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ORIGINAL ARTICLE

Changes in the incidence, patterns and outcomes of graft failure following hematopoietic stem cell transplantation for Hurler syndrome

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Hematopoietic stem cell transplantation (HSCT) is the standard of care in children with Hurler syndrome (HS) as it is the only therapy that can arrest disease progression. We examined the incidence, patterns and outcomes of graft failure in all HS children undergoing first HSCT at the Royal Manchester Children's Hospital or the University of Minnesota Children's Hospital from 1983 to 2016. Implementation of busulfan pharmacokinetic monitoring started in 2004 in both institutions. Two hundred and forty HS children were included in this analysis (historical era (pre-2004), $n = 131$; current era (post 2004), $n = 109$). The proportion of patients with graft failure was significantly lower in the current era compared with the historical era (37.2% vs 10.1%, respectively). Of 49 patients with graft failure in the historical era, 1 had aplasia and 48 had autologous reconstitution. All the 11 graft failures of the current era occurred in recipients of cord blood transplants (7 aplasia and 4 autologous reconstitution). The outcomes of second transplant in these patients has improved, with 89% of such patients alive and engrafted in the current era compared with 58% in the historical era. The pattern of graft failure has changed from autologous reconstitution, likely secondary to inadequate myelosuppression in the historical era, to aplasia in the current era, likely due to imperfect immunosuppression.

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INTRODUCTION

Hurler syndrome (HS), the most severe phenotype of mucopolysaccharidosis type I, is characterized by α-L-iduronidase deficiency and has an estimated incidence of 1 in 100 000 newborns.[1] HS is a severe, life-threatening and multi-system disorder and its morbidity is substantial, as affected children exhibit cardiac, respiratory, skeletal, central nervous system, auditory and ophthalmologic dysfunction. Without therapy, most affected children die in the first decade. Hematopoietic stem cell transplantation (HSCT) is the mainstay of treatment in HS, often augmented by peri-transplant intravenous enzyme replacement therapy (ERT). Together, they aim to reverse the physical features, ameliorate organ dysfunction and arrest neurodegeneration resulting from the disease. The remarkable ability of donor-derived hematopoietic cells to secrete functional α-L-iduronidase for the metabolic cross-correction of both central nervous system and somatic disease makes HSCT the standard-of-care intervention in HS. Although ERT has emerged as an effective way to address some somatic disease, the currently available recombinant enzyme product is unable to effectively cross the blood–brain barrier.[2,3]

Since the first successful HSCT for HS in 1980, more than 600 children with HS have been transplanted worldwide; it remains the most common metabolic indication for HSCT. Initially, the major obstacles of HSCT in HS were high rates of graft failure and transplant-related mortality. In early reports, 5-year overall survival (OS) rates ranged between 50 and 80%, while 5-year engrafted survival ranged between 30 and 80%.[4–7] However, transplant outcomes in HS have improved dramatically over the past 10 years. In a recent series of 62 children with mucopolysaccharidosis (most with HS) transplanted at two expert European centers, the OS and engrafted survival estimates at a median follow-up of 3 years were 95.2% and 90.3%, respectively.[8] Collaborative studies identified the risk factors for graft failure and led to improved transplant strategies for this patient population such as the abandonment of *ex vivo* allograft T-cell depletion and the adoption of busulfan pharmacokinetic monitoring in myeloablative regimens.[9–11] In addition, it has previously been shown that the use of cord blood as an alternative donor cell source is associated with a lower incidence of mixed chimerism in engrafted recipients.[10,12] Finally, mounting evidence suggests favorable engrafted survival outcomes with the addition of peri-transplant ERT.[8,13]

In light of the significant improvements and shifts in transplant care for HS over time, this retrospective study aimed to examine the changing incidence and patterns of graft failure and its risk factors in the modern era of HSCT for HS.

PATIENTS AND METHODS

From January 1983 to December 2015, a total of 240 HS children underwent first HSCT at the Royal Manchester Children's Hospital or the University of Minnesota Children's Hospital. The diagnosis of HS was confirmed by an increase in urinary GAG excretion, a deficiency or absence of α-L-iduronidase in the peripheral blood leukocytes and the clinical phenotype. The clinical and laboratory data were retrieved from

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transplantation databases at Royal Manchester Children's Hospital and University of Minnesota Children's Hospital, patients' medical files and laboratory records. Written informed consent was obtained from the parents or legal guardians of the patients. We defined and compared two eras of patients. The historical era comprised patients transplanted before 2004. Thereafter, busulfan pharmacokinetic monitoring was introduced in each institution and a current era was defined as patients transplanted since 2004. From 2004 onwards, pre-transplant ERT was administered at 100 U/kg (0.58 mg/kg), typically at least 6 weeks before the transplant and continued until engraftment was established.

