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Haematopoietic stem cell transplantation for Diamond Blackfan anaemia: a report from the Italian Association of Paediatric Haematology and Oncology Registry

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Summary

Allogeneic haematopoietic stem cell transplantation (HSCT) is the only curative option for patients with Diamond Blackfan anaemia (DBA). We report the transplantation outcome of 30 Italian DBA patients referred to the Italian Association of Paediatric Haematology and Oncology Registry between 1990 and 2012. This is one of the largest national registry cohorts of transplanted DBA patients. Most patients (83%) were allografted after 2000. A matched sibling donor was employed in 16 patients (53%), the remaining 14 patients (47%) were transplanted from matched unrelated donors. Twenty-eight of the 30 patients engrafted. One patient died at day +6 due to veno-occlusive disease without achieving neutrophil recovery and another patient remained transfusion-dependent despite the presence of a full donor chimerism. The 5-year overall survival and transplant-related mortality was 74·4% and 25·6%, respectively. Patients younger than 10 years as well as those transplanted after 2000 showed a significantly higher overall survival and a significantly lower risk of transplant-related mortality. No difference between donor type was observed. Our data suggest that allogeneic HSCT from a related or unrelated donor was a reasonable alternative to transfusion therapy in young and well chelated DBA patients.

Keywords: BMT, congenital red cell aplasia, Diamond-Blackfan anaemia, iron overload, neoplasm.

Diamond-Blackfan anaemia (DBA) is a rare, congenital or early-onset, pure red cell aplasia observed in 5–10 per million live births. Ninety per cent of patients are diagnosed by 1 year of age, presenting with macrocytic anaemia and reticulocytopenia (Vlachos *et al*, 2008). As with other inherited bone marrow failure syndromes, DBA is associated with congenital abnormalities in approximately 50% of patients and an increased incidence of cancer (Vlachos *et al*, 2012). A subset of DBA patients (15% to 50%) will develop other cytopenias over time (Giri *et al*, 2000; Campagnoli *et al*, 2004). Ribosomal protein (RP) gene abnormalities have been described in 65% of patients. Point mutations or deletions of 10 RP genes (*RPS7, RPS10, RPS17, RPS19, RPS24, RPS26, RPL5, RPL11, RPL26,* and *RPL35A*) have been demonstrated (Boria *et al*, 2010; Gazda *et al*, 2012).

Corticosteroids remain the mainstay of initial therapy in DBA, with a response rate of approximately 80% but only 20% of patients achieve remission. Some 40% require

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continued therapy with steroids, which can have significant side effects, and a further 40% remain transfusion-dependent and at risk for iron overload (Vlachos *et al*, 2008; Roggero *et al*, 2009).

Steroid-intolerant or transfusion-dependent patients may be considered for haematopoietic stem cell transplantation (HSCT), which is the only definitive treatment for the haematological manifestations of DBA. The first HSCT for DBA was reported in 1976 confirming DBA as a transplantable disease (August *et al*, 1976). After this initial case, several authors reported successful transplantations in DBA patients (Iriondo *et al*, 1984; Wiktor-Jedrzejczak *et al*, 1987; Lenarsky *et al*, 1988; Gluckman *et al*, 1989, 1997; Zintl *et al*, 1991; Mori *et al*, 1992; Greinix *et al*, 1993; Saunders *et al*, 1993; Lee *et al*, 1995; Mugishima *et al*, 1995; Wagner *et al*, 1996; Bonno *et al*, 1997; Ladenstein *et al*, 1997; Morimoto *et al*, 1997; Vettenranta & Saarinen, 1997; Willig *et al*, 1999). However, these reports are limited by low numbers of patients. The first cohort of DBA patients who underwent HSCT was reported by the North American DBA Registry (DBAR) in 2001 and updated in 2006 (Vlachos *et al*, 2001a; Lipton *et al*, 2006). Lipton *et al* (2006) described 36 DBA patients, 21 of whom were transplanted from human leucocyte antigen (HLA)-matched sibling donors, and 15 were transplanted from alternative donors. Survival for an allogeneic sibling donor *and* alternative donor transplant was 72·7% vs. 19·1% at over 5 years from HSCT ($P = 0{\cdot}01$) (Lipton *et al*, 2006). The largest report to date from the International Bone Marrow Transplant Registry showed similar results in a series of 61 DBA patients who underwent HSCT between 1984 and 2000; the 3-year probability of overall survival (OS) was 64%; 76% after sibling donor transplantation and 39% after alternative donor HSCT ($P = 0{\cdot}01$) (Roy *et al*, 2005).

