from an HLA-identical sibling. *N Engl J Med* 2000; 342: 1846-54.
- Locatelli F, Rocha V, Reed W, et al. Related umbilical-cord blood transplant in patients with Thalassemia and Sickle Cell Disease. *Blood* 2003; 101: 2137-43.
- Michel G, Rocha V, Chevret S, et al. Unrelated cord blood transplantation for childhood acute myeloid leukemia: a Eurocord Group analysis. *Blood* 2003; 101: 351-7.
- Reed W, Smith R, Dekovic F, et al. Comprehensive banking of sibling donor cord blood for children with malignant and non-malignant disease. *Blood* 2003; 102: 4290-7.
- Rubinstein P, Carrier C, Scaradavou A, et al. Outcomes among 562 recipients of placental-blood transplants from unrelated donors. *N Engl J Med* 1998; 339: 1565-77.
- Ohnuma K, Isoyama K, Nishihira H. Cord blood transplantation from HLA-mismatched unrelated donors. *Leuk Lymphoma* 2002; 43: 1029-43.
- Kurtzberg J, Laughlin M, Graham ML, et al. Placental blood as a source of hematopoietic stem-cells for transplantation into unrelated recipients. *N Engl J Med* 1996; 335: 157-66.
- Wagner JE, Barker JN, DeFor TE, et al. Transplantation of unrelated donor umbilical-cord blood in 102 patients with malignant and nonmalignant diseases: influence of CD34 cell dose and HLA disparity on treatment-related mortality and survival. *Blood* 2002; 100: 1611-18.
- Styczynski J, Cheung YK, Garvin J, et al. Outcomes of unrelated cord blood transplantation in pediatric recipients. *Bone Marrow Transplant* 2004; 34: 129-36.
- Yu LC, Wall DA, Sandler E, et al. Unrelated cord blood transplant experience by the pediatric blood and marrow transplant consortium. *Pediatr Hematol Oncol* 2001; 18: 235-45.
- Staba SL, Escolar ML, Poe M, et al. Cord blood transplants from unrelated donors in patients with Hurler's syndrome. *N Engl J Med* 2004; 350: 1960-9.
- Benito AI, Diaz MA, Gonzalez-Vincent M, et al. Hematopoietic stem-cell transplantation using umbilical-cord blood progenitors: review of current clinical results. *Bone Marrow Transplant* 2004; 33: 675-90.
- Gluckman E, Rocha V, Arcese W, et al. Eurocord Group. Factors associated with outcomes of unrelated cord blood transplant: guidelines for donor choice. *Exp Hematol* 2004; 32: 397-407.
- Migliaccio AR, Adamson JW, Stevens CE, et al. Cell dose and speed of engraftment in placental/umbilical-cord blood transplantation: graft progenitor cell content is a better predictor than nucleated cell quantity. *Blood* 2000; 96: 2717-22.
- Iori AP, Cerretti R, De Felice L, et al. Pre-transplant prognostic factors for patients with high-risk leukemia undergoing unrelated cord blood transplantation. *Bone Marrow Transplant* 2004; 33: 1097-105.
- Stevens CE, Gladstone J, Taylor PE, et al. Placental/umbilical-cord blood for unrelated-donor bone marrow reconstitution: relevance of nucleated red blood cells. *Blood* 2002; 100: 2662-4.
- Rocha V, Cornish J, Sievers E, et al. Comparison of outcomes of unrelated bone marrow and umbilical-cord blood transplants in children with acute leukemia. *Blood* 2001; 97: 2962-71.
- Barker JN, Davies SM, DeFor T, et al. Survival after transplantation of unrelated donor umbilical-cord blood is comparable to that of human leukocyte antigen-matched unrelated donor bone marrow: results of a matched-pair analysis. *Blood* 2001; 97: 2957-61.
- Sanz G, Rocha V. Umbilical-cord blood transplantation: current status and future directions. *Curr Opin Organ Transpl* 2003; 8: 109-17.
- Moscardo F, Sanz GF, Sanz MA. Unrelated-donor cord blood transplantation for adult hematological malignancies. *Leuk Lymphoma* 2004; 45: 11-8.
- Koh LP, Chao NJ. Umbilical-cord blood transplantation in adults using myeloablative and non-myeloablative preparative regimens. *Biol Blood Marrow Transplant* 2004; 10: 1-22.
- Laughlin MJ, Barker J, Bambach B, et al. Hematopoietic engraftment and survival in adult recipients of umbilical-cord blood from unrelated donors. *N Engl J Med* 2001; 344: 1815-22.
- Sanz GF, Saavedra S, Planelles D, et al. Standardized, unrelated donor cord blood transplantation in adults with hematological malignancies. *Blood* 2001; 98: 2332-8.
- Ooi J, Iseki T, Takahashi S, et al. Unrelated cord blood transplantation for adult patients with de novo acute myeloid leukemia. *Blood* 2004; 103: 489-91.
- Ooi J, Iseki T, Takahashi S, et al. Unrelated cord blood transplantation for adult patients with advanced myelodysplastic syndrome. *Blood* 2003; 101: 4711-3.

