

# blood

2010 116: 3715-3723  
Prepublished online July 22, 2010;  
doi:10.1182/blood-2010-02-251090

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## How I treat

# How I treat Diamond-Blackfan anemia

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**Diamond-Blackfan anemia (DBA) is characterized by red cell failure, the presence of congenital anomalies, and cancer predisposition. In addition to being an inherited bone marrow failure syndrome, DBA is also categorized as a ribosomopathy as, in more than 50% of cases, the syndrome appears to result from haploinsufficiency of either a small or large subunit-associated ribosomal protein.**

**Nonetheless, the exact mechanism by which haploinsufficiency results in erythroid failure, as well as the other clinical manifestations, remains uncertain. New knowledge regarding genetic and molecular mechanisms combined with robust clinical data from several international patient registries has provided important insights into the diagnosis of DBA and may, in the future, provide new treat-**

**ments as well. Diagnostic criteria have been expanded to include patients with little or no clinical findings. Patient management is therefore centered on accurate diagnosis, appropriate use of transfusions and iron chelation, corticosteroids, hematopoietic stem cell transplantation, and a coordinated multidisciplinary approach to these complex patients. (*Blood* 2010;116(19):3715-3723)**

## Introduction

Diamond-Blackfan anemia (DBA; Mendelian Inheritance in Man #105650), one of a rare group of inherited bone marrow failure syndromes (IBMFSs), is characterized by red cell failure, the presence of congenital anomalies, and cancer predisposition. DBA is now also categorized as one of an emerging group of disorders known as ribosomopathies.<sup>1</sup> The molecular biology of DBA is being extensively explored and, in more than 50% of cases, the syndrome appears to result from haploinsufficiency of either a small or large subunit-associated ribosomal protein.<sup>2-8</sup> Nonetheless, the exact mechanism by which ribosomal protein haploinsufficiency results in erythroid failure, as well as the other clinical manifestations, remains uncertain. During the past year, a great deal of the molecular biology of DBA has been clarified, leading to a rudimentary understanding of the relationship between ribosomal protein haploinsufficiency, nucleolar function, translational surveillance, erythroid failure, and cancer predisposition.<sup>9</sup> This explosion in knowledge combined with robust clinical data from several international patient registries has provided important insights into the diagnosis and treatment of DBA. Indeed, diagnostic criteria have been expanded to include "patients" with few or no clinical findings.<sup>10</sup> We also have the opportunity to provide at-risk couples with information regarding reproductive choices. These choices are complex, expensive, and, for many, controversial. However, for those who choose it, preimplantation genetic diagnosis may provide the option of conceiving unaffected children. Furthermore, families may avail themselves of the additional step of conceiving a human leukocyte antigen (HLA)-compatible stem cell transplantation donor for an affected sibling.<sup>11,12</sup>

The science of DBA is burgeoning. Apoptotic pathways, presumably leading to erythroid failure, are becoming relatively well understood. In the near future, an article such as this will probably include treatment options that avail themselves of the ability to modulate these cell death pathways. Until then, patient management is centered on accurate diagnosis, appropriate use of transfusions and iron chelation, corticosteroids, hematopoietic

stem cell transplantation (HSCT), and a coordinated multidisciplinary approach to these complex patients.

## How we make the diagnosis of DBA

The diagnostic criteria have expanded dramatically as a consequence of gene discovery and improved knowledge of DBA epidemiology.<sup>10</sup> The classic presentation of DBA includes a usually macrocytic, or occasionally normocytic, anemia with reticulocytopenia, essentially normal neutrophil and platelet counts, and a normocellular bone marrow with a paucity of erythroid precursors, in a child younger than 1 year.<sup>13</sup> Results obtained from the Diamond Blackfan Anemia Registry of North America (DBAR) show that 50% of patients are diagnosed by 3 months of age, 75% by 6 months, and 92% within the first year of life.<sup>14</sup> Only 10% of patients present with clinically significant anemia at birth, and hydrops fetalis is rarely seen. With the identification of mutations in 9 unique genes in patients with DBA (Table 1), a large number of nonclassic cases of DBA have been identified. These persons may have mild hematologic findings or DBA-associated congenital anomalies or may be completely hematologically and physically normal. Furthermore, patients may present beyond one year of age into late childhood, adolescence, or even adulthood. With the recognition of these nonclassic phenotypes, the number of autosomal dominant inherited cases has been estimated, for at least one genotype, to be approximately 45%.<sup>15</sup> The remainder of the mutations are sporadic and appear to represent new dominant cases, although autosomal recessive inheritance may yet be demonstrated.

