

Single centre experience of umbilical cord stem cell transplantation for primary immunodeficiency

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Summary:

Primary immunodeficiencies (PID) are an important cause of childhood mortality. Haematopoietic stem cell transplantation (HSCT) is the best treatment for many PID. Umbilical cord stem cells are an alternative source of HSC. There is little data regarding outcome of umbilical cord stem cell transplantation (UCSCT) for PID. Our single centre experience is reported. A retrospective study of 14 of 148 patients transplanted for PID, who have received 15 UCSCT was performed, with specific regard to graft-versus-host disease (GvHD) and immune reconstitution. Eight patients with severe combined immunodeficiency (SCID), and six with other combined immunodeficiencies were treated. Of the patients, 12 received unrelated cords, and two had sibling transplants. Median age at transplant was 3.5 months, median nucleated cell dose was 0.8×10^8 /kg. All engrafted. Median time to neutrophil engraftment was 22 days, median time to platelet engraftment was 51 days. One developed significant grade III GvHD post transplantation. In total, 11 patients had full donor T and six full donor B-cell chimerism, six of nine patients >1 year post-BMT had normal IgG levels and specific antibody responses to tetanus and Hib vaccines; two are being assessed. Two patients died of multi-organ failure related to pre-existing infection and inflammatory complications respectively. UCSCT should be considered for patients requiring stem cell therapy for PID.

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Primary immunodeficiencies (PID) are a rare but important cause of mortality and morbidity in childhood. Untreated, the most severe disorders, known collectively as severe combined immunodeficiency (SCID), are invariably fatal within the first year of life. International registry data have demonstrated that other less immediately life-threatening PID have a poor long-term outlook.^{1–3} Haematopoietic stem cell transplantation (HSCT) is the best treatment for most SCID disorders, and is becoming increasingly used for other PID. HSCT using an HLA-matched sibling donor or from an HLA-matched volunteer gives good results, with over 80% cure for patients with SCID and over 70% cure for patients with other PID.⁴ For patients with SCID, haploidentical HSCT from a parent donor is increasingly successful, with almost 80% of patients cured.⁴ However, immune recovery is quicker if replete, rather than T-cell depleted marrow is used.⁵

Haematopoietic stem cells are usually harvested directly from bone marrow or from G-CSF-mobilised peripheral blood stem cells. Umbilical cord stem cells are an alternative source. Since the introduction of umbilical cord stem cell transplantation (UCSCT) in 1988, there have been more than 3500 adult and paediatric cord blood transplants from related and unrelated donors mainly for malignancy and other haematological disorders.⁶ Cord blood transplantation has several advantages including ready availability of the donor unit, no risk to donor, low rate of viral contamination, low risk of graft-versus-host disease (GvHD), tolerance of 1–2 HLA mismatch and ease of arranging a date for transplantation.⁷ The results of treatment of haematological disorders and malignancy are as good as those for which whole marrow is the stem cell source.⁸ There is little information regarding outcome of UCSCT for PID. Although PID's make up a small proportion of disorders amenable to treatment by HSCT, cord blood is an ideal source for many of these patients as many patients are small and so the limited stem cell dose in a cord blood unit is often adequate. Furthermore, transplants often need to be arranged urgently, particularly for SCID, and GvHD is an unwanted complication (unlike in haematological malignancy where a graft-versus-leukaemia effect is encouraged). A number of individual case reports have been published but only one small case series

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describing the outcome of UCSCT for primary immunodeficiency in eight patients.^{9–14} We report our 5-year single centre experience using UCSCT for patients with primary immune deficiency.

Methods

In total, 148 patients, with primary immune deficiency, 72 with SCID disorders, have been transplanted at our centre since 1987. Between May 2000 and December 2004, 14 of these patients have received 15 UCSCT (Table 1). We performed a retrospective study of all the cord blood transplants performed in our centre for PID.