32. Long GD, Laughlin M, Madan B, et al. Unrelated umbilical-cord blood transplantation in adult patients. *Biol Blood Marrow Transplant* 2003; 9: 772-80.

33. Rocha V, Arcese W, Sanz G, et al. Prognostic factors of outcome after unrelated cord blood transplant in adults with hematological malignancies. *Blood* 2000; 96: 587a (abstract).

34. Takahashi S, Iseki T, Ooi J, et al. Single-institute comparative analysis of unrelated bone marrow transplantation and cord blood transplantation for adult patients with hematological malignancies. *Blood* 2004; 104: 3813-20.

35. Rocha V, Labopin M, Sanz G, et al. Acute Leukemia Working Party of European Blood and Marrow Transplant Group; Eurocord-Netcord Registry. Transplants of umbilical-cord blood or bone marrow from unrelated donors in adults with acute leukemia. *N Engl J Med* 2004; 351: 2276-85.

36. Laughlin MJ, Eapen M, Rubinstein P, et al. Outcomes after transplantation of cord blood or bone marrow from unrelated donors in adults with leukemia. *N Engl J Med* 2004; 351: 2265-75.

37. Niehues T, Rocha V, Filipovich A, et al. Factors affecting lymphocyte subset reconstitution after either related or unrelated cord blood transplantation in children - a Eurocord analysis. *Br J Haematol* 2001; 114: 42-8.

38. Ballen K, Broxmeyer HE, McCullough J, et al. Current status of cord blood banking and transplantation in the United States and Europe. *Biol Blood Marrow Transplant* 2001; 7: 635-45.

39. Shpall EJ, Quinones R, Giller R, et al. Transplantation of ex vivo expanded cord blood. *Biol Blood Marrow Transplant* 2002; 8: 368-76.

40. Jaroszak J, Goltry K, Smith A, et al. Augmentation of umbilical-cord blood (UCB) transplantation with ex vivo-expanded UCB cells: results of a phase 1 trial using the Aastrom Replicell System. *Blood* 2003; 101: 5061-7.

41. McNiece I. Ex vivo expansion of hematopoietic cells. *Exp Hematol* 2004; 32: 409-10.

42. Peled T, Landau E, Mandel J, et al. Linear polyamine copper chelator tetraethylenepentamine augments long-term ex vivo expansion of cord blood-derived CD34+ cells and increases their engraftment potential in NOD/SCID mice. *Exp Hematol* 2004; 32: 547-55.

43. Barker JN, Weisdorf DJ, Wagner JE. Creation of a double chimera after the transplantation of umbilical-cord blood from two partially matched unrelated donors. *N Engl J Med* 2001; 344: 1870-1.

44. Barker JN, Weisdorf DJ, DeFor TE, Blazar BR, et al. Transplantation of 2 partially HLA-matched umbilical-cord blood units to enhance engraftment in adults with hematological malignancy. *Blood* 2005; 105: 1343-7.

45. Hamsa NS, Fanning L, Tary-Lehmann M, et al. High rate of graft failure after infusion of multiple (3-5) umbilical-cord blood (UCB) units in adults with hematological disorders: role of HLA disparity and UCB graft cell cross immune reactivation. *Blood* 2003; 98(abstract).