Donor selection, conditioning regimen, supportive care and monitoring at Royal Manchester Children's Hospital

Before 2005, the donor selection was based on donor availability from family members and registries. Following implementation of European group for Blood and Marrow Transplantation guidelines for HSCT in mucopolysaccharidosis patients in 2005, the donor hierarchy was as follows: (i) non-carrier HLA-matched family donor, (ii) matched unrelated cord blood, (iii) matched unrelated donor. If these donors were not available, a carrier HLA-matched family donor or a mismatched, unrelated cord blood were used. For matched sibling donors and matched unrelated donors, high-resolution matching at HLA-A, B, C, DRB1 and DQB1 was performed. For cord blood donors, matching is done at HLA-A and B at intermediate resolution and DRB1 at high-resolution level.

In the historical era, various conditioning regimens were used, with the majority of patients undergoing conditioning with oral busulfan (14–20 mg/kg) and IV cyclophosphamide (CY, 200 mg/kg). Busulfan with pharmacokinetic monitoring and CY were used from December 2004 to July 2009. Busulfan was administered IV for 4 consecutive days targeting an area under the curve (AUC) exposure of 80 to 90 mg · h/L based on therapeutic drug monitoring. CY was dosed at 50 mg/kg for 4 days and administered at least 24 h after busulfan. Thymoglobulin 2.5 mg/kg for 2 to 4 days was used with cord blood while alemtuzumab (0.2 mg/kg for 5 days in unrelated donor transplant; 0.1 mg/kg for 3 days in related donor transplant) was administered to recipients of marrow and peripheral blood grafts. From August 2009, the conditioning regimen was switched to busulfan and fludarabine (160 mg/m^2) with the same serotherapy protocol as the previous period. The switch to fludarabine was because of the potential and actually described cardiac toxicity associated with cyclophosphamide in HS and because of the reduced duration of neutropenia when fludarabine is substituted for cyclophosphamide.[14,15]

Standard GvHD prophylaxis consisted of cyclosporine A (CSA) in all patients. In recipients of cord blood transplant, prednisolone (1 mg/kg/day) was added from the day of transplant to Day +28. Methotrexate was added on days +3, +6 and +11 after HSCT in patients receiving no serotherapy as part of their conditioning regimen. Antimicrobial prophylaxis consisted of aciclovir, cotrimoxazole, itraconazole and penicillin. Chimerism monitoring was performed on whole blood after transplantation by variable number tandem repeats (1999–2002) analysis or short tandem repeats (since 2003) analysis.

Donor selection, conditioning regimen, supportive care and monitoring at University of Minnesota Children's Hospital

Before 2002, donor selection was based on donor availability from family members and registries. Since 2002, the donor hierarchy was generally as follows: (i) non-carrier HLA-matched family donor, (ii) matched unrelated cord blood, (iii) matched unrelated donor, (iv) mismatched unrelated cord blood and (v) carrier HLA-matched family donor or mismatched unrelated donor. For unrelated marrow grafts, donor–recipient matching was by allele-level typing at HLA-A, -B, -C and -DRB1. For unrelated cord blood grafts, matching was by antigen-level typing at HLA-A and -B, and allele-level typing at HLA-DRB1.

In the historical era, various conditioning regimens were used. From 1992 to 1997, patients were conditioned with oral busulfan (320 mg/m^2), IV CY (120 mg/kg) and TBI (750 cGy, unfractionated). From 1997 to 2003, patients received IV CY (120 mg/kg) TBI (1400 cGy, fractionated) and equine antithymocyte globulin (ATG, 90 mg/kg). From 2004 to 2014, patients generally received a conditioning backbone of serotherapy (equine ATG or alemtuzumab), IV CY (200 mg/kg) and IV busulfan every 6 h for a total of 16 doses with pharmacokinetic monitoring. First-dose AUC was calculated and subsequent doses were altered to target a total regimen AUC exposure of mg · h/L Subsequent busulfan dose AUC was generally not measured and so actual total regimen exposure was not tightly monitored. From July 2014, the conditioning regimen was switched to busulfan, fludarabine and thymoglobulin (10 mg/kg IV, divided days −8 to −5). Fludarabine 40 mg/m^2 and busulfan were each administered once daily IV for 4 days. Busulfan AUC was monitored following the first three doses in all the patients, and subsequent doses were adjusted to target a total regimen AUC exposure of 90 mg · h/L.

Before 2005, GvHD prophylaxis regimens were variable but consisted primarily of *ex vivo* T-cell depletion, CSA/methotrexate or CSA/prednisolone. In 2005, GvHD prophylaxis shifted almost exclusively to CSA/mycophenolate mofetil. From 2014, the recipients of cord blood grafts were administered CSA/prednisolone for GvHD prophylaxis. The patients received antimicrobial prophylaxis according to existing institutional guidelines at the time of transplant. Donor hematopoietic chimerism monitoring was performed according to the available standard clinical assay at the time of assessment. Over time, practice evolved to monitor chimerism on marrow aspirates, to unfractionated blood, to the myeloid ($CD15^+$) selected fraction of blood.