When we suspect DBA, our initial laboratory evaluation includes a complete blood count, reticulocyte count, and if possible, fetal hemoglobin (HbF) level and an erythrocyte adenosine deaminase (eADA) activity. Persons with consistent physical anomalies or family history, found to have a hypoproliferative anemia, or an otherwise unexplained macrocytosis or elevated

**Table 1. DBA genes**

Gene	Approximate percentage of cases	Locus	Gene product
<i>RPS19</i>	25	19q13.2	RPS19
<i>RPS24</i>	2	10q22-q23	RPS24
<i>RPS17</i>	1	15q25.2	RPS17
<i>RPL35a</i>	4	3q29-qter	RPL35a
<i>RPL5</i>	7	1p22.1	RPL5
<i>RPL11</i>	5	1p36-p35	RPL11
<i>RPS7</i>	1	8q21.11	RPL7
<i>RPS26</i>	6	12q13	RPS26
<i>RPS10</i>	3	6p21.1-21.3	RPS10
Total	54		

HbF, even without anemia, should be investigated further. The majority of patients have macrocytosis, with an elevation in HbF.<sup>13</sup> eADA activity is elevated, for reasons not entirely understood, in 80% to 85% of DBA patients.<sup>16,17</sup> Transfusion within the previous 8 to 12 weeks may result in a false normal eADA activity. If the eADA activity cannot be obtained before transfusion, selected laboratories may be able to determine activity in reticulocytes. eADA activity usually remains elevated, even in patients who have achieved a remission or are hematologically stable on corticosteroids.

A bone marrow aspirate is required for diagnosis and usually reveals a normocellular marrow with normal myeloid maturation, adequate megakaryocytes, and a selective paucity of red cell precursors. A bone marrow biopsy is also recommended to assess cellularity. Cellularity has been noted to decrease disproportionately to the usual age-related decrement.<sup>18</sup> A routine karyotype is necessary to identify any major chromosomal abnormalities. Indeed, 2 of the “DBA genes” have been identified through the evaluation of a translocation involving the gene encoding *RPS19*<sup>2</sup> and a large deletion on chromosome 3q involving the coding region for *RPL35a*,<sup>6</sup> respectively.

If the diagnosis of DBA is not definitive by genotyping and/or diagnostic criteria, then we screen patients for the other IBMFS, as these too may present with macrocytic anemia or isolated macrocytosis. Chromosomal breakage analysis is done to screen for Fanconi anemia, especially if the eADA activity is not elevated. A complete medical history is taken with particular attention to diarrhea and malabsorption, suggestive of pancreatic insufficiency and a possible diagnosis of Shwachman-Diamond syndrome, especially if neutropenia is present. Careful examination of the bone marrow to search for vacuolization in hematopoietic precursors is important to identify Pearson syndrome. Testing for mitochondrial DNA deletions may be necessary if clinical signs and symptoms of Pearson syndrome, such as metabolic acidosis and/or exocrine pancreatic dysfunction along with anemia, are evident. The most common misassignment of patients referred to the DBAR has been in those later demonstrated to have Shwachman-Diamond syndrome, followed by Pearson syndrome. Telomere length is assayed to rule out the diagnosis of dyskeratosis congenita. This is especially important in the younger child presenting with macrocytosis who may not yet exhibit the characteristic cutaneous or oral manifestations of dyskeratosis congenita. Patients who present with macrocytosis and pure red cell aplasia should be evaluated for myelodysplastic syndrome (MDS), including flow in situ hybridization for 5q-, monosomy 7, and trisomy 8 and 9. One class of MDS (ie, 5q- syndrome) has now been shown to be the result of an acquired *RPS14* mutation.<sup>19</sup> Although not yet described, the presentation of this syndrome in childhood is

plausible. If the bone marrow morphology is consistent with giant, multinucleated erythroblasts and pronormoblasts, or if there is no genetic evidence supporting the diagnosis of DBA, particularly in atypical presentations, viral titers and evaluation for viral genome are performed to rule out parvovirus B19 as a cause.

Mutation analysis for the known “DBA genes” is also performed in the patient for whom there is a clinical suspicion of DBA. To date, commercial genotyping laboratories can evaluate the patient for 6 of the 9 known mutated DBA ribosomal protein genes. The presence of a gene mutation confirms the diagnosis of DBA. However, these mutations currently are found in only 50% of the patients. In addition, genotyping is not available everywhere and is quite expensive. Thus, a gene mutation may not be identified to confirm the diagnosis, but the diagnosis will depend on the clinical and laboratory evaluation. For these patients, genotyping must be the goal, as a precise knowledge of the genetics is currently required not only for reproductive counseling but also for transplantation donor selection. In the future, screening strategies may emerge as certain genotypes may correlate with cancer predisposition or other risks. Gene discovery research efforts are ongoing to identify additional mutations in other ribosomal protein genes or in genes encoding nonribosomal proteins. Clinicians are encouraged to make these studies available to their patients, but diagnostic caution must be observed until new mutations are verified in clinically certified laboratories.

## How we evaluate and manage congenital anomalies

Congenital anomalies are not an insignificant problem for patients with DBA, and their management requires the coordination of diverse caretakers. Of patients enrolled in the DBAR, approximately 50% have congenital anomalies. The most common anomalies are craniofacial (50%), skeletal, mostly upper limb and hand (39%), genitourinary (38%), and cardiac (30%; Table 2). More than one anomaly was found in 21% of the patients. Short stature was found in many patients but was excluded in the analysis as a congenital anomaly. The etiology of short stature is multifactorial and complex and may be the result of the disease itself, chronic anemia, the result of chronic steroid therapy starting at an early age or severe transfusion-acquired iron overload, leading to growth hormone deficiency. Echocardiogram and renal ultrasound are performed to identify any “silent” congenital anomalies. Congenital anomalies may be the only clue, once