46. Fernandez MN, Regidor C, Cabrera R, et al. Unrelated umbilical-cord blood transplants in adults: Early recovery of neutrophils by supportive co-transplantation of a low number of highly purified peripheral blood CD34+ cells from an HLA-haploididentical donor. *Exp Hematol* 2003; 31: 535-44.

47. Kim DW, Chung YJ, Kim TG, et al. Co transplantation of third-party mesenchymal stromal cells can alleviate single-donor predominance and increase engraftment from double cord transplantation. *Blood* 2004; 103: 1941-8.

48. Mazurier F, Doedens M, Gan OI, et al. Rapid myelo erythroid repopulation after intra femoral transplantation of NOD-SCID mice reveals a new class of human stem-cells. *Nat Med* 2003; 9: 959-63.

49. Castello S, Podesta M, Mendifito VG, et al. Intra-bone marrow injection of bone marrow and cord blood cells: an alternative way of transplantation associated with a higher seeding efficiency. *Exp Hematol* 2004; 32: 782-7.

50. Hagglund J, et al. Intraosseous compared to intravenous infusion of allogeneic bone marrow. *Bone Marrow Transplant* 1998; 21: 331-51.

51. Barker JN, Weisdorf DJ, DeFor TE, et al. Rapid and complete donor chimerism in adult recipients of unrelated donor umbilical-cord blood transplantation after reduced-intensity conditioning. *Blood* 2003; 102: 1915-9.

52. Chao NJ, Koh LP, Long GD, et al. Adult recipients of umbilical-cord blood transplants after non-myeloablative preparative regimens. *Biol Blood Marrow Transplant* 2004; 10: 569-75.

53. Del Toro G, Satwani P, Harrison L, et al. A pilot study of reduced intensity conditioning and allogeneic stem-cell transplantation from unrelated cord blood and matched family donors in children and adolescent recipients. *Bone Marrow Transplant* 2004; 33: 613-22.

54. Talvensaari K, Clave E, Douay C, et al. A broad T-cell repertoire diversity and an efficient thymic function indicate a favorable long-term immune reconstitution after cord blood stem-cell transplantation. *Blood* 2002; 99: 1458-64.

55. Montagna D, Locatelli F, Moretta A, et al. T lymphocytes of recipient origin may contribute to the recovery of specific immune response toward viruses and fungi in children undergoing cord blood transplantation. *Blood* 2004; 103: 4322-9.

56. Klein AK, Patel DD, Gooding ME, et al. T-cell recovery in adults and children following umbilical-cord blood transplantation. *Biol Blood Marrow Transplant* 2001; 7: 454-66.

57. Hamsa NS, Lisgaris M, Yadavalli G, et al. Kinetics of myeloid and lymphocyte recovery and infectious complications after unrelated umbilical-cord blood versus HLA-matched unrelated donor allogeneic transplantation in adults. *Br J Haematol* 2004; 124: 488-98.

58. Barker JN, Martin PL, Coad JE, et al. Low incidence of Epstein-Barr virus-associated post transplantation lymphoproliferative disorders in 272 unrelated-donor umbilical-cord blood transplant recipients. *Biol Blood Marrow Transplant* 2001; 7: 395-9.

59. Sashihara J, Tonaka-Taya K, Tanaka S, et al. High incidence of human herpes virus 6 infection with a high viral load in cord blood stem-cell transplant recipients. *Blood* 2002; 100: 2005-11.

60. Tomonari A, Iseki T, Takahashi S, et al. Ganciclovir-related neutropenia after preemptive therapy for cytomegalovirus infection: comparison between cord blood and bone marrow transplantation. *Ann Hematol*. [in press]

61. Tomonari A, Takahashi S, Iseki T, et al. Herpes simplex virus infection in adult patients after unrelated cord blood transplantation: a single-institute experience in Japan. *Bone Marrow Transplant* 2004; 33: 317-20.

62. Tomonari A, Iseki T, Takahashi S, et al. Varicella-zoster virus infection in adult patients after unrelated cord blood transplantation: a single institute experience in Japan. *Br J Haematol* 2003; 122: 802-5.

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