A better survival rate in unrelated donor bone marrow (BM) recipients was observed in the Japanese DBA registry series (Mugishima *et al*, 2007).

Recently, Vlachos and Muir (2010) reported a better outcome after HSCT using HLA-matched sibling donors in patients 9 years of age or younger even though a significant improvement in survival with alternative donors has been observed since the year 2000.

In order to investigate the impact of allogeneic HSCT in Italian DBA patients, we carried out a retrospective, multicentre study of all Italian DBA patients who received either related or unrelated-HSCT reported to the Italian Association of Paediatric Haematology and Oncology (AIEOP)-DBA and -HSCT Registries.

Methods

Patients and allogeneic haematopoietic stem cell transplantation

As of 31 December 2012, 173 Italian patients from 162 families have been enrolled in the AIEOP-DBA Registry. Of these 173 DBA patients, 30 received allogeneic HSCT in nine AIEOP transplant centres. Diagnosis of 'classical' or 'probable' DBA was made using the criteria suggested by the DBA International Clinical Consensus Consortium (Vlachos *et al*, 2008). All the patients were screened for mutations in several RP genes using sequencing analysis or deletion detection by Multiplex Ligation Probe Amplification, as previously reported (Quarello *et al*, 2012).

Informed consent was obtained from all patients and/or their legal guardians.

In all donor-recipient pairs, histocompatibility was determined by serology for HLA-A and HLA-B antigens and by DNA typing for HLA-DRB1 locus. In all patients transplanted from an unrelated donor, HLA-DRB1 typing was performed by using a high-resolution allelic technique. Since 1998, all class I and class II (HLA-A, HLA-B, HLA-C, DRB1, DQα1, and DQβ1) HLA alleles have been typed by using a high-resolution technique.

Full donor chimerism was defined as the presence of more than 95% of donor cells; mixed chimerism was defined as the presence of more than 5% and <95% of donor cells, while autologous reconstitution was defined as the presence of <5% of donor cells. The methods used for chimerism analyses varied according to each centre (polymerase chain reaction-based assay, analysing selected polymorphic short tandem repeat loci, HLA typing, cytogenetics and fluorescent *in situ* hybridization analysis).

End-points and definitions

Primary end points were neutrophil and platelet engraftment, acute and chronic graft-*versus*-host disease (aGvHD and cGvHD) incidence, overall survival (OS), and transplant-related mortality (TRM).

Neutrophil and platelet engraftment were defined as the first of three consecutive days with a neutrophil count $>0{\cdot}5 \times 10^9$/l and an unsupported platelet count $>50 \times 10^9$/l, respectively. Graft failure was defined as the absence of haematopoietic recovery at day 60, a second transplantation or autologous reconstitution.

Acute GvHD and cGvHD were diagnosed and graded according to established criteria (Shulman *et al*, 1980; Przepiorka *et al*, 1995). OS was defined as the interval between HSCT and either death or the date of the last follow-up. TRM was defined as death due to any cause related to HSCT procedures.

We analysed the impact of iron overload on OS and TRM using the pre-transplant ferritin level and thus categorized the patients in two groups, those with pre-transplant ferritin levels lower than 1000 μg/l and those with levels higher than 1000 μg/l.

Unfortunately, we do have no data on liver and/or cardiac iron concentrations to better define the iron overload for each of these patients.

Statistical analysis

Patient-, disease- and transplantation-related variables were expressed as medians and ranges, or as percentages, as appropriate. Patients were censored at time of death or last follow-up. OS was calculated according to the Kaplan–Meier method. The occurrence of aGVHD and cGVHD, as well as TRM, were expressed as cumulative incidence curves, in order to adjust the analysis for competing risks. Death from any cause and graft rejection were competing risks to estimate the cumulative incidence of aGvHD and cGvHD. The significance of differences between the OS curves was estimated by the log–rank test (Mantel–Cox) while, in univariate analyses, Gray's test was used to assess differences between TRM curves. A multivariate analysis was not performed because of the limited number of patients.

P values <0·05 were considered to be statistically significant. Statistical analysis was performed using NCSS (Hintze, 2001; NCSS PASS, Number Crunched Statistical System, Kaysville, UT, USA) and R 2.5.0 software packages.

Results

Patient and transplant characteristics

Table I details the patient characteristics, the transplant features and the clinical course after HSCT.

From 1990 to 2012, 30 DBA patients (17 males and 13 females) were reported to AIEOP- HSCT registry. Five (16·6%) received an allograft between 1990 and 1999, 11 (36·7%) between 2000 and 2004, and the remaining 14 (46·7%) between 2005 and 2012.

