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# How I manage children with Diamond-Blackfan anaemia

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## Summary

Diamond-Blackfan anaemia (DBA) is a rare inherited marrow failure disorder, characterized by hypoplastic anaemia, congenital anomalies and a predisposition to cancer as a result of ribosomal dysfunction. Historically, treatment is based on glucocorticoids and/or blood transfusions, which is accompanied by significant toxicity and long-term sequelae. Currently, stem cell transplantation is the only curative option for the haematological DBA phenotype. Whereas this procedure has been quite successful in the last decade in selected patients, novel therapies and biological insights are still warranted to improve clinical care for all DBA patients. In addition to paediatric haematologists, other physicians (e.g. endocrinologist, gynaecologist) should ideally be involved in the care of this chronic condition from an early age, to improve lifelong management of haematological and non-haematological symptoms, and screen for DBA-associated malignancies. Here we provide an overview of current knowledge and recommendations for the day-to-day care of DBA patients.

**Keywords:** Diamond-Blackfan anaemia, marrow failure disorder, hypoplastic anaemia, HSCT, cancer screening.

Diamond-Blackfan anaemia (DBA; Online Mendelian Inheritance in Man reference 105650) is a rare (6–7 per million live births) inherited bone marrow failure syndrome (IBMFS) characterized by hypoplastic anaemia, congenital anomalies and a predisposition to cancer (Vlachos *et al*, 2008). The majority of DBA patients carry haploinsufficient mutations in one of several ribosomal genes, classifying DBA as a "ribosomopathy" (Dianzani & Loreni, 2008). DBA was first described as "anaemia of early infancy" by Hugh W. Joseph in 1936, and was later recognized as a specific clinical entity by Louis Diamond and Kenneth Blackfan in 1938 (Diamond & Blackfan, 1938). Physical anomalies, including craniofacial defects, thumb deformities and short stature, occur in about 50% of patients, and some of the congenital malformations appear to be linked to specific ribosomal gene mutations (Vlachos *et al*, 2001a; Gazda *et al*, 2008; Boria *et al*, 2010; Arbiv *et al*, 2018). Whereas the increased risk of developing cancer is not as high as with other IBFMS (e.g. Fanconi anaemia, dyskeratosis congenita), DBA patients have a significantly elevated risk to develop cancer, in particular haematological malignancies [myelodysplastic syndrome (MDS), acute myeloid leukaemia (AML)], colon carcinoma, osteosarcoma, and urogenital malignancies in female DBA patients (Ruggero & Pandolfi, 2003; Vlachos *et al*, 2012, 2018). More than 65 years after the first report of their effectiveness, glucocorticoids (GC) are still the first line treatment for DBA, to which around 80% of patients show (some) response (Vlachos *et al*, 2008; Sjogren *et al*, 2015). Although many new potential drugs are currently undergoing preclinical trials, the existing alternative treatment options include chronic blood transfusions or haematopoietic stem cell transplantation (HSCT) in a selected group of patients. Given that DBA is a very heterogeneous disease and the course of DBA and DBA-treatment is characterized by complicated long-term sequelae, it is crucial to recognize and manage DBA-related issues from an early age.

Here, we provide an overview on where we stand in this disorder and on what we think the coming years will bring.

## Diagnosis

### *Laboratory evaluation*

In the majority of patients, DBA is diagnosed during early infancy (median age of diagnosis 2 months) based on signs of (persistent) severe anaemia (e.g. pallor, failure to thrive) and reticulocytopenia. Anaemia in DBA is macrocytic or normocytic and, according to the criteria presented in the landmark review (Diamond *et al*, 1976), classical DBA is further characterized by the absence of cytopenias in the other cell lines, and normal bone marrow cellularity with a paucity of erythroid progenitors. However, not all DBA patients present with severe anaemia at a very young age, and cytopenias in other lineages (and no signs of MDS/AML) have been described. In most of

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these cases, the diagnosis is based on genetic analysis or retrospectively, following exclusion of other diagnoses. Evaluation of a bone marrow aspirate can be used to distinguish DBA, typically characterized by normal cellularity, normal myeloid and megakaryocyte maturation, and a selective deficit of red cell precursors, from other hypogenerative anaemias and bone marrow failure disorders. To further support the diagnosis of DBA, in patients with no classical phenotype, additional laboratory evaluation (if feasible) includes erythrocyte adenosine deaminase (eADA) activity, which is elevated in 84% of DBA patients.(Fargo *et al*, 2013) In addition, it has been described recently that erythrocyte reduced glutathione (GSH) can potentially be used as a biomarker for DBA (Utsugisawa *et al*, 2016; Noguchi *et al*, 2017). However, as erythrocyte GSH levels have not been analysed in other IBMFS, it is not known whether this is DBA-specific. Elevated levels of fetal haemoglobin (HbF) and erythropoietin (EPO) further support failure of the erythroid lineage, but are not DBA-specific.