Definitions and end points

OS was defined as survival from first HSCT to last follow-up or death. Alive-and-engrafted survival was defined as survival from HSCT to last follow-up, death or graft failure following the first HSCT. Graft failure was sub-categorized into four types as defined in Table 1: primary aplastic, primary autologous reconstitution, secondary aplastic or secondary autologous reconstitution. Other end points assessed were as follows: (i) time to neutrophil recovery (first day of achieving a neutrophil count $\geqslant 0.5\times10^9$/L for three consecutive days); (ii) time to platelet recovery (platelet $>20\times10^9$/L without transfusions for 7 days); (iii) incidences of transplant-related complications as defined and graded according to existing institutional guidelines at the time of HSCT, including infections, hepatic veno-occlusive disease and GvHD; and (iv) degree of donor hematopoietic chimerism at the most recent assessment.

Statistical analysis

Medians and ranges were reported for continuous variables and percentages were calculated for categorical variables. Nonparametric tests were used to analyze continuous variables and the Chi-square test was used to compare categorical variables for historical and current eras. If the minimal expected frequency requirement for Chi-square test was not met, the Fisher's exact test was used. Univariate analysis was used to identify the predictors of overall and engrafted survival in the post-busulfan pharmacokinetic era, as well as predictors of graft failure in cord blood recipients. Characteristics assessed in univariate analysis included

Table 1. Sub-categories of graft failure

Term	*Definition*
Primary aplasia	No neutrophil engraftment by day +42
Primary autologous reconstitution	Neutrophil engrafted by day +42 but with $<20\%$ donor-derived hematopoiesis
Secondary aplasia	Sustained cytopenias following neutrophil engraftment with fully donor-derived hematopoiesis ($\geqslant95\%$)
Secondary autologous reconstitution	Falling donor chimerism (to $<20\%$) following neutrophil engraftment and adequate donor-derived hematopoiesis
Full-donor chimerism	$\geqslant95\%$ donor-derived hematopoiesis
Mixed chimerism	20–94% donor-derived hematopoiesis
Autologous reconstitution	$<20\%$ donor-derived hematopoiesis

Table 2. Patient and transplantation characteristics according to historical era and current era

	All patients	*Historical era*	*Current era*	P-*value*
Number of patients	240	131	109	
Patient characteristics				
Sex, male (%)	212 (50.4)	57 (51.1)	54 (49.5)	0.805
Age at transplant, months				
Median	15.1	16.3	14.0	0.002
Range	3.8–72.2	4.9–72.2	3.84–65.1	
Age at transplant, *n* (%)				0.003
<12 months	69 (28.7)	26 (19.8)	43 (39.4)	
12 to <24 months	138 (57.5)	86 (65.6)	52 (47.7)	
$\geqslant 24$ months	33 (13.8)	19 (14.5)	14 (12.8)	
Donor characteristics				<0.001
MFD (59 marrow; 7PB; 1CB)	67 (27.9)	42 (32.1)	25 (22.9)	
MUD (54 marrow; 6PB)	60 (25.0)	46 (35.1)	14 (12.8)	
Unrelated CB	91 (37.9)	22 (16.8)	69 (63.3)	
MMD (19 marrow; 3PB)	22 (9.2)	21 (16.0)	1 (0.4)	
Total nucleated cell dose				
Marrow/PB, $\times 10^8$/kg				<0.001
Median	3.6	3.0	8.2	
Range	0.05–34.0	0.05–15.0	1.47–34.0	
CB, $\times 10^7$/kg				
Median	9.8	6.3	10.6	<0.001
Range	0.5–36.0	0.5–19.0	3.9–36.0	
Transplant characteristics				
Conditioning regimen				<0.001
Bu/Cy $\pm$ serotherapy, *n* (%)	145 (60.4)	84 (64.1)	61 (56.0)	
Bu/Flu $\pm$ serotherapy, *n* (%)	40 (16.7)	0	43 (36.7)[a]	
Others, *n* (%)	55 (22.9)	47 (35.9)	5 (7.3)	
GvHD prophylaxis, *n* (%)				<0.001
CSA alone	43 (17.9)	21 (16.0)	22 (20.2)	
CSA+MTX	28 (11.7)	18 (13.7)	10 (9.2)	
CSA+Steroid	36 (29.6)	36 (27.5)	35 (32.1)	
CSA+MMF	46 (19,2)	4 (3.1)	42 (38.1)	
Others	52 (21.7)	52 (21.7)	0	
Ex vivo T depletion	43 (17.9)	43 (32.8)	0	<0.001

Abbreviations: Bu = busulfan; CB = umbilical cord blood graft; CSA = cyclosporine A; Cy = cyclophosphamide; Flu = fludarabine; MFD = HLA-matched family donor; MMD = HLA-mismatched donor; MMF = mycophenolate mofetil; MUD = HLA-matched unrelated donor; MTX = methotrexate; PB = peripheral blood stem cell graft. [a]3 Bu/Cy/TBI/Alemtuzumab.