**Table 2. Range of congenital anomalies observed in DBA**

Organ/system	Anomalies
Craniofacial	Hypertelorism, cleft lip and/or palate, high arched palate, microcephaly, micrognathia, microtia, low-set ears, low hairline, epicanthus, ptosis
Ophthalmologic	Congenital glaucoma, strabismus, congenital cataract
Neck	Short neck, webbed neck, Sprengel deformity, Klippel-Feil deformity
Musculoskeletal	Short stature, thumb (hypoplastic, duplex or bifid, triphalangeal) syndactyly, flat thenar eminence
Urogenital	Kidney (absent, horseshoe), duplicated collecting systems, hypospadias
Cardiac	Ventricular septal defect, atrial septal defect, coarctation of the aorta, patent foramen ovale, tetralogy of Fallot, complex cardiac anomalies

The list includes the anomalies that are most characteristic of DBA. Multiple anomalies are present in up to 25% of affected persons.

a proband is identified, to the recognition of nonclassic familial cases. To date, it appears that all the genotypes have associated congenital anomalies, although the nature and frequency of particular anomalies vary considerably among genotypes. In the DBAR, none of patients with mutations encoding *RPS19* has orofacial clefts, and all the patients with cleft palate who have been genotyped have *RPL5*<sup>7</sup> mutations. This genotype/phenotype correlation is the first such correlation made. Recently, 2 patients, identified to have mutations in *RPL11* and *RPS26*, respectively, also have orofacial clefts.<sup>8,20</sup> However, not all the patients with orofacial clefts have mutations in these *RP* genes. Patients with mutations in *RPL11* also have limb anomalies.<sup>7,20</sup>

We attempt to perform any corrective surgery before the start of steroid therapy, if possible, to facilitate adequate wound healing. If the patient is already on steroid therapy, surgical intervention can be delayed, where feasible, until the patient is off steroid therapy or on the lowest possible dose.

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## How we evaluate the family of the DBA patient

There is very strong evidence that DBA is inherited as an autosomal dominant with variable penetrance and expressivity. Some multiplex families have inheritance patterns suggestive of an autosomal recessive mode of inheritance, but this has not yet been proven. To date, 9 *RP* genes are found to be mutated in DBA patients: 6 of the small subunit (*RPS7*, *RPS10*, *RPS17*, *RPS19*, *RPS24*, and *RPS26*) and 3 of the large subunit (*RPL5*, *RPL11*, and *RPL35a*). (Other genes have been described in individual families only and as yet have not been confirmed.<sup>8,21</sup>) There have not been any patients with more than one mutation in a single gene encoding a ribosomal protein. We perform a complete blood count, reticulocyte count, hemoglobin F, and eADA activity on the parents and siblings of the DBA patient, searching for mild or asymptomatic carriers of the DBA gene. Macrocytosis and/or elevated eADA, with or without anemia, may be signs of familial DBA. Indeed, in the United Kingdom DBA Registry, the known *RPS19* mutation was found in 40% to 45% of family members.<sup>15</sup> These families consisted of the proband, family members with classic DBA, family members with no hematologic evidence of DBA, and those with congenital anomalies without anemia. Thus, we obtain a complete family history, including a history of congenital anomalies, any persistent or intermittent anemias (ie, anemia with pregnancy requiring transfusion), as well as a history of macrocytosis or presumed pernicious anemia and possible treatment with folate or vitamin B<sub>12</sub>. Any such findings prompt an extended family evaluation. If a genotype is identified in the proband, then genotyping is performed in the parents. Gonadal mosaicism has been reported; thus, siblings are evaluated even if genotyping does not reveal autosomal dominant inheritance.<sup>22</sup> Other “possibly affected” family members should also be evaluated, even if they have no hematologic manifestations at present, as they may become anemic in the future. They should be advised to seek routine follow-up and medical attention should symptoms arise. Detailed genetic counseling is required for the family. We carefully review inheritance, emphasizing the variability in expression of a mutated gene and counsel regarding reproductive choices when appropriate. In this process, we invite all at-risk extended family members for evaluation and counseling. There are no data as yet to advise the necessity of bone marrow evaluations in these “silent” patients, and we are not yet able to offer advice regarding the nature and frequency of cancer screening.

In vitro fertilization with preimplantation genetic diagnosis is also available for families with known genetic mutations allowing for the selection of unaffected embryos.<sup>12</sup> Parents who avail themselves of this option in the conception of subsequent children often do so while simultaneously selecting an HLA-identical donor for the DBA patient.<sup>23</sup> Parental genetic screening reduces the risk of dominant transmission, but gonadal mosaicism is possible. Autosomal recessive inheritance has not been demonstrated to date, but some pedigrees are consistent with that mode of inheritance. Families need to be adequately counseled as in vitro fertilization with preimplantation genetic diagnosis is physically and emotionally demanding, expensive, and not foolproof. Unavoidable errors can occur, and preimplantation genetic diagnosis for HLA typing alone, without an identifiable gene defect, can inevitably lead to an affected child in any genetic disorder.

