ORIGINAL ARTICLE

Unrelated Cord Blood Transplantation in Children – A 10-Year Experience from UMMC

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SUMMARY

Children who would benefit from a haematopoietic stem cell transplantation often lacked a compatible sibling donor. Unrelated cord blood transplantation was offered as an alternative donor source for patients with a variety of malignant and non-malignant diseases who had no further treatment options. Cord blood units were sourced from various international cord blood registries. The median nucleated and CD34+ cell doses were 8.7 x 10^7/kg and 2.6 x 10^5/kg respectively. In spite of adequate cell doses, a high rate of non-engraftment of 32% was observed. Acute graft-versus-host disease (GVHD) occurred in 14 out of the 15 patients who engrafted with 53% being grade III to IV GVHD. The five year disease free survival was 40.7% with infection and GVHD being the commonest causes of death. The five year disease free survival was 20.5% and 60.7% for malignant and non-malignant diseases respectively.

KEY WORDS:
Children, Unrelated cord blood transplantation, Survival

INTRODUCTION

Allogeneic haematopoietic stem cell (HSC) transplantation is an established mode of therapy for various malignant and non-malignant diseases[1-4]. Patients with malignant diseases who fail on conventional chemotherapy may be salvaged with high-dose chemotherapy and stem cell transplantation. It may offer the only chance of survival for patients born with errors of metabolism or congenital immunodeficiencies[5]. The lack of a suitable human leucocyte antigen (HLA) matched sibling donor is a major obstacle to patients who would benefit from HSC transplantation. In reality the majority of potential recipients would have to look for alternative unrelated donors. If bone marrow or peripheral blood stem cells are used, a full HLA match is often necessary whereas 1-2 antigen mismatches are allowable if cord blood is used[6-9]. Beyond increasing the donor pool, the use of cord blood as a source of HSC has other advantages including rapid availability, ease of procurement[10], the lack of risks to the donor, reduced risks of transmission of infections like Cytomegalovirus and lower risk of severe graft-versus-host disease (GVHD)[11,12]. The establishment of cord blood banks in various parts of the world has helped promote the use of cord blood (CB) as a source of HSC[13]. Recent studies suggest that outcome of transplants using unrelated CB is similar to that of bone marrow[14].

The HSCT programme for paediatric patients was first established in Malaysia in March 1987. Initially only matched sibling donor transplantations were performed. After mid 1997, unrelated cord blood transplantation (UCBT) was offered. We report our experience over the past 10 years.

MATERIALS AND METHODS

The University Malaya Medical Centre is a tertiary referral centre for paediatric haematology and oncology. It was the first centre to provide HSCT services in the country in 1987 with allogeneic matched sibling and autologous bone marrow transplantation services.

Patients with established indications for HSCT who did not have matched sibling donors were offered UCBT from July 1997 when equipment and facilities for cryopreservation became available. When the HLA profile of a recipient was known, preliminary searches for unrelated cord blood units (CBU) were made through the Bone Marrow Donor Worldwide Programme. Occasionally searches were also performed with individual cord blood registries. CBUs were selected on the basis of the closest HLA matching with preference for HLA DRB1 matching and the highest nucleated cell dose. Typing of HLA A and B loci were by serology or low resolution molecular techniques and HLA DRB1 by high resolution molecular techniques. Confirmatory typing was performed by the individual CB Banks with occasional typing performed at our centre. Once an appropriate CBU was identified and an indication to import the CBU into Malaysia was established, a screen for infectious markers would be performed on the unit by the Cord Blood Bank. An average cost of USD22,000 was charged for each CBU excluding transportation fees which could come to USD2000 – 4000. Permission to import the CBU had to be obtained from the Health Office, Kuala Lumpur International Airport and the Ministry of Health. Between 1997 till 2001, personnel from our own unit would travel to collect the CBUs but subsequently, international courier services were used. The CBUs were transported in a dry shipper in vapour phase nitrogen and then transferred into storage tanks with liquid nitrogen in our medical centre. CBUs were imported from various CB registries located worldwide (See Table I).