### *Molecular analysis*

The majority (60%) of all heterozygous genetic lesions in DBA involve genes encoding ribosomal proteins (RP), of which 19 (of 80 known RP) genes have been identified so far, including *RPS7, RPS10, RPS15A, RPS17, RPS19, RPS24, RPS26, RPS27, RPS28, RPS29*; *RPL5, RPL11, RPL15, RPL18, RPL26, RPL27, RPL31, RPL35,* and *RPL35A* (Da Costa *et al*, 2018). In addition, there is a rapidly growing list of new (candidate) genes, including *RPL9* (in review), *RPL17* (E.E. Davis, D.W. Reid, J. Liang, J.R. Willer, L. Fievet, Z.A. Bhuiyan, A.L. Wal, J.S. Beckmann, N. Katsanis, C.V. Nicchitta, F. Fellman, unpublished data), and genes identified within genetic deletions (e.g. *RPS8*, *RPS14*) (Gazda *et al*, 2010; Ulirsch *et al*, 2018). Herein, 55% are sporadic (*de novo*) mutations and 45% comprise familial mutations, concerning both affected family members as well as asymptomatic ("silent") carriers, reflecting the variable penetration of RP gene mutations. Given that *RPS19* is the most frequently mutated gene (25% of cases), genetic screening often begins with targeted Sanger sequencing of *RPS19*. However, as a consequence of improved accessibility to newly developed genetic methods, many laboratories nowadays use next generation sequencing (NGS, targeted or whole exome) to analyse gene panels of commonly mutated genes in DBA, or all genes that have been linked to DBA (Da Costa *et al*, 2018), including mutations in non-ribosomal genes, such as *GATA1*, *TSR2, ADA2*, and the recently described biallelic mutations in erythropoietin (EPO) (Sankaran *et al*, 2012; Parrella *et al*, 2014; Ulirsch *et al*, 2018). However, whereas mutations in *GATA1* and *TSR2* have been directly linked to ribosome biogenesis or function, and are therefore generally considered DBA-specific, it makes sense to classify mutations in other non-RP genes separately (Ludwig *et al*, 2014; Van Montfrans *et al*, 2016; Khajuria *et al*, 2018; Schutz *et al*, 2018).

In cases where no RP gene mutation can be identified, the next step in genetic screening is to exclude large gene deletions (missed by NGS) utilizing multiplex ligation-dependent probe amplification, or comparative genome hybridization array. To screen for mutations in non-coding DNA regions (e.g. intronic mutations), whole genome sequencing can be considered, however this is hardly ever done in clinical practice.

In patients with still no molecular diagnosis following extensive genetic testing, additional functional molecular analysis can be performed to further support (or exclude) the diagnosis of DBA based on the quantitative and qualitative analysis of ribosome composition (e.g. 28s/18s ratio) with the use of patient-derived Epstein–Barr virus-immortalized cell lines (LCL) (Aspesi *et al*, 2014, 2018; Quarello *et al*, 2016). However, results should be interpreted very carefully, because these functional tests have not yet been evaluated and validated prospectively. In conclusion, the diagnosis of DBA in a patient without a known genetic mutation is based on the clinical phenotype in combination with functional assays, after the exclusion of alternative diagnoses presenting with hypoplastic anaemia.