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## How we treat DBA

Our current mainstays of treatment are red cell transfusions, corticosteroid therapy, and HSCT. Data reported to the DBAR regarding initial corticosteroid response do not differ substantially from the literature. Approximately 80% of patients will initially respond to corticosteroid therapy, whereas 20% do not respond and require chronic red cell transfusion therapy. However, cross-section analysis of the DBAR reveals that only 37% of patients remain on steroid therapy, 31% receive chronic transfusion therapy, 13% are in remission, 9% have undergone HSCT, and 9% are deceased.<sup>24</sup> At presentation, the majority of patients require stabilization with red cell transfusion. Until recently, all anemic DBA patients received corticosteroids on diagnosis. With the recognition that corticosteroids have a profound effect on linear growth as well as physical and neurocognitive development in infants,<sup>25,26</sup> we delay the start of steroids, if possible, and maintain infants on chronic transfusion therapy until one year of age. If the blood product supply in any locale is deemed unsafe or if maintaining venous access sufficient for chronic transfusions is problematic and requires placement of a venous access device, we recommend that steroids be started earlier. If a patient is having growth difficulties despite being on transfusion therapy, we occasionally delay the onset of steroid therapy further. Both steroid and transfusion therapy are aimed at maintaining a stable hemoglobin, adequate for physical growth and cognitive development. As it is not necessary to suppress ineffective erythropoiesis, as in thalassemia, we transfuse at hemoglobin (Hb) levels of 8 g/dL with 10 to 15 mL/kg of leukocyte-depleted, packed red blood cells. Thus, hemoglobin levels are generally maintained between 8 and 11 g/dL. Immediate family members are disqualified as blood donors as allosensitization should be avoided so as to not jeopardize a future HSCT.

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## Steroid therapy and toxicity

Our corticosteroid starting dose is 2 mg/kg per day, usually as prednisone or prednisolone, for a maximum trial of 4 weeks. Generally, we commence treatment 1 to 2 weeks after a transfusion (when the Hb is 9-10 g/dL) and follow weekly complete blood count and reticulocyte count. In this way, there is sufficient erythroid drive, not blunted by a high Hb level. A meaningful steroid response usually will occur within the first few weeks. We define an adequate response as a Hb level greater than 9 g/dL, without the need for transfusion. If the hemoglobin continues to

decrease and the patient needs a subsequent transfusion, then the steroid trial is considered a failure and is discontinued. Neither higher doses of steroids nor treatment beyond 4 weeks is recommended as the institution of either is probably predictive of an inability to taper to a safe and effective dose. Occasionally, a patient may have a partial response with stable Hb between 8 and 9 g/dL. If the steroid taper leads to transfusion requirement, then steroid treatment is again considered a failure. If the patient can be weaned and is comfortable and growing, this level may be acceptable. If a patient has no response or an ineffective partial response to steroid therapy, a second trial is attempted after 12 to 18 months, as some children may subsequently become responders. Once the patient has a response, then a steroid taper should ensue. We prefer a low-dose, alternate-day regimen. The steroids should be maintained at the lowest dose at which the patient has a sustained response. This target maintenance dose should not exceed 0.5 mg/kg per day or 1 mg/kg every other day.<sup>10</sup> If the patient is not able to be weaned to this low, acceptable dose, then chronic transfusion therapy should be reinstated. The speed of the taper, once a "safe" dose is achieved, needs to be very slow; seemingly homeopathic dosing may be achieved in some patients. Some patients require doses only twice or thrice weekly to maintain an adequate hemoglobin level.

The target steroid dose may change as more data become available regarding the toxicity of therapy. There are no other diseases for which patients receive potentially life-long steroid therapy beginning in infancy. Thus, even at low doses, there may be significant side effects. Pathologic fractures, cataracts, and avascular necrosis have been noted at alarming rates in DBA patients.<sup>10,24</sup> Therefore, we perform periodic bone densitometry determinations on DBA patients. A baseline evaluation is done as soon as the test can be accomplished without sedation, usually by the age of 5 years. If the result shows significant demineralization, then we generally start a steroid taper with probable conversion to transfusion therapy. We also institute a steroid hiatus in those patients with pathologic fractures.<sup>24</sup> Treatment with bisphosphonates may be necessary, but many experts only recommend therapy for patients with reduced bone mass and recurrent fractures. Current data are inadequate to support the use of these agents in children to treat reductions in bone mass/density alone. We are awaiting more definitive safety and efficacy data before making such a recommendation.<sup>27,28</sup> Ophthalmologic examinations are necessary in steroid-treated DBA patients to diagnose and treat cataracts and glaucoma before significant damage to vision occurs. Hypertension and diabetes are also possible but are less probable as long as patients are not maintained on high doses of steroids. We cannot emphasize enough the need for constant monitoring even for patients on seemingly "safe" doses of corticosteroids.

It is imperative that an accurate growth chart be maintained for each patient, with intervention of the steroid dose for failure to maintain adequate growth. Growth during the first year of life and during puberty is essential to achieving the patient's height potential. In DBA, the patient's final adult height can be influenced by (1) chronic anemia, (2) long-term steroid use, (3) iron overload leading to endocrinologic-mediated growth failure, and (4) constitutional factors resulting from the underlying DBA diagnosis. Optimizing therapy by finding the lowest possible steroid dose that affords the best hemoglobin or completely weaning the steroids ("steroid hiatus") is advisable during puberty in general, and in particular, if growth is compromised.