pre-transplant factors (gender, age at HSCT) and transplant factors (conditioning regimen intensity, serotherapy exposure, GvHD prophylaxis strategy, graft-recipient HLA disparity, graft-recipient ABO compatibility, stem cell source, graft total nucleated cell dose and CD34+ cell dose). All variables in univariate analysis with a *P*-value <0.25 were included in a multivariate logistic regression to assess their independent contribution to the outcome. All *p*-values quoted are two-sided, with a level of significance of 0.05. Probabilities of OS and engrafted survival were calculated using the Kaplan–Meier estimate and the two-sided log-rank test was used for univariate comparisons. Statistical analyses were performed with Statistical Package for Social Sciences, statistical software package (version 22.0, IBM).

RESULTS

Comparison of patient and transplantation characteristics between historical and modern eras

A total of 240 HS children were included. Patient and transplantation characteristics are shown in Table 2. A greater fraction of patients was transplanted at less than 12 months of age in the current era ($P=0.003$). One hundred two (93.6%) patients in the current era received pre-transplant ERT. Nearly all patients (n = 104, 92.7%) in the current era underwent a busulfan-based myeloablative regimen in combination with either CY or fludarabine. Cord blood (63%) was the main blood stem cell source in the current era ($P<0.001$, difference between relative proportions of stem cell source used in the two eras). No patients received *ex vivo* T-depleted grafts in the current era. Patients in the current era received a higher total nucleated cell dose.

OS, engrafted survival, transplant-related complications and mortality

OS has significantly improved from 60.8% (historical era) to 85.2% (current era; $P<0.001$), although follow-up is clearly shorter. Similarly, engrafted survival has almost doubled from 41.2 to 76.3% ($P<0.001$; Table 3 and Supplementary Figure 1). On multivariate analysis, age was the only predictor for OS (odds ratio: 1.07, 95% confidence interval: 1.01–1.14, $P=0.03$). In the current era, predictors of engrafted survival in patients receiving a busulfan-based myeloablative regimen ($n=104$) are shown in Table 4. The use of cord blood was associated with inferior engrafted survival in this era (odds ratio: 0.26, 95% confidence

Table 3. Primary and second end points according historical era and current era

	All patients	*Historical era*	*Current era*	P-*value*
Number of patients	240	131	109	
Duration of follow-up, years				< 0.001
Median	7.3	14.7	2.7	
Range	0.10–29.4	0.5–29.4	0.1–10.9	
Primary end points				
5-year OS, %	70.8	60.8	85.2	< 0.001
5-year EFS, %	57.5	41.2	76.3	< 0.001
Number of patients with graft failure, *n* (%)	60 (25.0)	49 (37.4)	11 (10.1)	< 0.001
Secondary end points				
Days to neutrophil recovery				
Median	16	17	14	0.020
Range	7–45	7–45	7–42	
Number of patients with hepatic VOD (%)	25 (10.4)	6 (4.6)	19 (17.4)	0.001
Number of patients with acute GvHD (%)	92 (38.3)	57 (43.5)	35 (32.1)	0.070
Grade I–II	70 (29.1)	41 (31.3)	29 (26.6)	
Grade III–IV	22 (9.2)	16 (12.2)	6 (5.5)	
Number of patients with chronic GvHD (%)	14 (5.8)	13 (9.9)	1 (0.9)	0.004
Causes of death, *n* (%)	74 (30.8)	60 (45.8)	14 (12.8)	0.003
Infection	21 (8.8)	17 (13.0)	6 (5.5)	
GvHD	11 (4.6)	10 (7.6)	1 (0.9)	
VOD	3 (1.3)	0	3 (2.8)	
Pulmonary failure	19 (7.9)	17 (13.0)	2 (1.8)	
Others	11 (4.6)	16 (12.2)	2 (1.8)	

Abbreviations: EFS = event-free survival; OS = overall survival; VOD = veno-occlusive disease.

interval: 0.03–0.96, $P = 0.04$). Deaths due to infection, GvHD and pulmonary failure have decreased significantly in the current era (Table 3). There were more patients with veno-occlusive disease in the current era (17.4% vs 4.6%, $P = 0.001$), and three patients in this era died from this complication of transplant.

Extent, type, outcomes and predictors of graft failure

The proportion of patients with graft failure decreased in the current era (10.1% vs 37.4%, $P < 0.001$; Table 3) and the pattern of graft failure dramatically changed from an overwhelming predominance of autologous reconstitution (primary or secondary) in the historical era to predominantly aplasia (primary or secondary) in the current era ($P < 0.001$, Figure 1). All graft failures in the modern era occurred in cord blood recipients. Of these 11 patients with graft failure in the current era, 10 received busulfan-based myeloablative conditioning regimen, whereas one patient received a non-myeloablative regimen. Seven of the 10 myeloablative conditioning recipients had aplastic-type graft failure and the remaining 3 as well as the patient that received non-myeloablative had autologous reconstitution. In contrast in the historic era, of the 49 graft failures only 1 was aplastic.