Patients

Patients were considered for unrelated donor cord blood transplantation if an HLA-matched sibling donor was not available. HLA matching between patient and umbilical cord blood donor was based on serological class I HLA typing and high-resolution molecular class II typing.

Conditions treated with UCSCT included adenosine deaminase deficiency (4), T-B + NK + SCID (undefined molecular defect) (2), reticular dysgenesis (1), chronic granulomatous disease (1), Wiskott-Aldrich syndrome (one patient, two cords), MHC class II deficiency (1), Omenn's syndrome (1), IPEX syndrome (1), undefined SCID (1), and severe immunodeficiency with CHARGE association (1).

Cytoreductive conditioning regimen and GvHD prophylaxis

The conditioning regimens used were in accord with the guidelines of the European Blood & Marrow Transplant Group and EBMT Working party for Inborn Errors. Seven patients received Busulphan 4 mg/kg/day from D–10 to D–7 (total dose 16 mg/kg) and Cyclophosphamide 50 mg/kg/day from D–5 to D–2 (total dose 200 mg/kg) with Cyclosporine from D–1. Two patients received reduced intensity conditioning with alemtuzumab 0.1 mg/kg/day (total dose 0.5 mg/kg), Fludarabine 30 mg/m²/day from D–7 to D–3 (total dose 150 mg/m²) and melphalan

Table 1 Characteristics of patients undergoing UCSCT for PID

Patient sex	Age at BMT (months)	Diagnosis	Infection	Conditioning	HLA match source	Nucleated cell dose × 10 ⁸ /kg	CD34+ cell dose × 10 ⁶ /kg	GvHD (Grade)
1 M	3	OS	PCP	Alemtuzumab Flu/Mel	10/10 MUD	3.1	1.45	Skin, gut (II) VOD
2 F	1.5	T-B + NK + SCID	Nil	Bu16/Cy200	10/10 MUD	0.8	0.16	Nil
3 F	7	MCH II	PCP	Alemtuzumab Flu/Mel	9/10 A mismatch MUD	0.447	0.242	Nil
4 M	1.5	RD	Nil	Alemtuzumab Bu16/Cy200	10/10 MUD	1.6	1.2	Nil VOD
5 F	2.5	ADA	PFIII	Nil	6/6 MUD	2	0.6	Skin, gut (III)
6 M	1.5	ADA	PCP	Nil	12/12 MUD	1.32	0.36	Nil
7 M	10	WAS	AdV I	ATG Bu16/Cy200	10/10 MUD 6/6 MUD	0.578 0.41	0.09 0.017	Skin (I)
8 M	36	CGD	Nil	Bu16/Cy200	12/12 Sibling	0.16	0.07	Nil
9 F	4	ADA	SRSV	Nil	12/12 MUD	1.25	0.7	Nil
10 M	0.25	T-B + NK + SCID	Nil	Nil	12/12 Sibling	0.6	0.17	Nil
11 M	7	IPEX	Nil	Alemtuzumab Bu16/Cy200	9/10 C mismatch MUD	1.8	0.6	Nil
12 M	3	ADA	Nil	Bu16/Cy200	9/10 C mismatch MUD	0.58	0.29	MFE
13 F	5	Undefined SCID	Nil	Alemtuzumab Bu16/Cy200	10/10 MUD	0.36	0.067	Pneumonitis
14 M	4	CHARGE association	Nil	Alemtuzumab	10/10 MUD	2.1	1.8	Nil

OS = Omenn syndrome; SCID = severe combined immunodeficiency; MHC II = MHC class II deficiency; RD = reticular dysgenesis; ADA = adenosine deaminase deficiency; WAS = Wiskott-Aldrich syndrome; CGD = Chronic Granulomatous disease; IPEX = immunodysregulation, polyendocrinopathy, enteropathy X-linked; PCP = pneumocystis carinii pneumonia; PFIII = parainfluenzae type III; AdV 1 = adenovirus serotype 1, SRSV = small round structured virus; Flu/Mel = fludarabine/melphalan; Bu = busulphan; Cy = cyclophosphamide; MFE = maternofetal engraftment.