We institute trimethoprim/sulfamethoxazole for *Pneumocystis jirovecii* (formerly *P carinii*) pneumonia prophylaxis (or pentami-

dine for patients with low neutrophil counts at baseline) after the first month of high-dose steroids. This is usually continued until the patient is on "low" dose, alternate-day therapy. ("Low or moderate" dose is considered by *Red Book* as "receiving < 2 mg/kg per day of prednisone or its equivalent, or < 20 mg/day if the patient weighs more than 10 kg."<sup>29</sup> We consider low dose as < 0.5 mg/kg per day.) Other oral agents, such as dapsone or atovaquone, may be used.<sup>30</sup> There have been deaths resulting from *Pneumocystis* pneumonia in young patients reported to the DBAR, and these are largely preventable with adequate prophylaxis. To avoid gastric upset, we use twice-daily steroid dosing and start a proton pump inhibitor, such as lansoprazole, or an H2 blocker, such as ranitidine, concurrently. We are also mindful of stress steroid dosing should patients need surgery or have a severe infection while on therapy. Patients must be carefully monitored for infections while on therapy. We recommend a normal vaccination schedule for DBA patients. This is greatly facilitated by delaying the initiation of steroid therapy until one year of age. If steroid therapy was begun before completion of the initial series of vaccinations, we check titers to confirm immunity, as revaccination may be necessary. Immunization with live viral vaccines is also recommended before the initiation of steroid therapy, if possible. If steroid therapy has commenced, then immunization with live vaccines should be not be given when the patient is on high steroid doses. If the patient is on high doses of steroids, live vaccines should be given only if clinically warranted, as in high endemic areas.<sup>29</sup>

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## Red cell transfusion and iron chelation

Patients receiving chronic red cell transfusion therapy generally require transfusions of 10 to 15 mL/kg every 3 to 5 weeks to maintain a hemoglobin level more than 8 g/dL. We monitor patients for growth and development and individualize transfusion therapy as needed. Some patients, especially younger children, require higher hemoglobin levels for daily functioning and adequate growth. For these children, we will transfuse to keep the hemoglobin greater than 9 g/dL for a period of time. The major problem with transfusion therapy is iron overload, which can lead to cardiac hemosiderosis and insulin-dependent diabetes mellitus, hepatic cirrhosis, hypoparathyroidism, hypothyroidism, and delayed puberty. Other than transplantation-related mortality, transfusion-associated iron overload is the leading cause of death for patients with DBA. Thus, we carefully screen transfused patients for iron overload and begin chelation when the patient has received 10 to 20 transfusions at 10 to 15 mL/kg per transfusion. Serum ferritin has traditionally been used as a surrogate marker for total body iron burden. However, serum ferritin determination can be unreliable as a measure of total iron burden.<sup>31</sup> Although following ferritin trends may be helpful over time, we follow multiple parameters, including biopsy-determined liver iron concentration (LIC) and magnetic resonance determinations of hepatic, cardiac, and pancreatic iron burden. Liver biopsy is an invasive procedure; however, it has the advantage of identifying significant histology, such as fibrosis and cirrhosis. An LIC of less than 3 mg/g, dry weight is considered "normal"; the acceptable LIC for a transfusion-dependent patient is between 3 and 7 mg/g, dry weight. We strive for the lowest level possible. An LIC of greater than 15 mg/g, dry weight has been associated with clinically significant cardiac iron overload and risk of cardiac death<sup>32</sup> in patients with thalassemia major. However, with advances in

imaging, hepatic iron appears to be a poor surrogate for cardiac iron burden in general,<sup>33,34</sup> and in patients with DBA in particular.<sup>35</sup>

The relaxation rates R2 (1/T2) and R2\* (1/T2\*), as measured by magnetic resonance imaging and verified by liver biopsy,<sup>36</sup> are directly proportional to iron burden. The normal myocardial T2\* is in the range of 40 ms and has a nonparametric distribution such that normal subjects do not have values less than 20 ms. Therefore, iron overload in the heart is “defined” as a T2\* less than 20 ms.<sup>37</sup> There is a correlation between decreasing ejection fraction and T2\* values less than 20 ms<sup>38</sup> and heart failure and T2\* less than 10 ms.<sup>39</sup> Therefore, we consider myocardial T2\* values less than 10 ms as severe and an indication for urgent iron chelation. Cardiac siderosis can manifest as a toxic cardiomyopathy or fatal arrhythmia and has been the cause of death in 9 patients reported to the DBAR (A.V., unpublished data, September 2010). The onset of cardiomyopathy is insidious, and the diagnosis is usually confounded by left ventricular EF in the normal or near-normal range.<sup>37</sup> The heart and the pancreas, as opposed to the liver, share a high susceptibility to nontransferrin bound iron. Thus, the appearance of pancreatic iron may be a better surrogate for cardiac iron burden than hepatic iron.<sup>40</sup> The recent finding of increased iron overload in DBA patients compared with transfusion-dependent  $\beta$ -thalassemia patients strongly support more careful monitoring.<sup>35</sup>