The characteristics of patients with graft failure in the current era are summarized in the Table 5. On univariate analysis, neither pre-transplant factors (age and sex) nor transplant factors (graft-recipient HLA disparity, total nucleated cell dose, CD34+ cell dose, graft-recipient ABO compatibility, conditioning regimen, exposure to serotherapy, GvHD prophylaxis regimen) correlated with graft failure in cord blood recipients receiving busulfan-based myeloablative conditioning in the current era (Supplementary Table 1).

Of the 60 patients with graft failure, 48 (39 and 9 in the historical and current eras, respectively) received a second transplant (Figure 2). Of the 39 patients undergoing second transplant in the historical era, 13 died and 5 had a second graft failure, while remaining 54% are alive and engrafted. Of the 13 deaths, 2 occurred more than 5 years post second transplant (1 aspiration pneumonitis; 1 pneumonia). Three patients underwent a third transplant and all died of transplant-related complications (one from GvHD, two from infection). Of the nine patients undergoing second transplant in the current era, eight are alive and engrafted (89%); the sole death was from late adenoviral infection. For all patients undergoing second HSCT, the estimated 5-year OS was 70.1% (historical era 66.7% vs current era 85.7%, $P = 0.24$)

Donor chimerism according to stem cell source in the current era

Chimerism data at last assessment were available in all 85 patients who were 'alive-and-engrafted' following first transplant in the current era (Table 6). Sixty-eight (80%) had full-donor chimerism while 17 (20%) had mixed-donor chimerism. Although cord blood recipients were more likely to experience graft failure, among survivors that were engrafted, the use of cord blood was significantly associated with a higher rate of full-donor chimerism ($P = 0.01$)

DISCUSSION

Graft failure remains a significant cause of treatment failure following HSCT for HS, and this is the first report specifically examining the evolution of its incidence and pattern over three decades. In this large series from two institutions, we demonstrate that transplant outcomes have improved immensely for children with HS in a modern era characterized by IV busulfan pharmacokinetic-guided myeloablative conditioning regimens, a detailed graft selection hierarchy, superior HLA matching technology, better cell-dosed grafts, the use of peri-transplant IV-ERT and the abandonment of *ex vivo* allograft T-cell depletion.[13] The incidence of graft failure has decreased nearly three-fold and mortality from transplant-related causes has decreased significantly, which may be a result of a greater availability of grafts, improved supportive care, more effective antimicrobial therapy and other factors.

We have shown a striking evolution in the pattern of graft failure from autologous hematopoietic reconstitution observed in recipients of transplantation before 2004 to aplastic forms observed in the modern era. The ability to dose adjust busulfan in the modern era more effectively achieved ablation of host hematopoiesis and there is little to no residual host hematopoiesis from which autologous reconstitution can proceed. In this study, the modern era graft failure is rejection of the graft by a residual, intact host immune system, and is therefore aplastic. We demonstrate therefore that graft failure is now a manifestation of inadequate host immune suppression.

We have also shown that this primary or secondary aplastic-type graft failure is more commonly observed following cord

Table 4. Univariate predictors of 'alive-and-engrafted' in patients receiving busulfan-based myeloablative conditioning regimens in the current era ($n = 104$)

	Yes	*No*	*OR*	*95% CI*	P-*value*
Number of patients, *n*	84	20			
Pre-transplant factors					
Sex, *n (%)*					
Male	41 (48.8)	10 (50.0)	1		
Female	43 (51.2)	10 (50.0)	1.05	0.40–2.78	0.92
Age at transplant, months					
Median	13.3	15.2	0.96	0.92–1.00	0.08
Range	4.1–34.5	3.8–65.1			
Donor					
Family, *n* (%)	23 (27.4)	2 (10)	1		
Unrelated, *n* (%)	61 (72.6)	18 (90)	0.30	0.06–1.18	0.82
HLA disparity					
Matched (8/8 for marrow/PB; 6/6 for CB), *n* (%)	68 (81.0)	15 (75.0)	1		
Mismatched (⩽7/8 for marrow/PB; ⩽5/6 for CB), *n* (%)	16 (19.0)	5 (25.0)	0.71	0.22–2.23	0.55
Stem cell source					
Marrow/PB, *n* (%)	34 (40.5)	3 (15.0)	1		
CB, *n* (%)	50 (59.5)	17 (85.0)	0.26	0.07–0.96	0.04
Blood group mismatch, ($n = 103$)[a]					
No, *n* (%)	39 (47.0)	8 (40.0)	1		
Yes, *n* (%)	44 (53.0)	12 (60.0)	0.75	0.28–2.03	0.57
Conditioning regimen					
BuCy-based, *n* (%)	51 (60.7)	12 (60.0)	1		
BuFlu-based, *n* (%)	33 (39.3)	8 (40.0)	0.97	0.36–2.63	0.95
Serotherapy ($n = 96$)[b]					
ATG, *n* (%)	34 (44.2)	11 (57.9)	1		
Alemtuzumab, *n* (%)	43 (55.8)	8 (42.1)	1.74	0.63–4.80	0.27

Abbreviations: ATG = antithymocyte globulin; Bu = busulfan; CB = umbilical cord blood graft; CI = confidence interval; Cy = cyclophosphamide; Flu = fludarabine; OR = odds ratio; PB = peripheral blood stem cell graft. [a]1 missing data. [b]2 received ALG = antilymphocyte globulin; 6 did not receive serotherapy.