140 mg/m² on D-2. Five patients did not receive cyto-reductive conditioning. Cyclosporin was commenced on D-1 in all patients as GvHD prophylaxis. A total of 10 patients received Methylprednisolone 2 mg/kg/day from D+5, continued until D+19 and subsequently weaned by 25% every other day.⁸

Two patients received direct sibling UCSCT, 12 received unrelated donor UCSCT. Eight cord units were well matched (four at 12/12, six at 10/10 [A, B, C, DR, DQ] HLA loci). Two units were matched at 6/6 (A, B, DR) HLA loci. Three of the cord units had a single major antigen mismatch (9/10, 1 HLA-A and 2 HLA-C locus mismatches). The cord blood cells were thawed and washed following standard procedures.¹⁵

Chimerism studies

T and B cells were separated from whole blood using the AutoMACS[®] system (Miltenyi Biotec, Cologne). Following extraction of DNA, short tandem repeat (STR) length polymorphism on four different STR markers was measured using multiplex PCR with fluorescent primers. Quantitation was determined by assessment of the area under the peak on a fragment analyser, with a lower limit of detection of 1-2% of the minor allele.

Results

In 12 patients, where an unrelated cord was used, the median time lag between identifying a suitable cord and requesting the stored cord for transplant was 8 days (range 2-50 days). In one patient, where two UCSCTs were used, the time lag between requesting and infusing the second UCSCT was 2 days. Median age at transplant was 3.5 months (range 7 days-3.5 years). Nucleated cell doses ranged from 0.16 to 3.1 × 10⁸/kg (median 0.8 × 10⁸/kg). CD 34+ stem cell doses ranged from 0.017 to 1.8 × 10⁶/kg (median 0.3 × 10⁶/kg). Median time to neutrophil engraftment (neutrophils >0.5 × 10⁹/l) was 22 days

(range 12-37). Median time to platelet engraftment (platelets > 50 × 10⁹/l) was 51 days (range 22-175). Median time to T lymphocyte engraftment (T cells >200 cells/μl) was 61 days (range 10-222) (Table 2). All patients had evidence of donor alleles on molecular genetic studies post transplantation.

One patient developed significant (Grade III-IV) biopsy proven GvHD post transplantation with skin and gut GvHD grade III (patient 5). Additionally, one had skin and gut GvHD grade II (patient 1). Both had received fully HLA-matched UCSCT (10/10 and 6/6 respectively). Patient 1 developed a post-GvHD enteropathy and required parenteral nutrition; patient 5 died from multi-organ failure related to pre-existing viral infection and GvHD.

In all, 11 patients had full donor T-cell chimerism. Six had full donor B-cell chimerism, and three had mixed B-cell chimerism. Four patients with conditions difficult to engraft, namely MHC II deficiency, reticular dysgenesis, Omenn's syndrome and IPEX syndrome, have engrafted successfully, the latter three having full donor chimerism in all cell lineages. Chimerism has been stable to date with a median follow-up of 2 years (range 0.5-6).

Six of eight patients who discontinued IVIG >1 year post-BMT had normal IgG levels and normal specific antibody responses to tetanus and Hib vaccines; two are currently being assessed. One currently remains on IVIG after receiving treatment for GvHD. Three continue to receive IVIG as they have been transplanted recently. In all, 12 of the 14 patients (86%) achieved immune reconstitution and cure of the underlying immunodeficiency.

Two patients (patients 1 and 4) developed severe veno-occlusive disease, which resolved with treatment with defibrotide. Two patients with late diagnosed ADA-deficient SCID died. One (patient 5) was admitted on mechanical ventilation with respiratory failure secondary to parainfluenza 3 pneumonitis died post transplant of multi-organ failure related to pre-existing infection and GvHD. The other (patient 12) died of multi-organ failure related to pre-existing inflammatory complications.

