Reduced toxicity conditioning for hematopoietic stem cell transplantation in children with Diamond-Blackfan anemia

Diamond-Blackfan anemia (DBA) is a rare inherited bone marrow failure disorder that typically presents as macrocytic hypoproliferative anemia during infancy.[1] Patients usually present with macrocytic anemia but can develop multilineage cytopenias and immunodeficiency over time.[2] Constitutional anomalies, such as craniofacial, skeletal, and cardiac defects, are observed in ~50% of the patients.[1] The mainstay of DBA therapy includes chronic red blood cell (RBC) transfusions and corticosteroid therapy, both of which can be associated with substantial adverse effects and poor quality of life.[1] Allogeneic hematopoietic stem cell transplant (HSCT) is the only hematopoietic cure for DBA. The recommended indications for HSCT in DBA include transfusion dependence due to either non-responsiveness to corticosteroids or requiring more than 0.3 mg/kg/day of corticosteroids, toxicities from treatment, multilineage cytopenias and evolution to hematologic malignancy.[3] The current consensus is to use myeloablative conditioning (MAC) regimen for HSCT in DBA patients.[1,3] Use of reduced-intensity/toxicity conditioning (RIC/RTC) has the potential to reduce transplant-related toxicities and potentially preserve fertility in these patients. However, there is limited information on the use of RTC in DBA patients. Here, we describe the clinical course of four children with DBA undergoing successful allogeneic HSCT using RTC regimen.

The four patients were diagnosed with DBA at the median age of 3.5 (range, 3.5-8) months and underwent HSCT at the median age of 8.5 (range, 6-14) years (Table 1). Pathogenic mutations or deletions were identified in *RPS17*, *RPS26*, and *RPS19* genes. With exception of bilateral club foot in patient 3, no other physical malformations were documented in our cohort. All patients previously failed two trials of standard dose corticosteroids (2 mg/kg/day) and underwent HSCT for transfusion dependence complicated by iron overload. Prior to HSCT, the median white blood cell count, absolute neutrophil count, absolute lymphocyte count, and platelet count were $5.54x10^9$/L (range, 4.11-10.8), $1.665x10^9$/L (range, 1.650-6.912), $2,7x10^9$/L (range, 1.940-4.307) and $240x10^9$/L (range, 224-414), respectively. All four patients had normal bone marrow morphology except for erythroid hypoplasia and normal karyotype before HSCT, with marrow cellularity ranging from 30% to 90%.

The peak liver iron concentration (LIC) measured by T2* MRI ranged from 7.04 mg iron/g to 14.06 mg iron/g of dry liver tissue. Cardiac iron measured by T2* value was within normal limits for all patients and there was no indication of pancreatic iron overload. Intensified iron chelation using various iron chelators was given for a median of 21 (range, 7-43) months leading to the reduction of median LIC to 3.58 (range, 2.23-5.75) mg/g of dry liver tissue, prior to HSCT