With regard to the preparative regimen, 20 patients received a busulfan-based conditioning (combined with fludarabine and thiotepa in 15 patients, cyclophosphamide in four patients, and thiotepa and cyclophosphamide in one patient), and a further four patients received a treosulfan-based conditioning (in combination with fludarabine and thiotepa). The patient who received HSCT for a myelodysplastic evolution received a preparative regimen based on busulfan-cyclophosphamide and L-phenylalanine mustard (L-PAM). No data were available for the remaining five patients.

The major indication for HSCT was transfusion-dependence; only two patients underwent HSCT for the evolution of trilineage cytopenia or myelodysplasia (Patients 17 and 30, Table I). Data on steroid treatment were available for 25 patients, all of whom received steroids before transplantation; 19 (76%) were steroid non-responders from diagnosis and 6 (24%) were responders. All but one of the responding patients required cessation of steroid therapy before HSCT due to significant steroid-related side effects. Patient 30 stopped steroids after 3 months of treatment with a partial response (haemoglobin level between 80 and 90 g/l without needing a red blood cell transfusion) and he underwent HSCT for evolution of myelodysplasia at the age of 4 years.

Forty-one per cent (11/27) of patients showed somatic malformations, data was not available for three patients. Fourteen patients carried a RP gene mutation. A high pre-transplant ferritin value (>1000 μg/l) was detected in 75% of patients.

A matched sibling donor (MSD) was employed in 16 patients (53%), while the remaining 14 patients (47%) were transplanted from a matched unrelated donor (MUD).

The median age for all patients at transplant was 6 years (range 1·3–19·8). No difference between age at HSCT and donor (MSD *versus* MUD) was observed.

The median time from the diagnosis of DBA to receiving the HSCT was 5·2 years (0·2–19·8).

The haematopoietic stem cell source was BM for 21 patients (70%), BM and cord blood (CB) for five patients (17%), CB for three patients (10%), and granulocyte colony-stimulating factor [G-CSF]-mobilized peripheral blood (PB) for one patient (3%).

Of the 16 HLA-matched sibling transplants, five were carried out using BM and CB as the stem cell source, nine used BM only and two used CB only. Of the 14 unrelated donor transplants, 12 used BM, one used CB and one used PB.

All HSCTs were performed using a radiation-free preparative regimen.

The vast majority of patients received a ciclosporin-containing GvHD prophylaxis regimen.

The median dose of transplanted nucleated cells was 5×10^8 and 3×10^7 per kg recipient body weight for BM and CB transplant, respectively (Table I).

Outcomes

Engraftment. Twenty-eight out of the 30 patients engrafted. One patient died on day +6 due to veno-occlusive disease without achieving neutrophil recovery and another patient experienced a primary graft failure and received a second BM HSCT from the same donor 112 d after the first one (Patient 28, Table I).

The median time to neutrophil engraftment was 17 d (range 11–27). The median time to platelet engraftment was 24 d (range 15–120).

Neutrophil engraftment was obtained after 17 (range 11–25) and 18 (range 12–27) d for MSD and MUD transplant recipients, respectively ($P = 0·38$). Platelet engraftment for MSD and MUD transplant recipients was reached after a mean time of 32 (range 16–66) and 26 (range 15–49) d, respectively ($P = 0·09$).

Chimerism results were available for 25 patients. Twenty-four patients reached full donor chimerism and one mixed chimerism (>90% donor). One patient (Patient 28, Table I) with primary graft failure underwent a second HSCT, reaching a full donor chimerism.

Table I. Patients characteristics, transplant features and clinical course.