### *Screening for congenital anomalies*

Based on data from international DBA registries, it has been estimated that approximately 50% of DBA patients have congenital anomalies, including craniofacial abnormalities, thumb deformities, and structural renal and heart defects (Vlachos *et al*, 2008). It is therefore recommended to perform echocardiogram and renal ultrasound to screen for silent congenital defects at diagnosis. Although the majority of patients have a short stature, and/or suffer from growth delay, it is generally not recorded as a congenital anomaly. There is still little understanding concerning the underlying mechanisms driving an intrinsic growth defect in DBA or other ribosomopathies, as growth delay is certainly multifactorial in patients with DBA as a result of chronic anaemia and its management. Skeletal deformities seem to play a role in at least some cases (Tentler *et al*, 2000; Watkins-Chow *et al*, 2013). Whereas, in line with other ribosomopathies, DBA has been associated with mental retardation, the incidence of neurocognitive conditions in DBA is largely unknown, and has been described most extensively in DBA subtypes involving genetic deletions as part of a clinical syndrome (Tentler *et al*, 2000; Campagnoli *et al*, 2004; Farrar *et al*, 2008).

In general, some of the congenital malformations appear to be linked to specific genotypes, such as an increased frequency of craniofacial and limb defects in patients carrying *RPL5* or *RPL11* mutations, yet no clear genotype-phenotype correlation has been described so far (Gazda *et al*, 2008; Quarello *et al*, 2010; Pospisilova *et al*, 2012; Smetanina *et al*, 2015; Van Dooijeweert *et al*, 2018).

### *Screening of family members*

It is generally assumed that DBA is inherited as an autosomal dominant disorder, although an autosomal recessive


 

inheritance pattern has been suggested in some families. As genetic penetrance is variable and the clinical spectrum is wide, affected family members can be easily missed clinically. It is therefore recommended to screen parents of DBA patients to search for silent carriers or asymptomatic patients, and to identify potentially affected siblings. In DBA patients with a known molecular defect, this can be done by genetic testing of the family members, whereas in patients with no known molecular defect, it makes sense to perform a complete blood count (including reticulocytes and mean cell volume) and eADA activity. Given that haematological manifestations can be intermittent, it is recommended that a complete family history should be obtained, including details of congenital anomalies, non-specific haematological findings and malignancies. While the initial work-up can be performed by the treating physician, it is recommended to involve the clinical geneticist to perform an extended family evaluation and genetic counselling.

## Differential diagnosis

In young children presenting with severe hypoplastic anaemia, transient erytroblastopenia of childhood (TEC) should be excluded, as well as other viral-induced and immune-mediated cytopenias (Vlachos *et al*, 2008; Van den Akker *et al*, 2014; Means, 2016). In some cases, patients with congenital dyserythropoietic anaemia (CDA) present in a similar way (Iolascon *et al*, 2013). As a result of improved molecular testing, functional analysis and increased insights in underlying pathophysiological mechanisms in DBA, distinct disease entities have been discriminated from DBA in recent years, including adenosine deaminase 2 deficiency (DADA2), which can resemble the DBA haematological phenotype, but includes immunological (e.g. hypogammaglobulinaemia), dermatological and neurological ("stroke") phenomena which are typical for DADA2 and very uncommon in DBA (Van Montfrans *et al*, 2016; Meyts & Aksentijevich, 2018). Whether genetic defects in *GATA1*, which do not directly involve a RP mutation, should be classified as classic DBA is debatable, although it has generally been considered as a DBA gene until now (Sankaran *et al*, 2012; Klar *et al*, 2014; Crispino & Horwitz, 2017).

## Treatment

For many years, three therapeutic approaches have been the cornerstone of DBA treatment: erythrocyte transfusions, GC and HSCT, the latter mostly as a salvage option. While HSCT is currently the only curative treatment for the haematological phenotype, it is not frequently performed, because of the risk of severe short term and long-term toxicity, and because spontaneous remission of anaemia has been known to occur in a significant number of DBA patients. In addition, spontaneous self-reverting mutations have been described, yet is most likely very rare (Venugopal *et al*, 2017).

### *Erythrocyte transfusions*

Given that bone marrow dysfunction in DBA generally only concerns erythrocyte production, red blood cell (RBC) transfusions are an effective option to treat anaemia in DBA. However, the toxicity associated with iron overload, concomitant with chronic transfusion regimens, is a limiting factor for lifelong transfusions. DBA patients generally require 10–15 ml/kg per RBC transfusion every 3–5 weeks to keep haemoglobin levels above 80 g/l. Transfusion schemes are further individualized based on growth, neurocognitive development, and general performance. In very young children (generally up to the age of 1 year) RBC transfusions are the first choice of treatment to avoid the toxicities of GC on neurodevelopment and growth (Stark *et al*, 2001; Crotty *et al*, 2012). As infants and young children often require higher haemoglobin levels to maintain adequate growth and development, and transfusion schemes in this group generally aim for haemoglobin levels above 90 g, clinical decisions should be based on the patient's individual requirements. Furthermore, chronic treatment with RBC transfusions is used for patients that show no response on treatment with GC, patients that require unacceptable high doses of GC or have severe GC-toxicity, including patients that are eligible for HSCT, but have no suitable donor available. RBC transfusions can also serve to temporarily interrupt GC use, or be periodically combined with low doses of GC.