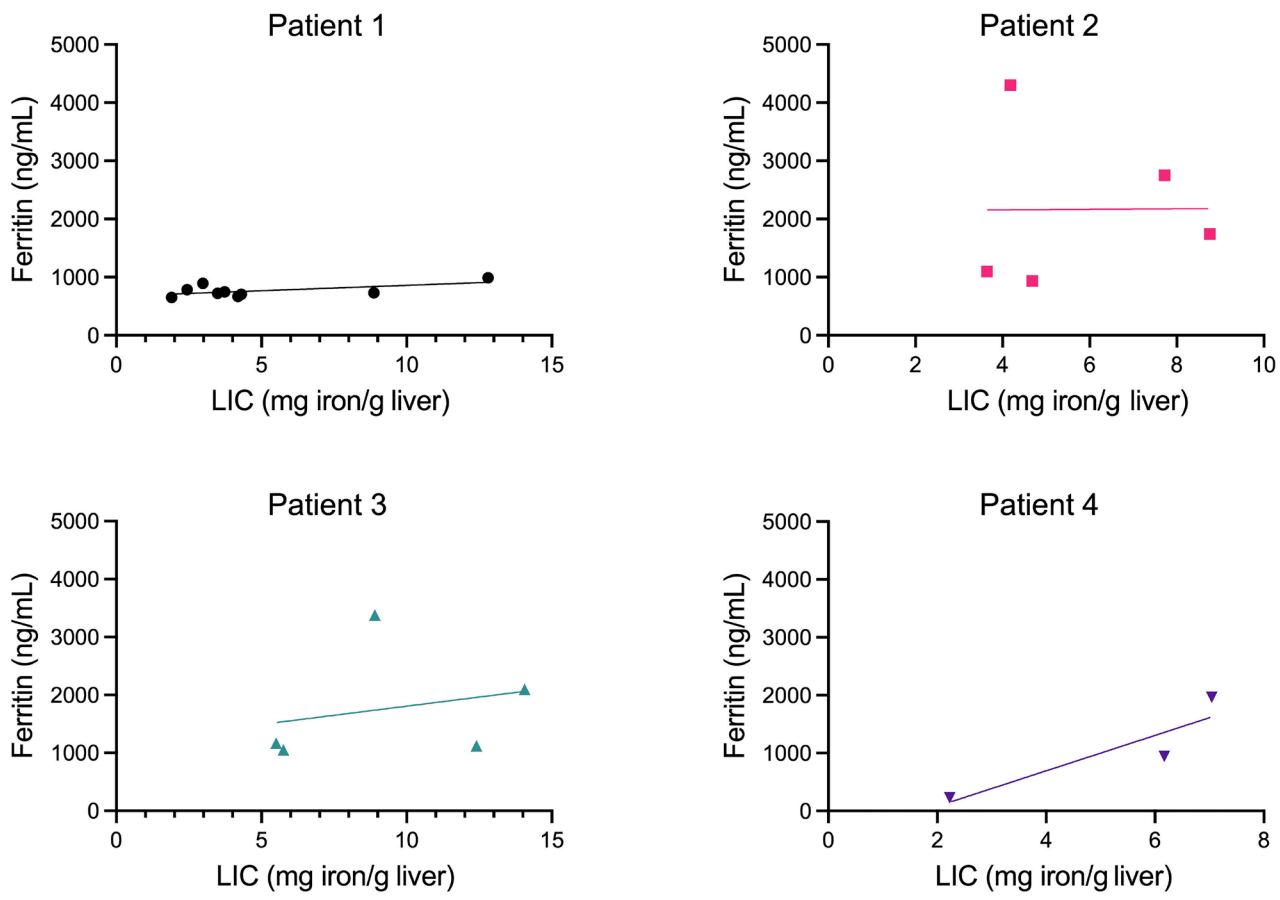

Figure 1. Ferritin levels *versus* liver iron concentration with linear regression line. LIC: liver iron concentration.

(Table 1). The duration of chelation-included interruptions in therapy due to insurance, compliance issues and transient acute tubular necrosis in patient 1. Interestingly, reduction in LIC did not correlate with ferritin levels in patients 1 and 2 compared to the other two patients (Figure 1). The exact reasons are unclear, but ferritin has been previously reported to be imprecise in estimating iron overload status in patients with hereditary anemias, including DBA.[4,5]

The median age at the time of transplant was 8.5 (range, 6-14) years. Donors were fully matched at the A, B, C, DR and DQ human leukocyte antigen (HLA) loci, with three being matched unrelated donor (MUD) and one sibling donor (MSD). All four patients received the same RIC regimen consisting of five doses of fludarabine at 30 mg/m^2/day, one dose of thiotepa at 10 mg/kg/day, and two doses of melphalan at 70 mg/m^2/day. Patients who received a graft from a matched unrelated donor (MUD) were given 7 mg/kg/ total dose of rabbit anti-thymocyte globulin (ATG) administered over 4 days. Graft-*versus*-host disease (GVHD) prophylaxis consisted of cyclosporine for at least 6 months, along with methotrexate. All patients received unmanipulated bone marrow grafts with the infused stem cell doses ranging from 5.4-7.7x10^6 CD34$^+$ cells/kg recipient weight.

Following HSCT, the median time for experiencing neutrophil engraftment was 16 (range, 14-20) days. In the post-HSCT course, patient 3 developed mild liver sinusoidal obstruction syndrome (SOS) which resolved with a short course of defibrotide while patients 3 and 4 had Epstein-Barr virus reactivation that resolved with rituximab. Patient 4 remains intravenous immunoglobulin-dependent at 1 year post transplant, while the other patients demonstrated normal immune reconstitution without any other infectious complications (Table 1). No other complications, including GVHD, were noted. At a median follow up of 16 (range, 11-21) months, all four patients remained transfusion-independent with 100% donor chimerism.

These four patients highlight that allogeneic HSCT in DBA patients using RTC regimen can lead to successful engraftment despite normocellular to hypercellular marrows as observed in patients 1 and 2 (Table 1). Outcomes after HSCT for DBA have improved considerably over the last 40 years, propelled by advances in high-resolution HLA typing/matching and supportive care.[6] Recent studies report overall survival (OS) of 80-90% for matched sibling donor (MSD) HSCT across the globe.[6] In comparison, outcomes after MUD HSCT are more variable with OS ranging from 70-90% although MUD HSCT outcomes are comparable to MSD HSCT in younger children (<10 years).[6,7] The current recommendation is to use myeloablative conditioning (MAC) regimen based on either busulfan or treosulfan for HSCT

Table 1. Clinical characteristics of Diamond-Blackfan anemia patients who underwent reduced toxicity conditioning hematopoietic stem cell transplantation.