Table 2 Immune Reconstitution of patients undergoing UCSCT for PID

Patient	Days to Neut >0.5	Days to platelet >50	Days to T cell >200	T-cell engrafted	B-cell engrafted	Myeloid engrafted	Igs	Tet/Hib	Outcome
1	+13	+105	+67	D	D	D	IVIG	IVIG	Engrafted
2	+21	+36	+10	D	D	D	Ceased	Eval	Engrafted
3	+18	+16	+89	M	M	M	Ceased	Eval	Engrafted
4	+19	+49	+168	D	D	D	N	N	Engrafted
5	NC	NC		D	R		IVIG	IVIG	Engrafted died
6	NC	NC	+95	D	R	D	N	N	Engrafted
7	+35/+27	+133/+125	+46/+38	D					Engrafted
8	+37	+51	+55	D	M	M	N	N	Engrafted
9	NC	NC	+12	D	M	M	Low IgA	N	Engrafted
10	NC	NC	+24	D	M	M	N	N	Engrafted
11	+22	+44	+222	D	D	D	IVIG	IVIG	Engrafted
12	NA	NA	NA	—	—	D	—	—	Died
13	+30	+59	+158	D	D	M	IVIG	IVIG	Engrafted
14	NM	NM	+46	D	R	R	IVIG	IVIG	Engrafted

NC = not conditioned; NM = non-myeloablative; NA = not available; D = donor; R = recipient; M = mixed; Eval = evaluating; N = normal.

Discussion

Umbilical cord stem cells are an alternative stem cell source for HSCT. Results of transplantation for nonPID disorders are widely published. There is little detailed information regarding the outcome of UCSCT for PID. In this single centre report, 9.5% of our patients transplanted for PID have received UCSCT, 86% from unrelated donors.

The advantages of UCSCT have been previously described, but speed of access to the cord unit and ease of arranging a bone marrow transplant are important considerations in the PID setting, where for SCID, a BMT should be performed as soon as possible. In this series, the median time between identifying a cord from the registry and requesting the cord to be transferred to the centre for use was 8 days, a time period considerably shorter than the 4–6 weeks that it takes to receive marrow from a matched unrelated donor.

Disadvantages of UCSCT include nonavailability of the donor for a boost BMT, lack of viral specific cytotoxic T cells, slower engraftment and small stem cell dose.¹⁶ The disadvantages of small stem cell dose or nonavailability of donor for a 'boost' infusion may be overcome by the use of more than one cord infusion – one patient in our series received 2 units 8 days apart, because of low stem cell dose in the initial unit in the face of disseminated viral infection.¹⁷ Although initially trimeric, one cord has subsequently predominated. While the use of multiple cord units given simultaneously will increase the stem cell dose for larger patients, and so aid engraftment,¹⁸ patients requiring a boost infusion or DLI for failing chimerism some weeks after the initial transplant cannot use the original donor. The *ex vivo* expansion of cord blood progenitor cells before UCSCT may overcome this, and further research in this area is urgently required.

Patients with SCID are ideal candidates for UCSCT as the stem cell dose from a UCSC unit is more likely to be adequate, given the presentation in infancy, often with associated failure to thrive secondary to viral enteritis and secondary malabsorption. Cord blood units tend to be used on the basis of total nucleated cell rather than CD34+ stem cell dose. However, the CD34+ stem cell dose may be much lower than suggested by the nucleated cell dose, particularly as nucleated red blood cells may falsely elevate the total nucleated cell dose. Recommended CD34+ stem cell doses for cord blood transplant are $\geq 1.7 \times 10^5$ /kg, as lower doses are associated with slow, poor engraftment.⁸ Despite this, all our patients engrafted in spite of extremely small stem cell doses in some.

The slow immune reconstitution is a potential risk, particularly in the face of persisting viral infection that is more likely in PID. It is recognised that lymphocyte reconstitution is slower following UCSCT than replete marrow.¹⁹ An advantage to this may be a more 'gentle' engraftment with less risk of organ inflammation due to primed T cells reacting against inflamed virally infected tissue, leading to pneumonitis and GvHD. However, a disadvantage in the setting of ongoing viral infection is a longer time to anti-viral T-cell activity, and hence slower viral clearance, and a longer time period in which pre-existing viral-induced end-organ damage may progress.