PT/ Sex	Molecular defect	Malformations	Response to steroids/PRE-HSCT status	PRE-HSCT FERRITIN (μg/l)	Age at HSCT (years)	Year of HSCT	Donor/SC source	Cell dose (†10^8/kg)	Conditioning regimen	Chimerism	Outcome
1/M	*RPS19*	Yes[a]	SE/TD	3300	9·98	2002	MSD/CB	0·53	BU+TT+FLU[1]	FD	ALIVE
2/F	NO	No	NR/TD	550	1·31	2004	MUD/BM	15	BU+TT+FLU[1]	FD	ALIVE
3/M	NO	No	NR/TD	790	11·73	2003	MUD/BM	6·1	BU+TT+FLU[1]	FD	ALIVE
4/F	*RPL35A*	No	NR/TD	890	4·88	2004	MUD/BM	5·7	BU+TT+FLU[1]	FD	ALIVE
5/M	*RPS17*	No	NR/TD	1750	9·31	2004	MSD/CB	0·28	BU+TT+FLU[1]	FD	ALIVE
6/F	*RPS19*	Yes[b]	NR/TD	2263	6·16	2007	MUD/BM	6·6	BU+TT+FLU[1]	FD	ALIVE
7/F	*RPL11*	Yes[c]	SE/TD	1468	4	2007	MSD/BM	11·4	TREO+TT+FLU[2]	FD	ALIVE
8/M	NO	No	NR/TD	3446	4·43	2008	MUD/PB	CD34 + =16·3 × 10^6	NA	MIXED*	ALIVE
9/F	NO	No	NA/TD	NA	4·60	2011	MSD/BM+CB	1·7 + 0·12	NA	NA	ALIVE
10/M	NO	Yes[d]	NR/TD	6457	8·10	2008	MSD/BM	5	BU+CY[3]	FD	ALIVE
11/F	NO	Yes[e]	NR/TD	920	3·16	2008	MSD/BM+CB	2·75 + 0·58	TREO+TT+FLU[2]	FD	ALIVE
12/F	*RPS19*	Yes[f]	NR/TD	1224	5·18	2010	MUD/BM	4·5	BU+TT+FLU[1]	FD	ALIVE
13/M	*RPS19*	No	NR/TD	1030	11·25	2008	MSD/BM+CB	10·7 + 0·3	BU+TT+FLU[1]	FD	ALIVE
14/F	*RPS26*	Yes[g]	NR/TD	3238	2·89	2010	MUD/BM	15·8	BU+TT+FLU[1]	FD	ALIVE
15/M	NA	NA	NA/TD	1970	7·84	2009	MSD/BM+CB	1·5 + 0·32	TREO+TT+FLU[2]	FD	ALIVE
16/F	NO	No	NR/TD	2790	16·69	2010	MSD/BM	2·9	TREO+TT+FLU[2]	FD	DEATH
17/F	NO	No	NR/TC	1930	6	2011	MSD/BM	5·7	BU+TT+FLU[1]	NA	ALIVE
18/M	*RPS19*	No	NR/TD	3388	1·98	2012	MUD/BM	10	BU+TT+FLU[1]	FD	ALIVE
19/M	NO	Yes[h]	NR/TD	1200	11·54	1990	MSD/BM	NA	BU+CY[3]	NA	DEATH
20/M	*RPS19*	No	SE/TD	960	2·12	2000	MSD/BM+CB	2·8 + 1	BU+TT+FLU[1]	FD	ALIVE
21/F	NO	No	NR/TD	2560	5·28	1997	MSD/BM	6·6	BU+CY[3]	FD	ALIVE
22/M	NO	No	NR/TD	5790	10·67	1994	MSD/BM	4	BU+CY[3]	NA	DEATH
23/F	*RPS26*	No	NA/TD	750	1·96	2000	MUD/BM	7·5	BU+TT+FLU[1]	FD	ALIVE
24/M	NA	NA	NA/TD	1570	14·31	2000	MUD/BM	7·5	BU+TT+FLU[1]	FD	DEATH
25/M	*RPL11*	Yes[i]	SE/TD	1736	10·32	1999	MUD/CB	0·3	NA	FD	DEATH
26/M	*RPL11*	Yes[j]	SE/TD	2520	11·84	1998	MSD/BM	4	BU+TT+CTX	FD	ALIVE
27/F	*RPS26*	Yes[k]	NR/TD	NA	19·8	2003	MUD/BM	NA	NA	NA	DEATH
28/M	NO	NA	NA/TD	2013	8·7	2002	MSD/BM	NA	NA	FR/FD†	ALIVE
29/M	NO	No	NR/TD	2540	2	2012	MUD/BM	9·9	BU+TT+FLU[1]	FD	ALIVE
30/M	NO	No	PR/TC	659	4	2004	MUD/BM	7·6	BU+CY+L-PAM[4]	FD	ALIVE

PT, patient; M, male; F, female; HSCT, haematopoietic stem cell transplantation; SE, side effects; NR, non responder; PR, partial responder; NA, not available; TD, transfusion dependence; TC, trilineage cytopenia; MD, myelodysplasia; MSD, matched sibling donor; MUD, matched unrelated donor; SC, stem cell; CB, cord blood; BM, bone marrow; PB, peripheral blood; BU, busulfan; TT, thiotepa; FLU, fludarabine; TREO, treosulfan; CY, cyclophosphamide; FD, full donor; FR, full recipient.

*Mixed chimerism (>90% donor), †Patient 28 experienced a primary graft failure and received a second BM HSCT reaching full donor chimerism.