Characteristics	Patient 1	Patient 2	Patient 3	Patient 4
Age at diagnosis in months/age at HSCT in years	3/14	3/8	4/5	8/8
DBA mutation	*RPS17* (whole gene deletion)	*RPS26* (p.R87*)	*RPS19* (p.Ala100Trpfs*12)	*RPS19* (exon 2-3deletion)
BM cellularity pre-HSCT, %	90	60-70	40-50	30
LIC: highest/pre-HSCT, mg/gm liver tissue	12.8/2.98	8.76/4.18	14.06/5.75	7.04/2.23
Ferritin pre-HSCT, ng/mL	895	4,301	1,054	227
Donor type/graft source	MSD/BM	MUD/BM	MUD/BM	MUD/BM
Conditioning regimen	Flu/Thio/Mel	Flu/Thio/Mel	Flu/Thio/Mel	Flu/Thio/Mel
GVHD prophylaxis	CsA/MTX	ATG/CsA/MTX	ATG/CsA/MTX	ATG/CsA/MTX
Neutrophil engraftment in days	20	17	14	15
HSCT complications	None	None	Mild SOS; EBV reactivation	EBV reactivation
GVHD	None	None	None	None
CD4/CD19 count at 1 year post-HCT, cells/μL	300/1,120	626/188	1,368/650	994/68
Last follow up in months post-HSCT	21	16	16	11
Chimerism at last follow up, %	100	100	100	100

DBA: Diamond-Blackfan anemia; HSCT: hematopoietic stem cell transplantation; MUD: HLA-matched unrelated donor; MSD: HLA-matched sibling donor; BM: bone marrow; Flu: fludarabine; Thio: thiotepa; Mel: melphalan; CsA: cyclosporine; MTX: methotrexate; GVHD: graft-*versus*-host disease; ATG: anti-thymocyte globulin; LIC: liver iron concentration; pRBC: packed red blood cell; SOS: sinusoidal obstruction syndrome; EBV: Epstein-Barr virus.

in DBA patients.[3] However, the outcome of older patients, especially those older than 10 years of age, undergoing MAC HSCT remains suboptimal.[6] Late effects and loss of fertility remain a matter of concern with MAC regimens. Reducing conditioning intensity can potentially reduce the risk of infertility as well as allow adolescent and young adult patients, who can undergo fertility preservation, to receive HSCT for DBA. HSCT is presumed to increase risk of cancer in DBA patients, although data is limited.[8] Long-term studies of larger cohort are needed to definitely test this hypothesis and determine if reducing conditioning intensity can alleviate the risk of cancer, if any.

Several observations provide a rationale for reducing the conditioning intensity without increasing the risk of graft failure. First, HSC function is impaired due to RPS19 deficiency in human and mice DBA models and *RPS19* haploinsufficient HSC are outcompeted by healthy HSC in a xenograft model.[9,10] Lentiviral vector encoding *RPS19* was able to rescue this HSC defect. Second, bone marrow cellularity is typically reduced in DBA patients over time.[2] Recent studies suggest RIC HSCT is well tolerated and eads to successful engraftment in several bone marrow failure disorders.[11-13] Wang *et al*. showed good safety and efficacy of RTC regimen in patients with various bone marrow failure disorders, including one 5-year-old patient with DBA who underwent cord blood transplant.[14] Another case series from Austria showed successful outcomes following RIC-based HSCT in three infants with DBA.[13] Similar results were reported with treosulfan-based RIC.[15] In a larger cohort of 15 DBA patients, Koyamaishi *et al*. reported excellent OS following RIC HSCT in very young children (median age 3.6 years).[12] The median age at HSCT in our case series was 8.5 years. Future studies can test safety and efficacy of RTC regimens in older children with DBA.

Iron overload and MAC regimen are associated with increased risk of SOS.[12] Only one of four patients developed mild, transient SOS that completely resolved following defibrotide; this patient was also noted to have the highest LIC prior to transplant. Despite lack of data in DBA, optimizing iron chelation and reducing conditioning intensity are believed to reduce the risk of SOS in HSCT recipients.

In summary, we report successful outcomes following HSCT using RTC regimen in transfusion-dependent DBA patients with a history of iron overload. Our encouraging results indicate that RTC regimen can be effective in DBA, particularly for children with history of iron overload. Lastly, these findings provide rationale for using RIC regimens for future gene therapy trials in DBA as gene-corrected HSC have survival advantage over DBA HSC.[9,10]

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Contributions

AQ and SS collected patient data. AQ, RM, AS, MWW and SB cared for the patients. All authors contributed to writing the manuscript. SB supervised the study. All authors reviewed and approved the final draft of the manuscript.

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Data-sharing statement

Data that support the study are available upon reasonable request to the corresponding author.

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