Reduced-intensity conditioning and stem cell transplantation in infants with Diamond Blackfan anemia

Diamond-Blackfan anemia (DBA) is a rare, inherited, pure red cell aplasia, associated with congenital anomalies[1] and predisposition to cancer.[2] Despite remarkable advances in our understanding of the underlying pathogenic mechanisms,[3] first-line treatment still relies on repeated red blood cell (RBC) transfusions and/or corticosteroid therapy.[4] While transfusion dependency, unresponsiveness to corticosteroids or development of additional cytopenias can justify the indication for hematopoietic stem cell transplantation (HSCT),[4] the time point for HSCT in DBA patients – one recommendation is between 3 and 9 years of age[1] - is less clearly defined due to variability of the disease course, including spontaneous remission.[1] The median age at HSCT in DBA series is 7 years (range, 2.6 – 14 years),[5-9] but the avoidance of iron overload and allosensitization from regular red blood cell transfusions may be reasons to consider HSCT in children younger than 3 years.[4,10]

In this report, we present the clinical course of DBA patients transplanted at less than 1.3 years of age following reduced intensity conditioning regimens (Table 1). Our retrospective analysis focuses on 3/16 children (19%) who were diagnosed with DBA at the two major pediatric hematology centers in Austria (St. Anna Children's Hospital, Vienna, N = 11; Department of Pediatrics, Medical University of Innsbruck, N = 5), between 1.1.1992 and 1.1.2016. The decision to perform HSCT was based on multilineage cytopenias associated with severe infections, non-response to steroids, and the availability of a HLA-matched donor.

Patient #1 was diagnosed with DBA at 5 weeks of age and treated with regular red blood cell transfusions at 4-6 week intervals (Table 1). At 5 months of age he developed trilineage aplasia and acute staphylococcal tonsillopharyngitis. Due to rapid bilateral extension to the submandibular tissues, intensification with broad-spectrum antibiotics and mechanical ventilation was required. After recovery, treatment with corticosteroids was initiated, but bilineage aplasia, including severe neutropenia and anemia, persisted. At the age of 9 months, the patient underwent HLA-identical and *RPS19* wild-type sibling bone marrow transplant. Acute graft *versus* host disease (GvHD) II° of the skin developed at day +12 after HSCT, and was successfully treated with a short course of steroids (Table 2).

Patient #2 was diagnosed at 4 weeks of age and substituted with red blood cell transfusions on a regular basis of 4 to 6 weeks (Table 1). He developed persistent neutropenia at 6 weeks of age, and a short interval of prednisone therapy was started; this attempt, however, was unsuccessful. At 7 months of age, allogeneic bone marrow transplant from unrelated matched donor was performed. The only complication post-transplant was a self-limiting grade I° skin acute GvHD reaction (Table 2).

In patient #3, DBA was diagnosed at the age of 2 months (Table 1). Pancytopenia with severe infection occurred at the age of 7 months. Due to transfusion-dependent anemia, which was non-responsive to steroids, and severe infection during pancytopenia, an allogeneic bone marrow transplant was performed at 13 months of age. With the exception of a grade II° skin acute GvHD reaction, the clinical course of the patient was uneventful (Table 2).

Forty years have elapsed since the first HSCT was performed on a patient with DBA.[11] Subsequently, in the 1980s and early 1990s, several authors reported successful transplantations in single DBA patients. Since then, HSCT has become a commonly accepted treatment option for patients with DBA unresponsive to steroids; three European DBA registries reported 13 HSCTs, 11/13 being successful.[5] HSCT-related deaths were due to toxicity, and were associated to severe iron overload at the time of transplant (16 and 18 years of age, respectively).[5] At the same time, a larger cohort from the DBA Registry (DBAR) reported 36 DBA patients, 21 of whom were transplanted with HLA identical donors, and survival was 72.7% at 5 years from HSCT *versus* 19.1% for those transplanted with mismatched donors (n = 15).[8] This observation was confirmed by the largest cohort to date from the International Bone Marrow Transplant Registry, showing 76% survival *versus* 39% for sibling and alternative donor HSCT, respectively.[6] Recently, Fagioli *et al.* reported a better outcome in patients under 10 years of age (100% survival *versus* 29.6% at 5 years post HSCT), though a significant improvement in survival has been observed since the year 2000 (86.6% *versus* 40%).[7]

In view of reduced transplant related mortality and improved HLA matching techniques, the DBA International Clinical Consensus Conference has suggested HSCT for patients younger than 10 years of age, if an HLA-identical donor is available.[4]

In our cohort, the indications for HSCT in 3 infants were multi-lineage cytopenias, the development of severe infections requiring intensive care treatment, and none-response of anemia to steroids. For all patients, a matched bone marrow donor was available, and HSCT was offered as second-line therapy.