Despite this, we have successfully transplanted one patient with disseminated adenovirus infection,¹⁷ which is historically associated with a poor outcome.

The most commonly used alternative haematopoietic stem cell source in patients who lack an HLA-matched sibling donor, particularly for those patients with SCID, is haplo-identical CD34+ stem cells, usually from a parent. Lymphoid reconstitution takes at least 120 days in this situation, before recent thymic emigrants are found in peripheral blood,⁵ leaving the patient vulnerable to pre-existing viral infection and end-organ damage. Comparison of our cohort of patients undergoing UCSCT compares favourably with previously published data on the outcome of transplantation using haplo-identical T-cell depleted marrow. In our historical published series of SCID patients receiving marrow stem cells, depleted of T cells using CAMPATH-1M and complement, 49% of 37 transplant episodes achieved engraftment, with 63% survival overall,²⁰ compared to 86% survival and cure in this report. Our current results also compare favourably with published pooled European data on the outcome of HLA-mismatched transplants for SCID between 1996 and 1999, with a survival of 77%.⁴

Of the two patients in this series who died, one was admitted on full mechanical ventilatory support for severe parainfluenza type 3 pneumonitis, and one had severe materno-fetal engraftment with GvHD and pneumonitis at presentation. These patients would fall into the high-risk group of patients with SCID in whom mortality has been historically very high. In contradistinction, two patients who were high risk for poor outcome, with Omenn syndrome and reticular dysgenesis, respectively, were successfully transplanted and have 100% donor chimerism.

The incidence of severe (Grade III–IV) transplant-related GvHD in our series is low (1/14). Interestingly, the three patients with a major HLA locus mismatch (1 A, 2 C locus mismatch) did not develop transplant-related GvHD. HLA matching is reported to be less important in UCBSCT, and GvHD less likely in the face of major HLA mismatches, compared with whole marrow transplants. However, in PID (unlike haematological malignancy), GvHD confers no benefit, and in the presence of pre-existing viral infection it contributes to a poorer outcome. For that reason, we have tended to use well-matched cord blood units, and our low GvHD results reflect this.

In T-cell immunodeficiencies, the goal of HSCT is to achieve full immune reconstitution. In six of our patients who have immuno-reconstituted sufficiently to evaluate, all have antibody responses to protein antigens. Donor chimerism has remained stable over time in all the patients, with no loss of graft. Results of HSCT for PID have been improving progressively – results for a matched unrelated donor transplant for SCID are 63%, and for other combined immunodeficiencies 42%.⁴ UCSC are a viable alternative stem cell source and although this series is small, our figures are at least comparable. Our policy for patients with SCID, in the absence of an HLA-identical sibling, is to look for a suitable umbilical cord stem cell unit through the national and international registries. Final decisions are based on the extent of pre-existing viral infection, end-organ damage, available stem cell dose/kg body weight and

degree of HLA matching. We now prefer a 10/10 HLA-antigen match, but are increasingly willing to accept a single major HLA class I antigen mismatch. In conclusion, cord blood stem cells are a suitable alternative to bone marrow and should be considered for patients requiring stem cell therapy for PID.