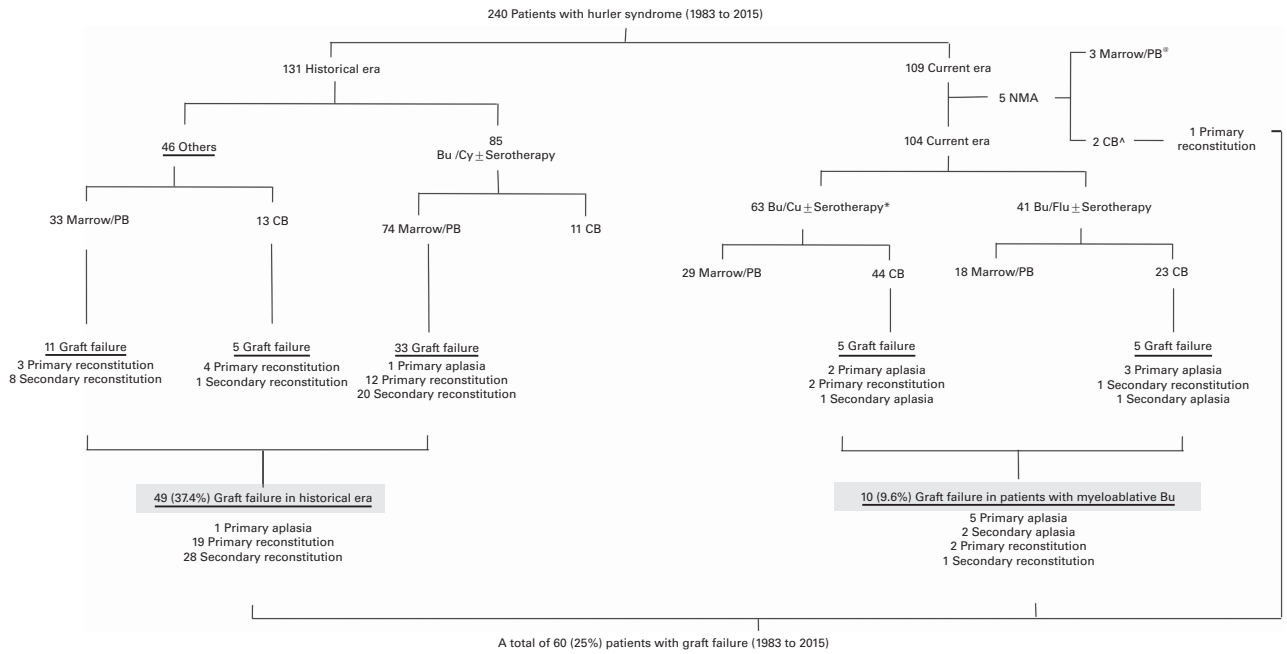

Figure 1. Changing pattern of graft failure from predominantly autologous reconstitution in historical era to predominant aplasia in the current era. NMA, non-myeloablative regimen; *three had Bu/Cy/TLI/ATG; ^two had Clofarabine/Melphalan/TBI/Alemtuzumab; @one had Flu/Treosulfan/Thiotepa/Alemtuzumab; one had Flu/Treosulfan; one Flu/Thiothepa/Alemtuzumab.