Transplantation

All patients (except patients with osteopetrosis) had back-up bone marrow harvested and cryopreserved. Conditioning regimen varied according to recipients' disease status. All but one patient received myeloablative regimen consisting of busulphan, cyclophosphamide +/- anti-thymocyte globulin

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(ATGAM) or total body irradiation (TBI) or melphalan or thiotepa. Before July 2004, GVHD prophylaxis consisted of cyclosporine A and methotrexate with cyclosporine given from Day -1 and methotrexate 5 mg/m2 on Days 1,3,6 and 11. Subsequent transplants used cyclosporine A +/- steroids as GVHD prophylaxis. Recipients were nursed in simple isolation rooms. Most patients received oral fluconazole or itraconazole as anti-fungal prophylaxis. Patients with positive serology to herpes virus received intravenous acyclovir from Days 0 till 20. Regular weekly intravenous immunoglobulin were administered for the first seven weeks post-transplant. CBUs were thawed following the method described by Rubinstein[15] for the first four transplants. In all subsequent UCBTs, thawing was carried out in a waterbath at 37°C followed by infusion over 5 to 10 minutes by intravenous push without further manipulation.

Patients were nursed in simple isolation rooms with air-conditioning. Post-UCBT fevers were treated with broad spectrum antibiotics with addition of systemic intravenous anti-fungals (Amphotericin B) when fever persisted beyond 72 hours. Granulocyte colony stimulating factor was given from Day 1 till neutrophil numbers exceeded 0.5 x 10^9/L for three consecutive days. Chimerism was evaluated qualitatively by sex karyotyping, blood group change or HLA typing on bone marrow samples. After May 2000, VNTR (variable number of tandem repeats) and later STR (short tandem repeats) testing became available and was the preferred method used for confirmation of engraftment[16].

Neutrophil engraftment was defined as the time to absolute neutrophil count (ANC) equal or greater than 0.5 x 10^9/L (first of three consecutive days). Evaluation of acute GVHD was based on clinical assessment +/- histological confirmation based on published criteria[17].

Statistical analysis:
Mann-Whitney and Extended Mantel-Haenszel chi-square test for analysis of trend were used where appropriate. The Kaplan-Meier method[18] was used to estimate survival rates with differences compared by the two-sided log rank test[19]. Event free survival (EFS) was calculated from the first day of transplant to the date of last contact or to the first event. Event was defined as death, resistant or refractory disease or graft rejection. Cases lost to follow-up were censored at the time of their withdrawal. Analyses were managed with SPSS 15.0 (SPSS, Chicago) and significance level was taken at 5%.

RESULTS

A total of 26 patients received unrelated CBUs from different CB Banks. Table I shows the relevant patient characteristics. The median age was 5.0 years (range 9 months to 13 years). There were equal numbers of males to females. The ethnic distribution was Malay 2, Indian 1 and Chinese 23. The median weight was 16.1kg (range 5.56 to 50.2kg). Indications for CBT were Acute Myeloid Leukaemia in second complete remission (CR) for three patients, Acute Lymphoblastic Leukaemia >CR2 5, Philadelphia chromosome positive Acute Lymphoblastic Leukaemia 1, Juvenile Myelo-Monocytic Leukaemia 3, Chronic Myeloid Leukaemia in chronic phase 1, Wiskott-Aldrich syndrome in 2, malignant osteopetrosis in three patients, Severe Aplastic Anaemia in one patient and thalassaemia major 7. Hence 13 (50%) patients had malignant disorders.

Engraftment was not evaluable in four patients who died of bacterial infection on Days -2 till Day +11. In the remaining 22 patients, engraftment was seen in 15 patients. The median nucleated cell dose was 8.7 x 10^7/kg (range 2.6 to 29.3) while the median CD34 cell dose was 2.6 x 10^5/kg (range 0.48 to 12.2). The median time to neutrophil engraftment was 28 days (range 12 to 36). There was no significant difference in the median nucleated cell doses between those who engrafted and those who did not. (See Table II and Figure 3). Only thirteen patients demonstrated platelet engraftment >20 x 10^9/l which occurred at a median of 49 days (range 33 to 116) whereas platelet engraftment >50 x 10^9/l occurred at a median of 53 days (range 39 to 141).