Malformations: [a]Bilateral glaucoma, inguinal hernia, thenar eminence hypoplasia, [b]Bifid uvula, [c]Triphalangeal thumb, palatoschisis, micrognathia, short stature, [d]Skeletal malformations, [e]Cafè au lait spots, short stature, [f]Supernumerary nipples, [g]Palpebral ptosis, skeletal malformations, [h]Anophthalmos, [i]Triphalangeal thumb, palatoschisis, micrognathia, short stature, [j]Hypospadia, cafè au lait spots, ectopic kidney, [k]Ventricular septal defect, skeletal malformations.

Conditioning regimen: [1]BU (16 mg/kg) + TT (6–10 mg/kg) + FLU (120–160 mg/m^2), [2]TREO (42 g/m^2) + TT (6–10 mg/kg) + FLU (120–160 mg/m^2), [3]BU (14–16 mg/kg) + CY (200 mg/kg), [4]BU (16 mg/kg) + CY (120 mg/kg) + L-PAM (L-phenylalanine mustard, 140 mg/m^2).

Graft-versus-*host-disease.* Acute GvHD was observed in 17 patients (57%), five patients had grade I (17%), five patients grade II (17%), six patients grade III (20%) and one patient developed grade IV aGvHD (3%).

The 100-d cumulative incidence of grade II-IV and III-IV aGvHD was 41% [95% confidence interval (CI), 26·6–63·6] and 24% (95% CI, 45·9–79·3), respectively. The cumulative incidence of III-IV GvHD was 25% (95% CI, 10·7–58·4) and 23·8% (95% CI, 8·5–62·2) for MSD and MUD transplant recipients, respectively (P = NS).

Five patients developed cGvHD, two patients presented limited cGvHD and three patients had extensive cGvHD. The overall cumulative incidence of cGvHD was 21% (95% CI, 9·6–46·2).

Overall survival. The 5-year OS rate was 74·4% (95% CI, 55·2–93·6).

In univariate analysis, factors influencing OS probability were: age at transplant (<10 vs. ≥10 years), year of HSCT (before 2000 vs. after 2000), and the occurrence of cGvHD.

Overall survival was remarkably better for children younger than 10 years of age at HSCT compared to those older than 10 [100% vs. 29·6% (95% CI, 0–61·7), $P < 0·00001$] and for patients who underwent HSCT after 2000 [86·6% (95% CI, 72·5–100) vs. 40% (95% CI, 0–83), $P = 0·03$]. No differences between a MSD and a MUD were observed (Figs 1–3).

Patients with extensive cGvHD showed a poor prognosis in comparison to patients with absent or limited cGvHD [0% vs. 95% (95% CI, 85·4–100) vs. 100%, $P = 0·01$].

The presence of a high pre-transplant ferritin level [63% (95% CI, 31·7–94·5) vs. 100%, $P = 0·1$] as well as the occurrence of grade II-IV aGvHD [62·8% (95% CI, 33·6–92) vs. 82·6% (95% CI, 59·1–100), $P = 0·11$] were negative prognostic factors although statistical significance was not reached.

Regarding HSC source, no impact on OS was observed, although the majority of patients received BM stem cells and only three and one patients received CB and PB, respectively. Specifically, the only patient who received MUD CB died after HSCT due to extensive cGvHD.

Other variables, such as gender and the presence of malformations, had no impact on OS (Table SI).

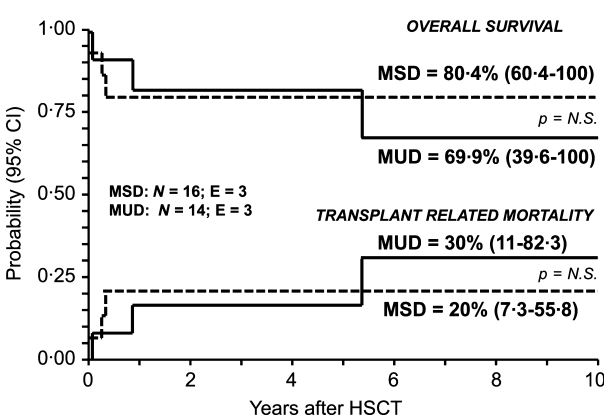

Fig 1. Overall survival and transplant-related mortality according to donor type. MSD, matched sibling donor; MUD, matched unrelated donor; HSCT, haematopoietic stem cell transplantation; 95% CI, 95% confidence interval.