For years, we have used the traditional chelation regimen of deferoxamine at 40 to 60 mg/kg per day given subcutaneously over 8 to 12 hours/night for 4 to 7 nights/week. New, more easily administered, oral chelators have permitted a potentially more viable option.<sup>41</sup> Deferasirox has been used at doses of 20 to 30 mg/kg per day, and more recently 40 mg/kg per day, with good results in  $\beta$ -thalassemia patients.<sup>42,43</sup> We have experienced better results with the higher dose, with limited toxicity. Deferiprone at 80 to 100 mg/kg per day has also been shown to be an effective iron chelator with some studies suggesting preferential unloading of cardiac iron.<sup>44-46</sup> However, agranulocytosis has been noted as a side effect of deferiprone administration,<sup>47-49</sup> with a fatal outcome in a patient with DBA.<sup>50</sup> We therefore reserve the use of deferiprone in DBA for extreme cases of cardiac iron overload and congestive cardiomyopathy. Deferasirox has been well tolerated and has not been associated with agranulocytosis in DBA patients thus far. Data regarding cardiac iron unloading with deferasirox is forthcoming. As well, combination trials of deferoxamine and deferasirox are underway; and once safety and efficacy are established, use in severe transfusion-associated cardiomyopathy may be warranted. Intravenous deferoxamine at 50 to 60 mg/kg per day over 24 hours has been able to rescue patients with severe cardiac and hepatic iron toxicity.

In summary, we start the transfusion-dependent DBA patient on chelation therapy after approximately 15 transfusions or after age 2. We generally start with deferasirox at 20 mg/kg per day to confirm tolerability and increase to 30 mg/kg per day within the first month of treatment, monitoring for rash, elevations in liver function tests, and renal dysfunction. We aim to keep the ferritin level between 1000 and 1500 ng/mL. Our maximum dose is 40 mg/kg per day. We monitor the ferritin every 3 months and adjust the dose for weight gain. If an increasing trend in ferritin levels is noted, then compliance is questioned and further workup to assess the iron overload is warranted. If the patient has been stable, then we usually conduct the MRI T2\* test when the child can perform the test without sedation. If the patient's ferritin is not stable and compliance is confirmed, then we will sedate the younger patient for an MRI. If the LIC is elevated more than expected, then we will confirm with a liver biopsy. If the patient is

considered a deferasirox failure, then we will start with deferoxamine subcutaneously at 50 to 60 mg/kg per day for 10 to 12 hours. If the patient has been noncompliant and has a greatly elevated LIC (> 12-15 mg/g, dry weight) or a markedly decreased cardiac T2\* (< 12 ms), we will institute deferoxamine at 50 to 60 mg/kg per day by continuous intravenous infusion over 24 hours each day. We will follow with repeat scanning in 3 to 6 months to look for improvement. We will monitor the patient with kidney and liver function tests at every transfusion (every 3-4 weeks). Once a patient is in the “safe zone” for hepatic and/or cardiac iron burden (LIC < 7 mg/g, dry weight and T2\* > 20 ms), then we can resume oral deferasirox. We reserve deferiprone for those patients with severe cardiac hemosiderosis, usually symptomatic.

Venous access may be problematic for patients who require monthly transfusion therapy. Often venous access devices are needed to alleviate this difficulty. We recommend the placement of plastic ports for transfusion-dependent patients. Nonmetal containing (plastic) ports are compatible with MRI scanners and therefore allow future screening for iron overload with the aforementioned tests. If inadequate iron chelation has resulted in severe iron overload, then intravenous chelation with deferoxamine can also be accomplished through a port device.

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## Alternative therapies

Historically, the lack of truly effective and safe therapy has prompted the investigation of several other agents, including high-dose corticosteroids,<sup>51,52</sup> intravenous immunoglobulin,<sup>53,54</sup> high-dose erythropoietin,<sup>55</sup> interleukin-3,<sup>56-59</sup> androgens,<sup>60</sup> and others. These modalities have achieved, at best, anecdotal success. Immunosuppressive therapy with cyclosporine A has been studied in DBA patients with no theoretical basis. Only very marginal success has been demonstrated.<sup>61-63</sup> Antithymocyte globulin has also had very limited use in DBA.<sup>64</sup> Metoclopramide has also shown to be effective in DBA. Abkowitz et al<sup>65</sup> described a 33% hematologic response rate in a small group of patients with DBA using metoclopramide, a dopamine antagonist that induces the release of prolactin from the pituitary gland. It was proposed that prolactin probably improves erythropoiesis by stimulating cells in the microenvironment of erythroblasts. Unfortunately, other studies in the United States and Europe did not confirm these responses but showed an overall 10% response rate.<sup>66,67</sup> Most recently, leucine and lenalidomide have been contemplated for use in DBA. Recently, leucine has been tried in one patient with DBA and reported to have attained a remission.<sup>68</sup> Others have been reported to the DBAR as having had responses as well, although not complete. The DBAR is embarking on a treatment protocol to evaluate the efficacy of leucine in patients with DBA.

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## Remission

Patients with DBA can also enter a state of remission. The DBAR defines “remission” as an adequate hemoglobin level without any treatment, lasting 6 months, independent of prior therapy. The actuarial likelihood of remission is 20% by age 25 years, with 72% experiencing a remission during the first decade of life.<sup>24,69</sup> Some patients have experienced more than one remission in their lifetime. Therefore, we challenge our patients who are on low, twice-weekly steroid dosing to determine whether discontinuation of the steroids is possible, as they may be in a remission. Relapses

are possible, often after a seemingly innocent viral illness. Women also may relapse during pregnancy.<sup>70</sup> The biology of remission is unknown but has been noted in patients of all genotypes evaluated.