### *Glucocorticoids (GC)*

Glucocorticoids have been the only drugs proven to be of use for ineffective erythropoiesis in DBA. While approximately 80% of patients respond to an initial course of GC treatment, the exact mechanism by which they exert a positive clinical effect is still not well understood (Vlachos *et al*, 2008). Hypotheses include an anti-apoptotic effect on early erythroid progenitors, involving p53 (also termed TP53) activity, and improvement of cell-cycle progression and proliferation of erythroblasts (Von Lindern *et al*, 1999; Sjogren *et al*, 2015). Treatment with GC is not recommended in patients less than 1 year old, but can be considered in children for whom venous access for transfusions is a problem. In general, treatment with GC is started in an initial dose of 2 mg/kg/day prednisone for a maximal trial of 4 weeks. It is recommended to start treatment with GC around 2 weeks after transfusion, aiming for Hb levels low enough to prevent suppression of erythropoiesis, and high enough to induce a haematological response (increase of reticulocytes and haemoglobin), which can be expected within a few weeks. After 4 weeks, in case of a response, slow tapering (in particular below doses of 1 mg/kg/day) is indicated to the lowest effective doses. There is a published consensus guideline on

 

tapering GC from 2 mg to 1 mg/kg/day in 8–12 weeks (Vlachos *et al*, 2008). However, in our experience this is accompanied by considerable steroid toxicity, often necessitating an attempt to taper more rapidly. An adequate response is defined as a Hb level >90 g/l in combination with transfusion independency. In general doses up to 0·5 mg/kg/day or 1 mg/kg on alternate days are considered acceptable with regards to long term toxicities (Vlachos *et al*, 2008; Vlachos & Muir, 2010). If transfusions are still needed, the trial is considered a failure. Nevertheless, in selected patients, an acceptable GC dose in combination with erythrocyte transfusions at a very low frequency (a few times per year) can be considered. In patients that do not respond to GC treatment, it is generally recommended to do a second attempt after 12–18 months; a third attempt is not considered to be useful. Some patients require very low maintenance doses of prednisone to keep Hb levels between adequate, and sometimes individually adjusted, levels (mean 80–90 g/l), but cannot stop completely. It is advised to carefully taper low maintenance doses to prevent overshooting the individual minimal effective dose. In patients that are treated long term with GC, physicians should monitor for specific long-term toxicities, including cataract and osteoporosis, which will be further discussed below. In addition, in specific cases in which higher doses of GC (exceeding 0·5 mg/kg/day) are required for longer periods, appropriate preventive measures for infectious complications, especially *Pneumocystis jiroveci* pneumonia, should be considered.

### *Haematopoietic stem cell transplantation*

Based on the consensus recommendations of the Paediatric Working Party of the European Group for Blood and Marrow Transplantation (EBMT), standard indications for haematopoietic stem cell transplantation (HSCT) in DBA include resistance to GC treatment, chronic transfusion dependency and unacceptable GC toxicity (Peffault de Latour *et al*, 2015). Resistance to GC can be defined as no adequate reticulocyte increase after two attempts with a minimum dose of 1 mg/kg/day of prednisone (Peffault de Latour *et al*, 2015). In most guidelines, 0·3–0·5 mg/kg/day of prednisone is accepted as the highest acceptable level to avoid long term toxicities. Reports on HSCT in DBA are still limited, yet have yielded a change in approach in the last two decades based on improved overall long-term results with the use of both family and unrelated donors. A recent report from the Italian cohort describes 30 patients, transplanted between 1990 and 2012, including 14 transplants performed with unrelated donors. The best results were obtained in patients transplanted before the age of 10 years, and patients transplanted after 2000, the latter resulting in a 5-year overall survival (OS) of 86·6%. While donor choice (related *versus* unrelated) did not influence outcome, data suggest that HSCT with stem cells from umbilical cord blood (UCB) leads to inferior outcomes (Mugishima *et al*, 2007; Fagioli *et al*, 2014). Older studies also report a better outcome in children <10 years of age, which is at least partially the consequence of severe iron overload in older children following chronic RBC transfusions (Vlachos *et al*, 2001b).