Acute GVHD was seen in all but one patient who engrafted. There was a trend towards occurrence of acute GVHD with increasing HLA disparity. (See Table III). In three patients, their acute GVHD progressed to chronic GVHD involving the skin alone in two patients and skin and oral mucosa in the remaining. Their symptoms were treated successfully with oral steroids and mycophenolate mofetil.

There were 15 deaths in total. Causes of death included bacterial infection in six patients; fungal infection 1; multi-organ failure 1; acute renal failure 1; GVHD and hepato-renal failure two and primary disease four patients. The probability of survival at 5 years was 40.7% (Figure 1). Survival for patients with malignant disorders was 20.5% while survival for those with non-malignant disorders was 60.6% (p=0.09) (Figure 2). The median follow-up time was 38 months (range 18 to 90).

At the time of reporting 11 patients are alive. Both patients with Wiskott Aldrich syndrome are well with satisfactory immune function and normal platelet counts. Patient IUBMID 194 with chronic myeloid leukaemia failed to engraft following a non-myeloablative preparative regimen and a relatively low cell dose received autologous marrow infusion in an effort to save him from life-threatening bleeding episodes. He is alive with disease and presently treated with imatinib mesylate. Only two out of 12 patients with leukaemia are alive while 5 out of 7 patients with thalassaemia are cured of their disease. The patient who was transplanted successfully for severe aplastic anaemia had beta thalassaemia major as his underlying disease who had failed two matched sibling donor HSCT.

DISCUSSION

Experience from various centres show that CBT is a viable option in the unrelated donor setting[6-9]. The rapid availability and ease of procurement make UCBT particularly advantageous[10]. Malaysia is a developing country where its largest ethnic group, the Malays are not represented in most bone marrow donor registries across the world. The establishment of cord blood banks worldwide has made possible the use of 1 to 2 antigen mismatched cord blood units for this ethnic group. Chinese patients although more likely to find bone marrow donors in the large Tzu Chi Bone

Table I: Patient characteristics

IU BMID	Age(yr)	Disease	Conditioning	CB Bank	NC dose x 10^7/kg	CD34 dose x 10^6/kg	Day Engrafted	HLA No. MM	AGVHD grade	Outcome
133	3	Osteopetrosis	BU16 CY120 ATG90	New York	6.05	0.23	None	1	NA	Died of disease Day 286
194	12	CML in CP	FLU150 BU8 ATG40	Melbourne	3.24	0.048	None	2	NA	Alive with disease
197	2	WAS	FLU150 BU8 CY100 ATG40	Milan	8.29	0.129	32	2	3	Alive and well
203	1	JMML	BU21 ARA-C12 MEL140	Sydney	7.26	0.08	36	2	3	Died, renal failure after severe gastroenteritis Day 270
212	2	ALL CR2	CY120 TBI 13.2 Gy	Dusseldorf	10.32	0.33	28	3	4	Died Fusarium brain abscess Day 93
216	6	AML CR3	BU16 CY200	Singapore	5.18	0.21	NE	1	NA	Died Staphylococcal sepsis Day 9
225	8	JMML	CY120 TBI 13.2 Gy	San Diego	3.4	0.2	28	2	3	Died multi-organ failure Day 43
233	6	AML 2° MDS	BU16 MEL180 ATG90	Stemcyte	12.8	0.21	29	2	3	Alive in remission
235	10	AML refractory	BU16 CY200	New York	6	0.246	NE	1	NA	Died MRSA sepsis Day 8
236	0.7	WAS	BU16 BY120	Stemcyte	13.5	0.52	24	1	2	Alive and well
243	3	JMML	BU16 CY120 MEL140 ATG30	Stemcyte	12.59	0.27	none	2	NA	Died of disease Day 173
253	3	ALL Ph' chrom	CY120 TBI 13.2 Gy	Stemcyte	14.35	1.22	12	2	3	Died Pseudomonas sepsis Day 84
256	7	ALL CR2	CY120 TBI 13.2 Gy	Stemcyte	7.85	0.177	none	3	NA	Relapse Day 117 Died Day 166
258	6	ALL CR2	CY120 TBI 13.2 Gy	Stemcyte	4.00	0.10	36	2	4	Died Hepatorenal syndrome, MRSE Sepsis Day 40
263	10	ALL CR2	CY120, ATG90, TT10, TBI12 Gy	Tokyo	2.6	0.06	25	2	4	Died Hepatorenal syndrome aGVHD Day 56
269	3	Osteopetrosis	BU18 CY120, ALG30, TT10	Stemcyte	29.3	0.143	None	0	NA	Died Pulm haemorrhage MRSA sepsis Day 39
270	0.8	Osteopetrosis	BU16 CY120, ALG30, TT10	Stemcyte	17.6	0.42	None	2	NA	Died of disease Day 82
275	5	Thalassaemia major	BU18 CY200 ATG120	Stemcyte	3.3	0.2	17	1	1	Alive and well
279	1	Thalassaemia major	BU18 CY200 ATG120	Stemcyte	9.36	0.4	15	2	2	Alive and well
280	8	Thalassaemia major	BU18 CY200 ATG120	Stemcyte	10.7	0.41	None	2	NA	Alive with disease
231	8	Severe Aplastic Anaemia	FLU20 ATG90	Stemcyte	7.48	0.26	34	0	none	Alive and well
290	6	Thalassaemia major	BU18 CY200 ATG120	Stemcyte	NA	NA	NA	1	NA	Died Klebsiella pneumoniae sepsis Day -2
292	13	ALL CR3	CY120 ATG90 TBI12 Gy	Stemcyte	6.79	0.23	27	1	2	Alive and well
293	3	Thalassaemia major	BU18 CY200 ATG90	Stemcyte	5.2	0.11	23	2	2	Alive and well
299	5	Thalassaemia major	BU18 CY200 ATG120	Stemcyte	5.1	0.18	NE	1	NA	Died Pseudomonas sepsis Day 11
302	1	Thalassaemia major	BU18 CY200 ATG120	Stemcyte	5.2	0.11	14	2	2	Alive with AIHA