## Stem cell transplantation

Stem cell transplantation is the only definitive treatment for the hematologic manifestations of DBA.<sup>71</sup> Allogeneic matched sibling HSCT has been very successful.<sup>72</sup> The data from the DBAR reveal an overall survival of  $77.3\% \pm 8.3\%$  for sibling HSCT and  $31.5\% \pm 12.7\%$  for alternative donor HSCT ( $P = .012$ ). For sibling HSCT done before 9 years of age, survival was  $90.0\% \pm 9.5\%$ , and for those older than 9 years of age, was  $70.0\% \pm 11.6\%$  ( $P = .007$ ). Survival for all patients before 2000 and since 2000 was  $47.1\% \pm 11.0\%$  and  $72.4\% \pm 9.3\%$ , respectively ( $P = .041$ ). Survival for alternative donor HSCT was  $23.1\% \pm 11.7\%$  before 2000 and  $85.7\% \pm 13.2\%$  since 2000 ( $P = .047$ ). Thus, the best outcomes for HSCT occur when using HLA-matched sibling donors in patients 9 years of age or younger. We therefore recommend HLA-matched sibling transplantations for transfusion-dependent patients between 3 and 9 years of age. However, every sibling donor should be carefully screened, including genotyping when known, even when the donor has no evidence of any hematologic or physical manifestation of DBA. The hematologically normal donor must not carry the DBA gene found in the patient. Indeed, an inadvertent transplantation reported from an affected donor resulted in nonengraftment.<sup>73</sup> There has been significant improvement in survival with alternative donor HSCT since the year 2000, which may be a result of improved HLA-matching techniques.<sup>74</sup> The dramatic improvement in alternative donor transplantation suggests that this approach no longer needs to be reserved for secondary aplastic anemia, leukemia, or other severe hematologic complications but may be offered as front-line therapy in certain circumstances, if a molecular HLA match is identified. With the recognition of the very high risk of cardiac iron overload in DBA patients, this modality will probably provide an important option for select patients.

The majority of sibling donor HSCT have been performed using myeloablative chemotherapy regimens. These regimens generally include busulfan, cyclophosphamide, and an immunosuppressive agent, such as antithymocyte globulin. Alternative donor HSCT conditioning included total body irradiation when done before the year 2000, but the more recent HSCTs have been successfully performed without irradiation. Reduced intensity and nonmyeloablative conditioning regimens have also been successful, but we have reserved their use for adult patients with years of iron exposure or pediatric patients with significant organ toxicity. HSCT may be less successful in older patients because of their iron burden. Given the aforementioned increase in iron loading in DBA patients, we are very aggressive in achieving good iron balance before HSCT with the use of 24-hour continuous intravenous chelation with deferoxamine at 40 to 60 mg/kg per day. Deaths from veno-occlusive disease have been reported to the DBAR and seem probable in large part because of hemosiderosis. After HSCT, we use periodic phlebotomy, when the hemoglobin permits, to eliminate residual iron burden. A variety of stem cell sources, including related and unrelated donor bone marrow, peripheral blood stem cells, and cord blood, have been used successfully.<sup>53</sup> We therefore strongly recommend cord blood storage from subsequent pregnancies, keeping in mind the possibility of DBA-affected siblings. We carefully evaluate the therapeutic choices and work

with families to determine the best course of action when considering HSCT.

## How we monitor the patient

The frequency of blood count monitoring is dependent on patient status, treatment, and response. Transfusion-dependent patients have a blood count every 3 to 5 weeks based on their transfusion schedule. In contrast, steroid-maintained patients need blood counts every 3 to 4 months to monitor for occasional decreases in hemoglobin often seen with infection. Exposure to a known parvovirus contact should warrant a blood count to look for a more acute decrease in hemoglobin, and an acute Hb drop should result in testing for parvovirus. We also monitor blood counts for the development of neutropenia and/or thrombocytopenia. Some patients will exhibit neutropenia but only rarely need treatment with granulocyte-colony stimulating factor. However, if a patient develops clinically significant cytopenias, a thorough reassessment of the diagnosis should be made with bone marrow aspirate and biopsy and evaluation for other IBMFS, if not previously done. The incidence of neutropenia in DBA is currently unknown, but it is usually not clinically significant. The development of thrombocytopenia is more often indicative of marrow failure and warrants careful monitoring and evaluation. As mentioned, bone marrow cellularity appears to decrease with age more rapidly than in normal persons. There have been 5 DBA patients reported to the DBAR who developed severe aplastic anemia.<sup>10</sup> These patients have not responded to medical management for aplastic anemia with antithymocyte globulin, making HSCT the only treatment option. Acute leukemia and MDS have also been reported in DBA. Thus, we perform periodic bone marrow evaluations if there is a change in blood counts. We do not perform periodic marrow examinations in hematologically stable patients. Other follow-up evaluations will be discussed in the context of treatment.