Based on the available HSCT data in DBA, the following recommendations can be made:

*Donor selection.* Human leucocyte antigen (HLA)-matched family donors (MFD) are still the preferred donor type. However, siblings should be screened genetically for DBA to avoid an HSCT with an asymptomatic DBA carrier donor. Alternative diagnoses that can be present in family donors (e.g. DADA2) should be considered/excluded. In patients with no molecular or alternative diagnosis, and a (local) preference to use a sibling donor, it is recommended to thoroughly screen the potential HLA-identical sibling donor for physical and haematological abnormalities. If there is no MFD available, the best alternative would be bone marrow from a 10/10 allele-matched unrelated donor (MUD). In general, bone marrow (compared to peripheral blood-mobilized stem cells) less frequently leads to severe chronic graft-versus-host disease (cGVHD), an important determinant of HSCT outcome (Vlachos *et al*, 2001b; Fagioli *et al*, 2014). While in general, most studies show comparable results for 9/10 matched unrelated donors and 10/10 matched donors, this is not clear for all indications, and not supported by our own experience (M. Bartels, M. Bierings, unpublished data). In general, the use of a 9/10 MUD is not recommended in DBA.

*Stem cell source.* Data from HSCT studies utilizing UCB stem cells as a donor source have suggested an inferior outcome so far (Mugishima *et al*, 2007; Fagioli *et al*, 2014), indicating that UCB is currently not the preferred stem cell source. For many indications, and haemoglobinopathies in particular, haplo-identical transplantation is being explored, using either *ex-vivo* T-cell depletion or cyclophosphamide post-transplantation to deplete haplo-identical T cells.

*Conditioning.* An international expert panel recommended the use of either busulfan- or treosulfan-based myeloablative conditioning, for bone marrow failure disorders (Parikh *et al*, 2014; Peffault de Latour *et al*, 2015; Burroughs *et al*, 2017). All recommendations are based on small case series. While data concerning HSCT in DBA utilizing reduced intensity conditioning (RIC) regimen are still very limited, this approach can be considered in selected cases (Asquith *et al*, 2015; Crazzolara *et al*, 2017). Chronically transfused DBA patients, in addition to iron overload, often have HLA-antibodies following numerous RBC transfusions. Therefore, similar to patients with homozygous beta-thalassaemia or other transfusion-dependent haematological disorders, a modified preconditioning regimen can be considered as an additional pre-treatment in order to decrease the risk of graft-rejection (Shenoy & Thompson, 2016; Zaidman *et al*, 2016).


 

*Toxicity.* Short term toxicities of HSCT include the risk of (severe) GVHD as well as infectious complications. In addition, most relevant chronic toxicities include fertility issues and the risk for secondary malignancies, which may be of even greater relevance in DBA given that it is a cancer predisposition syndrome (Dietz *et al*, 2017a). Guidelines for the long-term follow-up of marrow failure patients after HSCT have recently been updated (Dietz *et al*, 2017b). Counselling for techniques aiming to preserve fertility prior to transplant is indicated, including ovarian preservation and/or oocyte or sperm cryopreservation (Bastings *et al*, 2012; Jensen *et al*, 2017; Stukenborg *et al*, 2018) irrespective of age and sex, and is currently offered to all HSCT patient in our centre before starting chemotherapy. In patients with severe iron overload, post-transplantation treatment with intermittent phlebotomy is indicated (and first-choice approach) as long as the graft tolerates this iron-depleting procedure (Kew *et al*, 2015).

### *Alternative treatments*

Various alternative therapeutic approaches have been attempted that have generally resulted in anecdotal reports of success. The best-known examples include erythropoietin, metoclopramide, cyclosporine and leucine supplementation. The use of metoclopramide in DBA, based on the effect of stimulating prolactin excretion from the pituitary gland, was started following an observation in a female patient with DBA, who significantly improved during the second and third semester of three pregnancies and during lactation. A positive effect of metoclopramide has been demonstrated in some patients, yet seems to be very limited in patients that are transfusion dependent (Abkowitz *et al*, 2002; Leblanc *et al*, 2007). As metoclopramide has no serious side effects, it can be considered in patients that are refractory to low doses of GC. Treatment with recombinant Interleukin 3 (IL3), a stimulator of erythropoiesis at the stem cell level, has been studied very extensively in DBA in the past, but was eventually discarded due to conflicting results and undesired side effects, such as deep venous thrombosis and hypereosinophilia (Gillio *et al*, 1993; Olivieri *et al*, 1994; Ball *et al*, 1995).