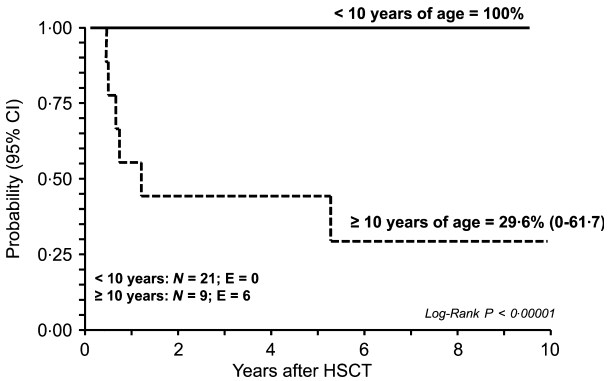

Fig 2. Overall survival according to age at haematopoietic stem cell transplantation (HSCT). 95% CI, 95% confidence interval.

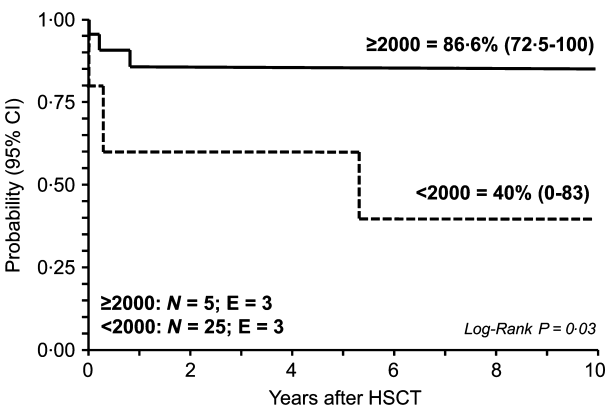

Fig 3. Overall survival according to year of haematopoietic stem cell transplantation (HSCT). 95% CI, 95% confidence interval.

Transplant-related mortality. The 5-year TRM was 25·6% (95% CI: 12–54%).

In univariate analyses, factors influencing TRM were age at transplant (<10 vs. ≥10 years), year of HSCT (before 2000 vs. after 2000), and the occurrence of cGvHD.

Patients older than 10 years showed a significantly higher risk of TRM [70% (95% CI, 44·6–100) vs. 0%, $P = 0·0001$] as well as those transplanted before 2000 [60% (95% CI, 29·3–100) vs. 13·6% (95% CI, 4·7–39·2), $P = 0·04$]. As expected, the occurrence of extensive cGvHD was associated with a higher TRM compared to absent or limited cGvHD [33% (95% CI, 6·7–100) vs. 5·2% (95% CI, 7·8–35·4), vs. 0%, $P < 0·0001$].

The presence of a high pre-transplant ferritin level [36·4% (95% CI, 15·8–86·4) vs. 0%, $P = 0·09$] as well as the occurrence of grade II-IV aGvHD [37·1% (95% CI, 16·9–81·6) vs.

17·4% (95% CI, 4·4–67·3), $P = 0·1$] were associated with a higher TRM although statistical significance was not reached.

Other variables, such as gender, somatic malformations, HSCT donor and source had no impact on TRM (Fig 1; Table SI).

Follow-up and causes of death. Twenty-four of the 30 transplanted patients are alive and red-cell transfusion independent. The median-observation time after transplantation for surviving patients was 46 months.

Of the 16 MSD transplants, 13 patients are alive, 11 of whom are in good clinical conditions and transfusion independent. One patient (Patient 28, Table I), who received a second HSCT from the same donor after a primary graft failure is alive but transfusion dependent despite the presence of a full donor chimerism.

Another patient (Patient 1, Table I) developed a grade 3 malignant osteosarcoma 10 years after HSCT. Eleven of the 14 unrelated donor transplant patients are alive and transfusion independent.

All deaths occurred in patients older than 10 years at the time of transplant (six out of 9 patients older than 10 years at HSCT) and with severe pre-transplant iron overload (Table I). Specifically, among those patients who received an HLA-matched sibling transplant, two deaths were related to infections (one bacterial and one fungal infection at +96 and +124 d from HSCT) and one death to veno-occlusive disease at day +6 after HSCT.

Among the patients who were transplanted from an unrelated donor, two deaths were related to extensive cGvHD and one to grade IV aGvHD (+29 d after HSCT). In the two patients with extensive cGvHD, one patient showed severe fungal pneumonia (5 years after HSCT) and the other an Epstein-Barr virus-associated lymphoproliferative disorder (11 months after HSCT).