## How we manage the nonhematologic manifestations of DBA

Appropriate management of the systemic manifestations of DBA along with the side effects of our present treatments allows the DBA patient to have a long and productive life. Once treatment is instituted, we advise monitoring by appropriate subspecialists. We advise regular ophthalmologic examinations for steroid-dependent patients and for transfusion-dependent patients every 6 months to one year based on dosage or chelation schedule, respectively. For patients receiving chelation therapy, audiologic examinations are also recommended every 6 months to one year. Transfusion-dependent patients should be monitored by cardiology with yearly echocardiogram and electrocardiogram. As the patient ages, Holter monitoring should also be done as arrhythmias are a significant problem in severely iron-overloaded patients. We refer all DBA patients to an endocrinologist by 5 years of age as endocrinologic surveillance for growth issues and diabetes is necessary, keeping in mind that HbA<sub>1c</sub> determination has no utility in a chronically transfused patient. Transfusion-dependent patients with iron overload are also at high risk of developing thyroid and parathyroid problems as well as hypogonadism. If endocrine abnormalities are detected early, then appropriate management not only can be instituted by the endocrinologist, but management of the iron overload can become more aggressive.

Management of adult DBA patients is becoming more and more complex, and these patients also require the coordination of multiple specialists. Ophthalmologic, endocrinologic, reproductive, hepatic, and cardiac issues increase the complexity of DBA management. Patient education with regard to the disorder, its treatment, and its side effects must be presented early to the patient to maximize patient compliance with therapy. The keys to better outcomes in adults with DBA include exquisite care during childhood, the early recognition of nonclassic DBA presentations, and awareness of the disorder by hematologists treating adults.

Data from the literature and patient registries strongly support DBA as a cancer predisposition syndrome. Leukemias and solid tumors have been reported in the literature and to the DBAR.<sup>10</sup> Acute myeloid and lymphoid leukemias have been reported along with solid tumors, such as osteogenic sarcoma, soft tissue sarcoma, lymphoma, breast, hepatocellular, gastric, and colon cancer. DBA patients with cancer appear to have a poor prognosis. Untoward side effects from chemotherapy with increased toxicity and prolonged cytopenias have been characteristic.<sup>10</sup> More data are required before we can make any recommendations regarding cancer surveillance in this population.

Pregnancies in women with DBA, or when there is a possibility of a DBA-affected fetus, should be treated as high-risk. A survey of the French and German DBA registries found complications in 66% of pregnancies.<sup>70</sup> There were no correlations found between pregnancy outcome and features of either maternal or child DBA. DBA-affected women who are pregnant must be monitored for the probable event of worsening of anemia, often requiring transfusion therapy, as well as vascular-placental issues and preeclampsia. Special precautions should be taken to check for possible intrauterine growth retardation and/or anomalies, and even fetal death.

Overall actuarial survival, as reported by the DBA Registry, is 75.1% ± 4.8% at 40 years of age, 86.7% ± 7.0% for steroid-maintainable patients ( $P = .08$ ), and 57.2% ± 8.9% for transfusion-dependent patients ( $P = .007$ ).<sup>24</sup> One important caveat is that many of the transfusion-dependent patients died of complications of HSCT, and not just from transfusion-associated complications, such as iron overload. There have been 54 deaths reported to the DBAR (A.V., unpublished data, September 2010). Of these, 67% are treatment-related (58% from stem cell transplantation-related complications, 25% from iron overload complications, 14% from infections, and 3% from venous access device complications). A total of 22% of the deaths were DBA-related (83% from malignancy and 17% of severe aplastic anemia). Two patients (4%) died of pulmonary embolism. The causes of 7% of the deaths were not reported.

In conclusion, clinical and laboratory science regarding DBA is evolving rapidly. We are hopeful that these advances will lead to a better understanding of the disorder and to potential new therapies. In the meantime, we are looking to improvements in the management of steroid therapy and red cell transfusion support as well as improved results for HSCT. The understanding of the biology of DBA suggests that, in the future, the manipulation of cell death pathways as well as the promise of gene therapy may be realized. However, scientists must be mindful of the pitfalls of this type of therapy, namely, increasing the risk of malignancy in a population with a known predisposition. Patient registries have played an important role in these improved outcomes as well as providing well-characterized patient samples for laboratory investigation. We have attempted, herein, to synthesize our approach to the DBA patient that is based on science and experience.

## Acknowledgments

The authors thank Jeffrey M. Lipton for his continued advice and support and Eva Atsidaftos who is more than dedicated to the DBA patients and our efforts; Marie and Manny Arturi and the Daniella Maria Arturi Foundation for all their unwavering support for our research and beyond; the Diamond Blackfan Anemia Foundation; and all the physicians and patients for participating in the Diamond Blackfan Anemia Registry.

This work was supported by the National Institutes of Health (R01 HL079571; A.V.), the Centers for Disease Control and Prevention (Cooperative Agreement U27/CCU 224942; A.V., E.M), the Feinstein Institute for Medical Research at the North Shore-Long Island Jewish General Clinical Research Center (M01 RR018535; A.V.), and the Diamond Blackfan Anemia Gene Discovery and Resequencing Project of the National Heart, Lung, and Blood Institute Resequencing & Genotyping Service (R109MOHLKE; A.V.).

## Authorship

Contribution: A.V. and E.M. wrote the manuscript.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

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