Leucine, an essential amino acid that plays an important role in the regulation of protein synthesis, and an activator of the mechanistic target of rapamycin (mTOR), has been studied in animal models of *RPS19*- or *RPS14*-deficient DBA, and tested clinically in a small number of patients and as part of a pilot phase I/II study performed by the North American Diamond-Blackfan Anemia Registry (DBAR) study partners (NCT 01362595) (Pospisilova *et al*, 2007; Jaako *et al*, 2012; Payne *et al*, 2012; Ovsyannikova *et al*, 2015). Based on the effects in other hypoplastic bone marrow failure syndromes, ciclosporin and androgens have been tested in DBA for a long time. The use of these drugs is now discouraged because the positive effects on erythropoiesis were very limited and generally transient (reviewed in (Narla *et al*, 2011). None of the alternative therapies discussed here are included in the current DBA guidelines (reviewed in Narla *et al*, 2011).

### *Novel treatment options*

It is frustrating for physicians, researchers and patients that, more than 40 years after their discovery, GC are still the only effective drugs in DBA. Therefore, searching for therapeutic alternatives, new techniques and bioinformatics approaches integrating high throughput data are used in order to better understand the biological networks in DBA. This can lead to the identification of novel therapeutic targets and/or result in improved, and potentially predictive biological models (Khan *et al*, 2018). Based on a chemical compound screen on reprogrammed haematopoietic progenitors from patient-derived induced pluripotent stem cells, SMER28, a small-molecule inducer of autophagy, was recently identified as a potential new drug for DBA, and its therapeutic potential will be further investigated in (pre)-clinical trials in the coming years (Doulatov *et al*, 2017). In line with other monogenetic disorders, gene therapy would be an attractive therapeutic option for DBA patients in whom the underlying genetic defect can be identified. Given that 25% of patients carry a pathogenic mutation in *RPS19*, most studies currently focus on gene editing in haematopoietic stem cells in this molecular subgroup. The results of these pre-clinical studies, using retroviral vectors, lentiviral vectors or Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) technology, are promising for the future, demonstrating correction of severe anaemia and bone marrow failure in *RPS19*-deficient DBA mouse models (Jaako *et al*, 2014; Debnath *et al*, 2017; Bak *et al*, 2018).

## Comorbidities

### *Iron overload*

Iron overload is a large concern in patients that require chronic blood transfusions. In addition to HSCT-related mortality, transfusion-associated iron overload is a leading cause of mortality in DBA patients (Lipton *et al*, 2006). Whereas serum ferritin levels can be used as an indicative parameter for iron overload, and to evaluate the effect of chelation therapy, it is not reliable to determine total body iron burden. The best, and most feasible way to analyse iron overload is to perform magnetic resonance imaging (MRI)-based measurements of hepatic, cardiac and pancreatic iron burden. Due to technical improvements in the last decade, MRI has replaced liver biopsy in the majority of patients (Hernando *et al*, 2014; Viprakasit *et al*, 2018). From MRI images, the liver iron concentration (LIC, normal <3 mg/g dry weight, acceptable 3–7 mg/g), and myocardial T2* (normal $\approx$ 40 ms, never <20 ms) can be calculated. The myocardial T2* is a surrogate for myocardial relaxation, and reflects the ejection fractions and signs of cardiac failure due to iron

 

overload. It is recommended to measure liver iron content every 12–18 months in patients on chronic RBC transfusion treatment. It is generally recommended to screen for iron overload and start chelation therapy after 10–20 RBC transfusion (of 10–15 ml/kg), or when the MRI-measured LIC reaches ≥6–7 mg/g. Alternatively, if MRI is not available or applicable, serum ferritin levels of ≥1000 μg/l and/or transferrin saturation levels ≥75% have been used as a starting point for chelation therapy. While chelating drugs are not registered yet for children younger than 2 years old (deferiprone <6 years), results of recent studies in younger children with transfusion-related iron overload are promising (Roggero *et al*, 2009; Berdoukas *et al*, 2013; Marsella & Borgna-Pignatti, 2014; Origa *et al*, 2016; Bellanti *et al*, 2017; Taher & Saliba, 2017; Elalfy *et al*, 2018).