Experience with HSCT in patients younger than 2

Table 1. Basic characteristics of DBA patients.

Patients	Age at diagnosis (months)	Mutations	Hemoglobin F (%)	Congenital anomalies
#1	6	c.-173G>A c.-1+36T>C c.71+81dupC c.71+89GYC c.356+14G>A c.357-90C>T c.388_389delGA	25.8	Pulmonary stenosis
#2	1	c.158_160 dupTCT	8.3	Hypospadias, pulmonary stenosis
#3	2	c.165_166ins	4.2	Ventricular septal defect

Table 2. Clinical characteristics of DBA patients before and after HSCT.

Patients	#1	#2	#3
Nadir of hemoglobin level before HSCT (g/L)	60	36	54
Number of RBC-T before HSCT	9	13	20
Ferritin level before HSCT (μg/L)	2184	1561	3021
Nadir of leukocyte count before HSCT (/μL)	300	1300	2260
Nadir of neutrophil count before HSCT (/μL)	20	160	30
Nadir of thrombocyte count before HSCT (/μL)	21000	34000	4000
Number of severe infections before HSCT	3	2	1
Growth percentile before HSCT	8.0	18.6	50.0
Age at HSCT (months)	9	7	13
Conditioning regimen	Flu/Thio/Threo	Flu/Thio/Threo	Flu/Thio
Donor	MSD	MUD	MUD
Take of neutrophils (days)	12	15	21
Take of thrombocytes (days)	18	14	15
Number of RBC-T after HSCT	0	0	0
Age at latest follow-up (years)	4	3.12	10.4
Chimerism at follow-up (of donor)	100%	100%	100%
Ferritin level at latest follow-up (μg/L)	605	140	359
Growth percentile at latest follow-up	10.0	15.5	50.0

RBC-T indicates red blood cell transfusions; Flu: fludarabine 8 mg/m² day-7; Thio: thiothepa 40 mg/m² day-6 to -3; Threo: threosulfan 14 g/m² day-6 to -3; MSD: matched sibling donor; MUD: matched unrelated donor; GvHD: Graft *versus* Host Disease; Take: persistent neutrophil count of over $500x10^6$/L and persistent thrombocyte count of over $20x10^6$/L.

years is very limited (the youngest reported patient undergoing HSCT was 1.7 years at HSCT). However, successful results in terms of safety, tolerability and efficacy have been reported in other non-malignant diseases, such as thalassemia and metabolic disorders, with transplant related mortality as low as 5% in young low-risk children transplanted from an HLA-matched sibling donor.[10] One major concern is the conditioning regimen-related acute toxicities and the toxic late effects of busulfan, mainly secondary malignancies and infertility.[12-14] In fact, the North American DBAR registry described two DBA patients who developed post-transplant neoplasms after conditioning with a total body irradiation (TBI)-containing regimen, and one patient after a busulfan-including regimen.[2] In thalassemia patients, busulfan has been replaced by threosulfan as a low toxic preparative agent.[10] The consequences of conditioning therefore have to be taken into account, but can be counterbalanced by the risks of developing severe infections or organ dysfunctions in non-transplanted DBA patients.

According to the DBA International Clinical Consensus Conference, the use of corticosteroids is the mainstay of treatment for patients who are transfusion independent. However, their use in infancy is not recommended; deaths have been reported on steroid therapy, as have severe infections.[15] In particular, the initiation of steroid therapy in infancy, prior to the acquisition of immunity to live vaccines, has to be taken into account; and growth retardation can be a concerning complication of steroid treatment, particularly in a disorder that is already associated with short stature. In our patients, steroid therapy was administered early, and some response was observed in myelo- and thrombopoiesis (patient 3); however, no increase in hemoglobin was noted within two to four weeks.

Our small series demonstrates that non-myeloablative conditioning and matched HSCT can be effective in very young patients who are at risk of severe complications, such as life-threatening infections. To the best of our knowledge, our patients are the three youngest patients with DBA to have successfully undergone HSCT. Though further studies with larger patient numbers are needed to critically evaluate its role, our observations are encouraging; thus, HLA-matched donor transplantation should be considered in selected young patients.

Roman Crazzolara,[1,2] Gabriele Kropshofer,[1] Oskar A. Haas,[3,4] Susanne Matthes-Martin[3] and Leo Kager[3,4]

Department of Pediatrics, Medical University Innsbruck; [2]Tyrolean Cancer Research Institute, Innsbruck; [3]St. Anna Children's Hospital, Department of Pediatrics, Medical University Vienna and [4]Children's Cancer Research Institute, Austria

Correspondence: roman.crazzolara@i-med.ac.at
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