References

- 1 Winkelstein JA, Marino MC, Johnston Jr RB *et al*. Chronic granulomatous disease. Report on a national registry of 368 patients. *Medicine (Baltimore)* 2000; **79**: 155–169.
- 2 Winkelstein JA, Marino MC, Ochs H *et al*. The X-linked hyper-IgM syndrome: clinical and immunologic features of 79 patients. *Medicine (Baltimore)* 2003; **82**: 373–384.
- 3 Sullivan KE, Mullen CA, Blaese RM, Winkelstein JA. A multi-institutional survey of the Wiskott-Aldrich syndrome. *J Pediatr* 1994; **125**: 876–885.
- 4 Antoine C, Muller S, Cant A *et al*. Long-term survival and transplantation of haemopoietic stem cells for immunodeficiencies: a report of the European experience 1968–99. *Lancet* 2003; **361**: 553–560.
- 5 Muller SM, Kohn T, Schulz AS *et al*. Similar pattern of thymic-dependent T-cell reconstitution in infants with severe combined immunodeficiency after human leukocyte antigen (HLA)-identical and HLA-nonidentical stem cell transplantation. *Blood* 2000; **96**: 4344–4349.
- 6 Cohen Y, Nagker A. Umbilical cord blood transplantation – how, when and for whom? *Blood* 2004; **18**: 167–179.
- 7 Gluckman E, Rocha V, Chevret S. Results of unrelated umbilical cord blood hematopoietic stem cell transplant. *Transfus Clin Biol* 2001; **8**: 146–154.
- 8 Wagner JE, Barker JN, Defor ET *et al*. Treatment of unrelated donor umbilical cord blood in 102 patients with malignant and non-malignant diseases: influence of CD34 cell dose and HLA disparity on treatment-related mortality and survival. *Blood* 2002; **100**: 1611–1618.
- 9 Knutsen AP, Wall DA. Umbilical cord blood transplantation in severe T-cell immunodeficiency disorders: two-year experience. *J Clin Immunol* 2000; **20**: 466–476.
- 10 Benito A, Diaz MA, Alonso F *et al*. Successful unrelated umbilical cord blood transplantation in a child with Omenn's syndrome. *Pediatr Hematol Oncol* 1999; **16**: 361–366.
- 11 Bonduel M, Pozo A, Zelazko M *et al*. Successful related umbilical cord blood transplantation for graft failure following T cell-depleted nonidentical bone marrow transplantation in a child with major histocompatibility complex class II deficiency. *Bone Marrow Transplant* 1999; **24**: 437–440.
- 12 Fagioli F, Biasin E, Berger M *et al*. Successful unrelated cord blood transplantation in two children with severe combined immunodeficiency syndrome. *Bone Marrow Transplant* 2003; **312**: 133–136.
- 13 Bhattacharya A, Slatter M, Curtis A *et al*. Successful umbilical cord blood stem cell transplantation for chronic granulomatous disease. *Bone Marrow Transplantation* 2003; **31**: 403–405.
- 14 Knutsen AP, Steffen M, Wassmer K, Wall DA. Umbilical cord blood transplantation in Wiskott-Aldrich syndrome. *J Pediatr* 2003; **142**: 519–523.
- 15 Rubinstein P, Dobrila L, Rosenfield RE *et al*. Processing and cryopreservation of placental/umbilical cord blood for unrelated bone marrow reconstitution. *Proc Natl Acad Sci USA* 1995; **92**: 10119–10122.
- 16 Filipovich AH. Unrelated cord blood transplantation for correction of genetic immunodeficiencies. *J Pediatr* 2001; **138**: 459–461.
- 17 Slatter MA, Bhattacharya A, Barge D *et al*. Use of two unrelated umbilical cord stem cell units in stem cell transplantation for Wiskott-Aldrich Syndrome. *Pediatr Blood Cancer* (in press).
- 18 Barker JN, Weisdorf DJ, DeFor TE *et al*. Transplantation of 2 partially HLA-matched umbilical cord blood units to enhance engraftment in adults with hematologic malignancy. *Blood* 2005; **105**: 1343–1347.
- 19 Dalal I, Reid B, Doyle J *et al*. Matched unrelated bone marrow transplantation for combined immunodeficiency. *Bone Marrow Transplant* 2000; **25**: 613–621.
- 20 Gennery AR, Dickinson AM, Brigham K *et al*. CAMPATH-1M T cell depleted bone marrow transplantation for severe combined immunodeficiency: long-term follow up of 19 children treated in the period 1987–1998 in a single centre. *Cytotherapy* 2001; **3**: 221–232.