Table 5. Characteristics of patients with graft failure in current era

No	Year	*Type of graft failure*	*Age at Tx (months)*	*HLA & stem cell*	*TNC ($\times 10^8$/kg)*	*Conditioning regimen*	*GvHD prophylaxis*	*Day N>0.5*	*First chimerism*	*Onset of GF*	*Chimerism at GF*	*Second transplant*						*Outcome*	*Latest chimerism*	*Duration follow-up (years)*
												Age (months)	*HLA& donor*	*Stem cell*	*TNC ($\times 10^8$/kg)*	*Conditioning regimen*	*GvHD prophylaxis*	*N>0.5*		
1	2005	Primary aplasia	22	6/6 CB	8.1	Bu/Cy/ATG	CSA+Pred	NR	2.2	Day+28	2	25	5/6 ^{D}CB	CB	11.7*	Flu/Treo/Alem	CSA+Pred	16	Died[a]	NA
2	2006	Primary reconstitution	63	5/6 CB	7.3	Bu/Cy/ATG	CSA+MMF	25	0	Day+23	0	No second transplant							Died[b]	NA	0.2
3	2007	Secondary aplasia	18	6/6 CB	6.4	Bu/Cy/ATG	CSA+MMF	31	95	Day+2841	68	111	8/8 ^{D}MUD	M	2.8	Cy/Flu/ATG/TBI	CSA+MMF	13	Alive	100	8.9
4	2008	Primary aplasia	7	6/6 CB	9.8	Bu/Cy/ATG	CSA+Pred	14[c]	100	Day+28	0	16	10/10 ^{D}MUD	PB	44.2	Flu/Treo/Alem	CSA+MMF	11	Alive	99.1	7.1
5	2012	Primary reconstitution	27	5/6 CB	9.8	Clof/Mel/TBI/ Alem	CSA+MMF	25	0	Day+27	0	30	5/6 ^{D}CB	CB	5.0*	Bu/Flu/TBI (NMA)	CSA+MMF	26	Alive	100	1.4
6	2012	Primary aplasia	6	6/6 CB	12.3	Bu/Cy/ATG	CSA+Pred	NR	31.9	Day+28	8	21	6/6 ^{D}CB	CB	9.2*	Flu/Treo/Alem	CSA+Pred	37	Alive	100	3.2
7	2013	Primary reconstitution	17	6/6 CB	15.6	By/Cy/Alem	CSA+MMF	42	1	Day+54	1	No second transplant							Died[d]	NA	1.8
8	2014	Primary aplasia	19	4/6 CB	15.6	Bu/Flu/ATG	CSA+Pred	NR	15	Day+42	0	20	4/6 ^{D}CB	CB	1.5*	Bu/Flu/TBI (NMA)	CSA+MMF	21	Alive	100	1.1
9	2014	Primary aplasia	15	6/6 CB	10.6	Bu/Flu/ATG	CSA+Pred	16	85.8	Day+85	10	19	10/10 ^{D}MSD	M	8.2	FTT/Alem	CSA	16	Alive	100	2.1
10	2014	Secondary aplasia	11	6/6 CB	24.0	Bu/Flu/ATG	CSA+Pred	14	96	Day+124	43	16	10/10 ^{D}MUD	M	10.5	Flu/Alem	CSA	9	Alive	100	1.6
11	2015	Secondary reconstitution	15	6/6 CB	13.4	Bu/Flu/ATG	CSA+Pred	12	99	Day+98	1	22	5/6 ^{D}CB	CB	1.8*	Bu/Flu/TBI (NMA)	CSA+MMF	15	Alive	100	0.7

Abbreviations: Alem = Alemtuzumab; ATG = antithymocyte globulin; Bu = busulfan; CB = cord blood; CSA = ciclosporin; Cy = cyclophosphamide; Flu = fludarabine; GF = graft failure; M = marrow; MMF = mycophenolate mofetil; NA = not available; NMA = non-myeloablative regimen; NR = never reached; PB = peripheral blood; Pred = prednisolone; TNC = total nucleated cell dose. [a]Cause of death: adenovirus. [b]Cause of death: acute respiratory distress syndrome. [c]Patient 4 had unsustained neutrophil engraftment, transfusion dependent and needed return of his autologous back-up. [d]Cause of death: influenzae A. [D]Different donor. *10^7/kg.

blood transplantation. No other transplant factors were found to be predictive of graft failure within the cohort receiving cord blood. Other recent communications in this field have highlighted that, although engrafted survival rates have improved significantly, graft failure is more commonly seen following cord transplant than marrow transplant.[8] In this study, there was no difference in the incidence of graft failure between patients receiving matched or mismatched HLA-matched cord grafts, busulfan/cyclophosphamide vs busulfan/fludarabine conditioning regimens, ATG vs alemtuzumab serotherapy or cyclosporine/steroid vs ciclosporin/mycophenolate mofetil GvHD prophylactic regimens. Notwithstanding this increased risk of graft failure using cord blood, if engraftment becomes established, then cord blood transplant has greater donor chimerism compared with a marrow donor.[9,12]

There are powerful reasons for using a cord blood as the preferred donor source in this disease. The greater chimerism of an engrafted cord blood recipient will deliver better enzyme levels to the patient and improve disease-related outcomes including better growth and a reduced need for surgical interventions to correct disease-related manifestations.[12] The time to transplant from diagnosis might also be expected to be reduced for a cord transplant recipient. However, the increased rate of graft loss that we describe and the consequent delay in sustained engraftment until after a second transplant undermines these benefits. It is important, therefore, that the reasons that underpin this increased rejection rate be recognized and addressed.