Abbreviations:
BU=busulphan, CY=cyclophosphamide, ATG=anti-thymocyte globulin, FLU=fludarabine, MEL=melphalan, ARA-C=cytosine arabinoside, TBI=total body irradiation, TT=thiotepa, MM=mismatch, NA=not applicable, NE=not evaluable, AIHA=autoimmune haemolytic anaemia.

Table II: Engraftment and Cell Doses

Groups Median (interquartile range)	Engrafted	Non-engrafted	p-value
NC dose x10^7kg	7.3 (4.0-10.3)	6.9 (5.2-13.8)	0.617
CD34+ dose x10^5kg	2.0 (1.1-3.3)	2.2 (1.7-3.0)	0.657

Table III: GVHD and HLA mismatch (n=15):

Acute GVHD	Number of HLA mismatch		
	0	1	≥2
Yes	0 (0%)	4 (100%)	10 (100%)
No	1 (100%)	0 (0%)	0 (0%)

p-value = 0.017 (Extended Mantel-Haenszel chi-square test for analysis of trend)

Table III: GVHD and HLA mismatch (n=15):

Chronic GVHD	Number of mismatch		
	0	1	≥2
Yes	0 (0%)	1 (25%)	3 (30%)
No	1 (100%)	3 (75%)	7 (70%)

p-value = 0.788 (Extended Mantel-Haenszel chi-square test for analysis of trend)

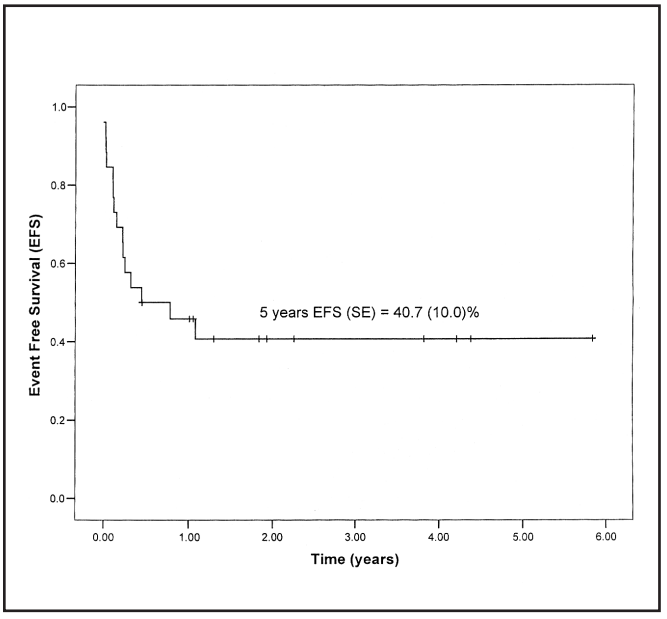

Fig. 1: Overall event free survival (EFS) of patients treated with unrelated cord blood transplantation