Interestingly, pancreatic iron burden highly correlates with cardiac iron, whereas hepatic iron burden in DBA does not predict cardiac iron burden, which is in contrast to what has been shown in patients with thalassaemia. Moreover, and further underlining the importance of closely monitoring iron overload in DBA, it has been demonstrated that iron overload in transfusion-dependent DBA is generally more severe than iron overload in beta-thalassaemia (Roggero *et al*, 2009; Taher & Saliba, 2017).

### *Endocrine dysfunction*

Endocrine problems are very common in DBA, even at a young age. Obviously, chronic treatment with GC and iron overload following chronic blood transfusions play an important role in endocrine dysfunction, including growth impairment, hypocortisolism, osteoporosis and avascular necrosis, diabetes and pubertal delay. In addition, hypogonadism and hypothyroidism have been related to iron overload (Lanes *et al*, 2000; Chen *et al*, 2005; Noetzli *et al*, 2012; Lahoti *et al*, 2016; Muir *et al*, 2017). Given that some endocrine abnormalities reflect significant and potentially irreversible organ damage, it is advised to regularly screen for endocrine dysfunction. This includes graphing height and weight (including body mass index) every 6 months and checking indicators for gonadal insufficiency (e.g. delayed puberty, impaired pubertal progression) in chronically transfused patients, and screening for hypocortisolism and diabetes in transfused patients and patients treated with corticosteroids. In addition, it is recommended to prescribe vitamin D supplementation in all DBA patients and perform periodic bone density measurements. In patients with reduced growth velocity or a short stature; bone age and indicators of growth hormone (GH) deficiency [insulin-like growth factor (IGF)1, IGF-B3] should be analysed. In selected patients, treatment with GH is indicated, but should be considered carefully in the context of the increased cancer risk in DBA patients (Alter, 2004; Scott *et al*, 2004; Howell *et al*, 2015; Lahoti *et al*, 2016). It is advised that patients aged >14 years, particularly transfused patients, are screened for hypothyroidism. Altogether, with regards to the complexity of (potential) endocrine dysfunction, it is therefore advised that an endocrinologist should be involved in the management of all chronically transfused patients and children that are treated with prednisone ≥0·3 mg/kg/day for more than a year (Chen *et al*, 2005; Lahoti *et al*, 2016).

## Transition to adulthood

DBA can be a capricious condition: spontaneous remissions of anaemia are well-known and can last for years. Recurrence of anaemia is however quite common, if not the rule. Therefore, patients should be clearly warned and monitored closely into and during adulthood. DBA is a lifelong condition that demands regular follow-up of haematological parameters, screening for primary and secondary endocrine dysfunction, early recognition of (pre)malignant conditions and specialized support of pregnancies. In addition, genetic counselling should be considered in certain cases. It is our experience that transition of care for children with DBA to the adult care settings is challenging. Explanations for this include the lack of knowledge and expertise of general haematologists, the unpredictability of the disease, both haematological and non-haematological, and the autonomy of adolescent and young adult patients that are no longer under the watch of their parents. In our opinion, the best model would be to offer care in a lifelong setting by a team of experts, integrating paediatricians, adult haematologists (and oncologists), endocrinologists, gynaecologists, as well as all the supportive staff. Ideally, this implicates that transition from paediatric to adult care should start at a relatively young age, preferably between 12–14 years of age. Similar models are used in patients with sickle cell anaemia and thalassaemia, and have proven to be effective in making the transition and in improving patient adherence.

## Fertility/pregnancy

Within the large variety of endocrine issues in DBA, recent clinical observational studies suggest that women with DBA have an increased incidence of delayed puberty, irregular menstrual cycles and decreased fertility (Lanes *et al*, 2000; Tufano *et al*, 2014; Lahoti *et al*, 2016). It has been demonstrated that pregnancies in DBA are decreased in numbers and more frequently complicated compared to the general population, reflected by a high prevalence of spontaneous abortions (Faivre *et al*, 2006; Alter *et al*, 2010). In agreement with this, recent studies have reported the outcome of pregnancies in women suffering from IBMFS, including DBA, illustrated by a high level of spontaneous abortions in DBA (Gansner *et al*, 2017; Giri *et al*, 2017). Moreover, pregnancies in mothers with IBMFS-affected fetuses have been identified as high-risk pregnancies, associated with prenatal IBMFS-symptoms and severe postnatal phenotypes, requiring professional counselling and monitoring of subsequent pregnancies.