Successful engraftment follows host T-cell suppression by conditioning therapy, including serotherapy, as well as ablation by the engrafting donor immune system. Children with metabolic disease are immune competent and their immune system is primed to reject the transplant graft compared with children that are heavily pre-treated for malignant diseases. Insufficient host immune suppression with conditioning agents including serotherapy, or excessive *in vivo* T-cell depletion of the cord graft by serotherapy, will both contribute to the risk of graft rejection. Importantly, the increased graft failure with cord blood in genetic diseases is not confined to HS but is well described in constitutional and acquired aplastic anemia and hemoglobinopathy.[16–19]

To improve transplant outcomes, we must now optimize pre-transplant immune suppression in patients with non-malignant diseases in the same way as we have optimized pre-transplant myelosuppression and improved the transplant outcomes of our patients. Additional pre-transplant immune suppression or the better use of our current agents, such as using pharmacokinetic-guided serotherapy.[20] Marrow transplant is less prone to aplastic graft failure, perhaps because of its larger and more predictable cell dose compared with cord blood grafts. Another candidate approach might be the *ex vivo* manipulation of a portion of the cord blood unit to expand the numbers of CD34+ cells, while retaining some cord-derived T-cells in an unmanipulated fraction.[21] Although all patients in this present study who underwent cord blood transplant in the modern era had adequate cell doses by conventional standards, it can be hypothesized that even higher cell doses may be able to overcome immunologic or other barriers in the recipient that contribute to aplastic failure.

We have also demonstrated in this study that survival rates after graft failure are good. Almost all patients remain alive and engrafted after a second transplant procedure, including a second cord transplant. This should encourage transplant teams to be proactive in individuals with poor blood count recovery or with later aplasia following a first transplant to consider expediently moving towards second transplant. In conclusion, we have shown in a large cohort of children undergoing HSCT for HS that the incidence of graft failure has fallen significantly in the modern era. In addition, we have highlighted the changing pattern of graft failure from insufficient myelosuppression to failure of

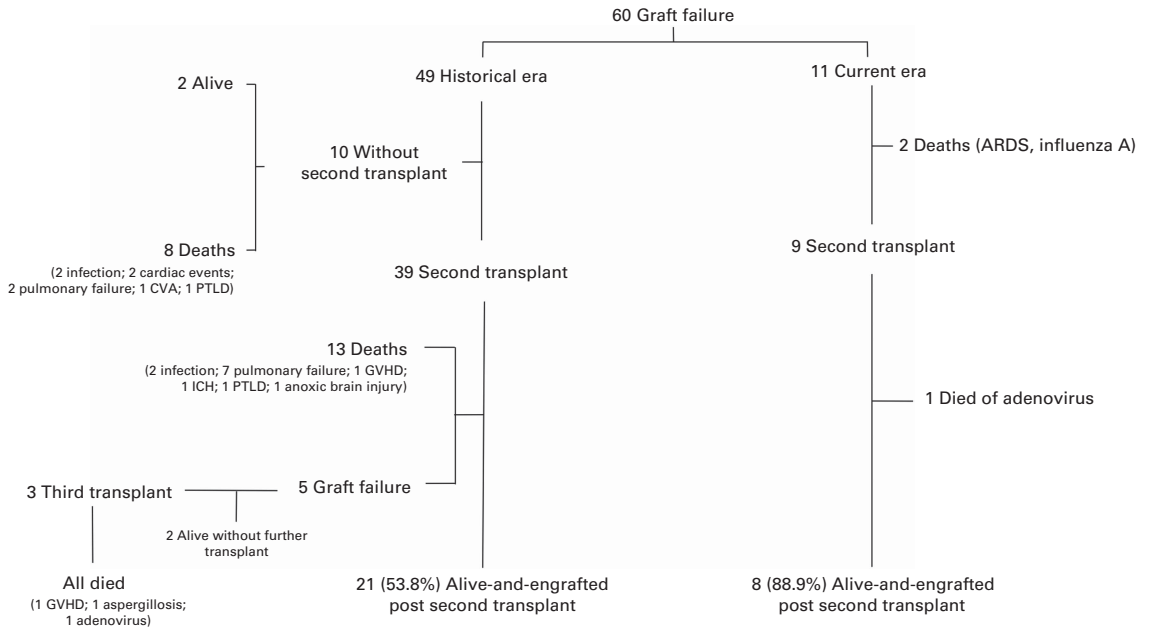

Figure 2. Outcome of patients with graft failure. ARDS, acute respiratory distress syndrome; CVA, cerebrovascular accident; ICH, intracranial hemorrhage; PTLD, post-transplant lymphoproliferative disease.

Table 6. Donor chimerism at last assessment according to blood stem cell source in 'alive-and-engrafted' patients after first transplant in the current era ($n = 85$)

	Marrow/PB n = *37 (%)*	*Cord blood* n = *48 (%)*	P-*value*
Interval between transplant and last assessment, months			
Median	15.9	12.0	0.36
Range	0.7–82.4	1.0–72.0	
Chimerism			
Full chimerism (>95%)	25 (68%)	43 (90%)	0.01
Mixed chimerism (20–95%)	12	5	

Abbreviation: PB = peripheral blood stem cell graft.

immunosuppression. Advances in graft selection, graft engineering or patient-tailored specific immunoablation may be critical to ensure that the next era of transplant in Hurler syndrome is even more successful.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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