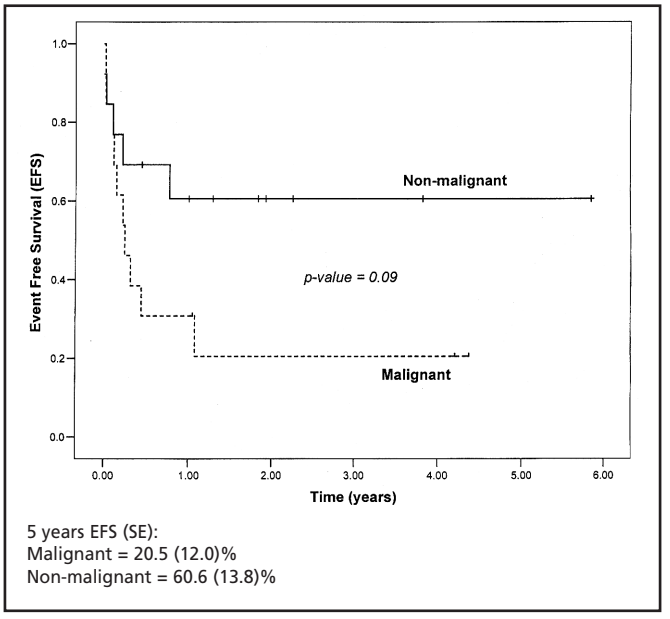

Fig. 2: 5-year Event free survival (EFS) of patients with malignant vs non-malignant conditions after UCBT

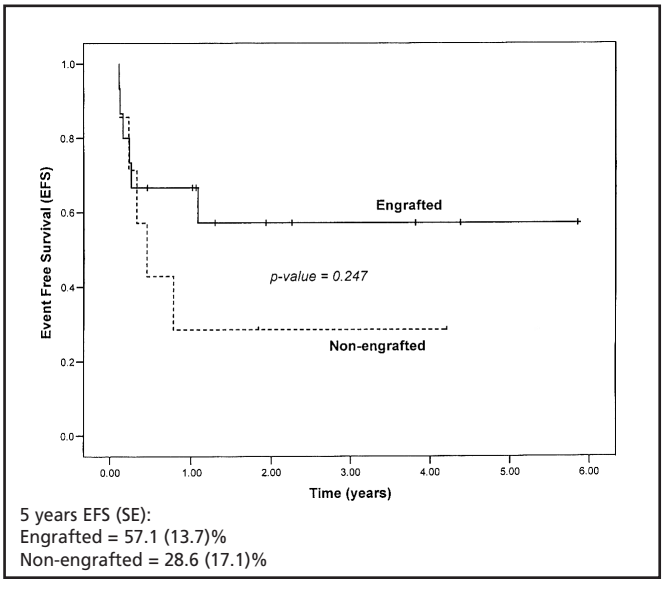

Fig. 3: 5-year Event Free Survival of Engrafted and Non-engrafted patients

Marrow Donor Registry in Taiwan or elsewhere in Singapore or Hong Kong, may not have the time needed to search for a well-matched bone marrow donor. Hence the availability of large cord blood registries of Chinese ethnicity as found in the Stemcyte Taiwan Registry and Tzu Chi Cord Blood Registry makes searches highly rewarding. Eleven out of the 13 patients who were transplanted for leukaemia were in their second or third clinical remission and there was great urgency to consolidate their remissions with transplantation.

One of the disadvantages of using CB as a source of stem cells is the rate of graft failure. Primary graft failure rates of 12 – 20% have been reported[20,21]. Risk factors for graft failure include inadequate cell dose, HLA incompatibility and non-myeloablative preparative regimen. Seven out of 22 (32%) evaluable patients failed to engraft. Three of these occurred in patients with osteopetrosis, a condition particularly associated with a high rate of non-engraftment when cord blood is the stem cell source[22]. The patient who received non-myeloablative conditioning failed to engraft and also failed to show autologous recovery.