 

## Cancer predisposition

Whereas the increased incidence of malignancies (both haematological malignancies and solid tumours) had been assumed for many years, based on a large number of case-reports, the real cancer incidence was first quantitatively assessed within the DBAR in 2012 and has very recently been updated. It was demonstrated that the incidence of malignancies was significantly increased in DBA patients, illustrated by an observed-to-expected (O/E) ratio of 4·84 ($P < 0·05$, all cancer types) with a median age at presentation of 35 years (range 11–70 years). In comparison with two other IBMFS, the O/E ratio was much lower (Fanconi anaemia O/E ratio 39–79; dyskeratosis congenita O/E ratio 11), suggesting that cancer predisposition is less pronounced in DBA (Alter *et al*, 2010; Vlachos *et al*, 2012). However, and more importantly, O/E ratios for the incidence of specific cancer types in DBA are striking, including for myelodysplastic syndrome (O/E ratio 352·1), acute myeloid leukaemia (O/E ratio 28·8), colon carcinoma (O/E ratio 45) and osteogenic sarcoma (O/E ratio 42·4). Overall, the cumulative incidence of evaluable cancers was 13·7% by age 45 years (Lipton *et al*, 2001; Vlachos *et al*, 2012, 2018).Whereas pathophysiological mechanisms underlying cancer development have been studied in animal models of DBA and other models involving RP (or acquired ribosomal defects), including 5q- MDS and leukaemia, predicting cancer risk in individual patients or patient subgroups (e.g. based on molecular defect) has been impossible until now (Ebert *et al*, 2008; MacInnes *et al*, 2008; Pellagatti *et al*, 2008; Girardi & De Keersmaecker, 2015; Fancello *et al*, 2017; Vlachos, 2017). Hopefully, based on updates from DBAR and other large patient registries (e.g. within the EuroDBA consortium) on the incidence, types and distribution of malignancies, we will have more insights into this in the upcoming years. Irrespective of the available data, it is recommended to closely monitor adult DBA patients and to regularly screen for malignancies. Whether this includes performing regular colonoscopies and bone marrow examination is still under debate.

## Future perspectives

Increased awareness will improve the early recognition and appropriate treatment of patients with rare diseases, including DBA. In recent years, rare genetic diseases have gained increased attention. As a result, this can speed up the implementation of innovative approaches for treatment of DBA. Large international collaborations, including DBAR (www.dbar.org) and the EuroDBA network (www.eurodba.eu), have proven to be crucial in the ongoing developments in clinical management of DBA and fundamental research. While both national and international collaborations can be challenging, impressive progress has been made with the DBA networks in recent years, and creating a global DBA network is starting to be established (Da Costa *et al*, 2018). We therefore encourage clinicians to identify and contribute to networks involved in the care for, and registration of, DBA patients and participate actively in these networks. Moreover, in line with other rare and very complex disorders, patients should be referred to centres of expertise for consultation and top-level treatment modalities, including HSCT. In addition, every day care and support in emergency situations, should be organized in a shared care setting in nearby hospitals. In particular, the transition to adult care and long-term follow-up, with a focus on early recognition of cancer, clearly needs improvement. The care of patients with rare diseases in adult medicine is behind on the improvement in paediatric care systems. We advocate the early initiation of transition from paediatric to adult care, to avoid abruptness and loss to follow-up in a vulnerable adolescent age group. Whether screening for DBA will prove (cost-)effective remains to be determined. Given the low incidence of the condition and the apparently easy recognition on clinical grounds (pallor, reticulocytopenic anaemia), this seems rather doubtful. Gene therapy and gene editing hold great promise for future treatments but are currently of limited impact for DBA. Current bioinformatic approaches integrating high throughput data to better understand biological networks and the use of new biological models may lead to the identification of novel potential therapeutic targets and the development of new therapeutic approaches (Doulatov *et al*, 2017; Khan *et al*, 2018).

## Authorship

MBa designed, wrote and reviewed the paper. MBi wrote and reviewed the paper.

## Conflict of Interest

Both authors have no conflicts of interest.

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