Discussion

Haematopoietic stem cell transplantation is the only definitive treatment for the haematological manifestations of DBA. Allogeneic matched sibling HSCT has already been reported to be successful in DBA patients, but poorer outcome has been described in HSCT from matched unrelated donors (Vlachos *et al*, 2001b; Roy *et al*, 2005; Lipton *et al*, 2006).

Here, we report one of the largest single national registry cohorts of DBA patients who underwent either related or unrelated HSCT, mostly performed after 2000.

Our data from the AIEOP HSCT Registry reveal an OS of 74·4%. As also observed by Vlachos and Muir (2010), an even higher OS was observed when comparing the patients transplanted after 2000 with those transplanted before the year 2000 (86·6% vs. 40%, $P = 0·03$). Unlike most data in the literature, in our cohort no significant difference between related and unrelated donors (80·4% vs. 69·9%, P = NS) was observed. This result is certainly influenced by the fact that the majority of our patients received HSCT after 2000 and it could be the result of improved HLA matching techniques in alternative donor HSCT.

Based on the DBA International Clinical Consensus Conference, patients with DBA, whether steroid-responsive or transfusion-dependent, may be considered for transplant prior to age 10 years, and preferably between the ages of 2 and 5 years, if an HLA-matched related donor is available. On the other hand the indications for HSCT for a patient without a MSD were limited and reserved only for bi- or trilineage cytopenia and/or the evolution to myelodysplasia or leukaemia (Vlachos *et al*, 2008).

The remarkable improvement in unrelated donor transplantation as well as the documented very high risk of severe iron overload in chronically transfused DBA patients suggest that this approach could be offered as front-line therapy if a HLA-matched donor is available (Mugishima *et al*, 2007; Roggero *et al*, 2009; Vlachos & Muir, 2010).

A variety of stem cell sources, including related and unrelated donor BM, peripheral blood stem cells and CB, have been used in DBA patients (Willig *et al*, 1999; Vlachos *et al*, 2001a; Roy *et al*, 2005; Mugishima *et al*, 2007; Bizzetto *et al*, 2011). More than one report described a worse outcome in unrelated CB transplant while the results of related CB HSCT were comparable with outcomes obtained with other haematopoietic stem cell sources (Vlachos *et al*, 2001b; Mugishima *et al*, 2007; Bizzetto *et al*, 2011).

The majority (70%) of patients included in our study received BM alone or BM combined with CB as the stem cell source. CB alone was used in three HLA-matched sibling transplants and in one unrelated transplant. On the basis of literature data, we strongly recommend CB storage from subsequent pregnancies, while the use of an unrelated CB donor should be considered on a case-by-case basis when individual circumstances justify the risk (Bizzetto *et al*, 2011).

In our cohort, the major indication for HSCT was transfusion dependence in patients who were unresponsive or intolerant to steroid treatment.

In general, as in patients with thalassaemia, DBA patients also had improved outcomes when transplantation was performed at a younger age, before multiple transfusions lead to iron overload and/or the development of significant allo-sensitization (Lucarelli *et al*, 2002; Vlachos *et al*, 2008; Vlachos & Muir, 2010).

Iron overload is a serious and early complication in regularly transfused DBA patients, and more frequent than that observed in regularly chelated thalassaemia patients (Roggero *et al*, 2009). This difference might be attributable to non-optimal chelation, or an unknown biological mechanism that leads to an early severe iron overload.

In this study, we demonstrated a remarkably better survival for children younger than 10 years of age at HSCT compared to those older than 10 (100% vs. 29·6%, $P < 0·00001$) for both related and unrelated donors. In

patients older than 10 years, the lower OS was mainly due to a significantly higher TRM (0% vs. 70%, $P = 0{\cdot}0001$).

Moreover, albeit not statistically significant, a worse survival rate and a higher TRM were observed in patients with higher pre-transplant serum ferritin levels.

We strongly suggest evaluating multiple parameters, including magnetic resonance determination of hepatic, cardiac and pancreatic iron burden in order to treat these patients with chelation therapy before HSCT to achieve a good iron balance and minimize the risk of iron overload. On the basis of these pre-transplant evaluations it may well be very useful to band DBA patients according to risk scores, as for thalassaemic patients (Lucarelli *et al*, 1993).

Like other bone marrow failure syndromes, DBA patients have a high risk of toxicities and long-term post HSCT complications, especially in terms of aGvHD, cGvHD and malignancies.

In our cohort, a remarkably lower survival rate was observed in patients who developed extensive cGvHD compared with patients with absent or limited cGvHD (0% vs. 95% vs. 100%, $P = 0{\cdot}01$). Furthermore, cGvHD results in decreased quality of life and it is a key risk factor for the development of post-transplantation late tumours (Socie *et al*, 1998).