It has been suggested that a CD34+ cell dose of <1.7 x 10^5/kg should be considered inadequate for transplantation[23] while a nucleated cell dose of >3.7 x 10^7/kg was associated with a lower transplant related mortality[24]. It is difficult to be certain what absolute minimum number of nucleated cells is needed for engraftment especially in relation to other factors like primary disease, HLA disparity and conditioning regimen. It would seem unwise to use a nucleated cell dose lower than 1.5 x 10^7/kg.[23]. In our cohort, four patients received a nucleated cell dose of <3.7 x 10^7/kg with the lowest dose of 2.6 x 10^7/kg but they all achieved engraftment apart from the one patient who received non-myeloablative conditioning.

Cord blood transplantation is also associated with a slower rate of engraftment translating to a higher incidence of infection and transplant related mortality. A study reported a 100-Day infection related mortality rate of 30%[25]. In our study 4 out of 26 patients (15%) died from bacterial infections within the first two weeks of transplantation while a further three patients died from infection in association with severe GVHD. It is uncertain if the lack of high efficiency particulate air filtration in our transplant environment contributed to this infection rate.

The risk of GVHD in UCBT is reported to be low despite HLA mismatching. In unrelated bone marrow transplantation using 0 and 1 antigen mismatched donors, grade III-IV acute GVHD was reported at 32% and 49% respectively[26]. By comparison, use of unrelated cord blood disparate at 0, 1 and 2 antigens resulted in similar grade acute GVHD occurring in only 11% of recipients in one study[23]. Grade II to IV acute GVHD occurring at 14 to 36%[11,27] have been reported with the use of UCB. We observed acute GVHD in all (93%) but one patient who engrafted. The solitary patient who did not experience GVHD received a 6/6 antigen matched CBU. Five patients experienced grade II GVHD of the skin which responded to systemic steroids. Eight patients developed grade III to IV GVHD of the skin and gastro-intestinal tract, necessitating treatment with systemic steroids, infliximab and mycophenolate mofitil. Graft versus host disease and its treatment contributed to the deaths in five out of these eight patients.

Rubinstein reported that chronic GVHD occurred in 25% of patients[9] but rates as low as 10% have also been observed[23]. We noted three patients with chronic GVHD (30%) out of the evaluable 10 who lived beyond 100 days post-transplantation but the grade of chronic GVHD was limited and easily controlled with oral steroids in two of these patients.

Results of unrelated cord blood transplantation for malignant disorders in our patients showed that the overall survival was only 20.5%. This could be attributed to the status of disease at the time of transplant as most of our patients were beyond their first clinical remission and were at high risk of relapse. Patients with poor-risk disease at transplantation consistently showed poorer survival compared with good risk disease[23, 28].

To improve outcome for patients with malignant disorders, we would have to identify patients with poor risk disease much earlier and offer them transplantation before they experience relapse. The five year disease free survival for patients with non-malignant disorders was 60.6%. Our results with thalassaemia patients showed that the use of 1 to 2 antigen mismatched unrelated cord blood donors was feasible, giving an overall survival of 71% and a cure rate of 57%. All the thalassaemia transplants were performed for Chinese patients where suitable CBUs were available from registries in Taiwan. In Malaysia where prenatal diagnosis and termination of pregnancy for fetuses affected by thalassaemia major is not allowed, the role of UCBT for thalassaemics without sibling donors is expected to increase. Experience with this source of stem cells remains limited with reports mainly from East Asian countries like Taiwan, Thailand and Singapore[29-31].

In conclusion, UCBT is a viable option for children with both malignant and non-malignant disorders. We encountered a high incidence of non-engraftment and acute graft-versus-host disease. Cord blood units with high nucleated cell doses and better HLA match would help reduce the risk of non-engraftment. Overall survival was affected by disease status at the time of transplantation hence patients with high risk leukaemia should be offered transplantation earlier while those with non-malignant diseases should be transplanted before complications from their primary diseases occur.

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