Secondary malignancies are an important and well-documented late effect after HSCT. Susceptibility varies widely depending on the underlying disease. DBA patients already have an increased risk for malignancies before HSCT even though this risk is lower compared to other hereditary bone marrow failure syndromes (e.g. Fanconi anaemia) (Vlachos *et al*, 2012).

The North America DBAR described two DBA patients who developed post-transplantation neoplasms. The first case was a DBA patient who underwent unrelated HSCT with a total body irradiation (TBI)-containing preparative regimen and who died from metastatic osteosarcoma on day +1571 post transplantation; the other patient developed a rectal cancer 15 years after HSCT (Vlachos *et al*, 2001a, 2012).

Taking into account the higher neoplastic risk for DBA patients, in our cohort all patients received a radiation-free preparative regimen. However, one patient who received a MSD HSCT with busulfan-based conditioning regimen developed an osteosarcoma 10 years after HSCT. The DBA patients' predisposition for malignancies needs to be further explored, especially when the consequences of transplant conditioning regimens are taken into account.

There are encouraging case reports of successful HSCT in DBA with reduced intensity fludarabine-based preparative regimens (Ostronoff *et al*, 2004; Berndt *et al*, 2005). Although successful, too few HSCT utilizing reduced intensity conditioning regimens have been reported.

In our cohort, the majority of patients received a busulfan-based conditioning regimen. In recent years the use of busulfan has been replaced by treosulfan as a low toxic preparative agent. The use of treosulfan in the field of non-malignant diseases is particularly interesting because of the reduced non-haematological toxicity compared to busulfan or TBI. Successful results in terms of safety, tolerability and efficacy have been reported in thalassaemias and metabolic disorders (Bernardo *et al*, 2008; Greystoke *et al*, 2008).

Any decrease in treatment-related toxicity is particularly welcome in these patients. However, these gains may be counterbalanced by the risk of non-engraftment.

In our cohort, 28 out of the 30 patients engrafted. One patient died on day +6 due to veno-occlusive disease without achieving neutrophil recovery whilst another patient experienced primary graft failure and received a second BM HSCT from the same familial donor 112 d after the first one with persistence of transfusion dependence despite the presence of a full donor chimerism.

A similar case was reported by Wynn *et al* (1999) who described a 10-year-old boy affected by DBA who had received a HSCT from his HLA-identical sister. The donor showed normal peripheral blood counts, reticulocyte count and marrow smear at the time of harvest. Despite cytogenetic evidence of complete donor haemopoietic stem cell engraftment there was selective failure of red cell engraftment and he remains red cell transfusion-dependent.

Recently, a growing number of apparently haematologically normal family members of DBA patients have been found to have a silent DBA phenotype by virtue of RP gene mutation or mild macrocytosis, elevated fetal haemoglobin and/or increased erythrocyte adenosine deaminase (eADA) activity.

The familial donor of our patient was haematologically normal but the eADA level was not available. Furthermore, as we did not identify a RP gene mutation in either our patient or his donor, the presence of a silent DBA phenotype cannot be ruled out.

There should be a note of caution regarding allogeneic donor selection. Each sibling donor has to be carefully screened. Screening should include a careful clinical and haematological evaluation (detection of classical DBA somatic malformations, eADA evaluation, RP molecular analysis) even when the donor has no phenotypical evidence of DBA. The risk of silent DBA-affected siblings has to be kept in mind also in the case of familial cord blood storage. Indeed, an allogeneic HSCT from an affected donor predictably resulted in no engraftment (Orfali *et al*, 1999; Wynn *et al*, 1999; Vlachos & Muir, 2010).

In conclusion, this report describes one of the largest national registry cohorts of transplanted DBA. Allogeneic HSCT from either related or unrelated donors is a reasonable alternative to transfusion therapy in young DBA patients without severe iron overload.

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Author contributions

Franca Fagioli performed transplantations, designed the study and wrote the manuscript. Paola Quarello collected and analysed the data, performed the statistical analysis and wrote the manuscript, Marco Zecca performed transplantations and collected the data. Edoardo Lanino, Paola Corti, Claudio Favre, Mimmo Ripaldi performed the transplantations. Ugo Ramenghi provided the AIEOP DBA Registry data. Franco Locatelli and Arcangelo Prete designed the study, performed transplantations and were responsible for critically revising the manuscript. The authors have no conflict of interest and do not possess any financial interests in the work submitted.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table SI. Univariate analyses of variables influencing the probability of overall survival and transplant related mortality.

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